Appetitive Associative Conditioning in the Basolateral Amygdala

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Declaration

(i) I, Claire Stirling Birch, hereby certify that this thesis, which is approximately 85,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

Date: 31st July 2004

Signature of candidate:

(ii) I was admitted as a research student in September 1997 and as a candidate for the degree of Ph.D. in September 1997; the higher study for which this is a record was carried out in the University of St Andrews between 1997 and 2001.

Date: 31st July 2004

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(iii) I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Ph.D. in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

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Abstract

This thesis is concerned with the neural basis of appetitive associative conditioning or 'reward learning' in rats. Research has implicated several brain structures within what is known as the cortico-striato-pallido-thalamic or 'limbic' loop in reward learning, including the nucleus accumbens and the amygdala. Wolfram Schultz and colleagues recently proposed that dopamine neurons, which project from the midbrain ventral tegmental area (VTA) to the ventral striatum (including the nucleus accumbens), signal expectancy of reward. They have shown that, not only do dopamine neurons markedly increase their activity at the presentation of unexpected rewards, but that with training this response transfers to stimuli predictive of those rewards. Moreover, once this transfer has occurred, if an expected reward does not in fact occur, there is a brief reduction in dopaminergic activity at the time that it should have occurred. The experimental work undertaken for the first part of this thesis describes the behavioural testing of an appetitive 'blocking' paradigm which was intended to explore and substantiate Schultz et al's proposal that dopamine signals a reward prediction error by assessing whether the transfer in dopaminergic activity is indeed due to contingency or merely to temporal contiguity. The results of the blocking paradigm were inconclusive. The amygdala, closely associated with the limbic loop, has long been associated with 'emotional' behaviour with damage leading to hypoemotionality in animals and to deficits in the perception of emotions in facial expression and impaired learning of and memory for emotional events in humans. It is becoming increasingly clear that the amygdala is also important for appetitive associative learning. The experimental work undertaken for the second part of this thesis investigates the relative contributions of the basolateral nucleus of the amygdala (BLA) and the central nucleus of the amygdala (CeN) to reward learning by assessing the performance of BLA- and CeN-lesioned rats on the Schedule Fraction Cue (SFC) task. In this task rats must make three, two, or one correct responses in order to receive reward, and are able to use information provided by cue lights to notify them as to their progress. Since previous research has suggested that the BLA is critical for the acquisition of positive incentive value by formerly neutral stimuli, and since it

has also been suggested that Pavlovian conditioned stimuli can exert a motivational influence on instrumental behaviour (Pavlovian-to-instrumental transfer), it was initially predicted that BLA-lesioned rats would be impaired in their performance of this task. The results of Experiment A suggested that this was not the case. More recent research has suggested that CeN lesions abolish Pavlovian-to-instrumental transfer, and so the experiment was repeated, using both BLA- and CeN-lesioned rats. The results of Experiment B showed very little impairment in performance in the BLA- or CeN lesioned rats. It is concluded that any impairments present in BLA- or CeN-lesioned rats are very subtle and will require further investigation.

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Chapter 1 - Introduction

1.1 Overview

Reward is of vital importance to the survival and well being of most animals. The term reward most readily brings to mind the idea of something pleasurable in its own right, but in a wider sense it also connotes the positive reinforcement of behaviours that enable an animal to survive and reproduce. Many factors influence just how 'rewarding' or desirable an animal finds a given reward, such as its intrinsic value, how accessible it is, what it is going to cost in terms of effort to obtain or consume, and how predictable it is. Other factors are extrinsic to the reward itself, such as how hungry or thirsty the animal is at the time that it encounters the reward, which in turn influences how motivated it will be to obtain the reward again in the future (Dickinson and Balleine 1994). All these factors influence learning about reward, whether it involves the association of given stimuli with reward, as in classical (Pavlovian) conditioning, or the engagement in certain behaviours which lead to reward, as in instrumental conditioning.

Research into the neural mechanisms underlying reward-learning is intensive, not least because they might be similar to those underlying drug addiction and such 'affective' psychopathologies as eating disorders, obsessive-compulsive disorder and depression. Given the diversity of factors involved, it is perhaps not surprising that multiple and dissociable neural systems are being found to mediate reward-learning. Early and powerful evidence that this is indeed the case was provided by intra-cranial self-stimulation studies. Animals will work to self-administer electrical stimulation to certain regions of the brain, thus making it possible to map the crucial structures involved in reward-processing and the systems with which they interact (Olds and Milner 1954; Wise 1998). Implicated structures include several within what is now known as the cortico-striato-pallido-thalamic or 'limbic' loop (Alexander, DeLong et al. 1986; Alexander and Crutcher 1990) such as the amygdala, nucleus accumbens, hippocampus, and prefrontal and cingulate cortices ***(Figure 1)***.

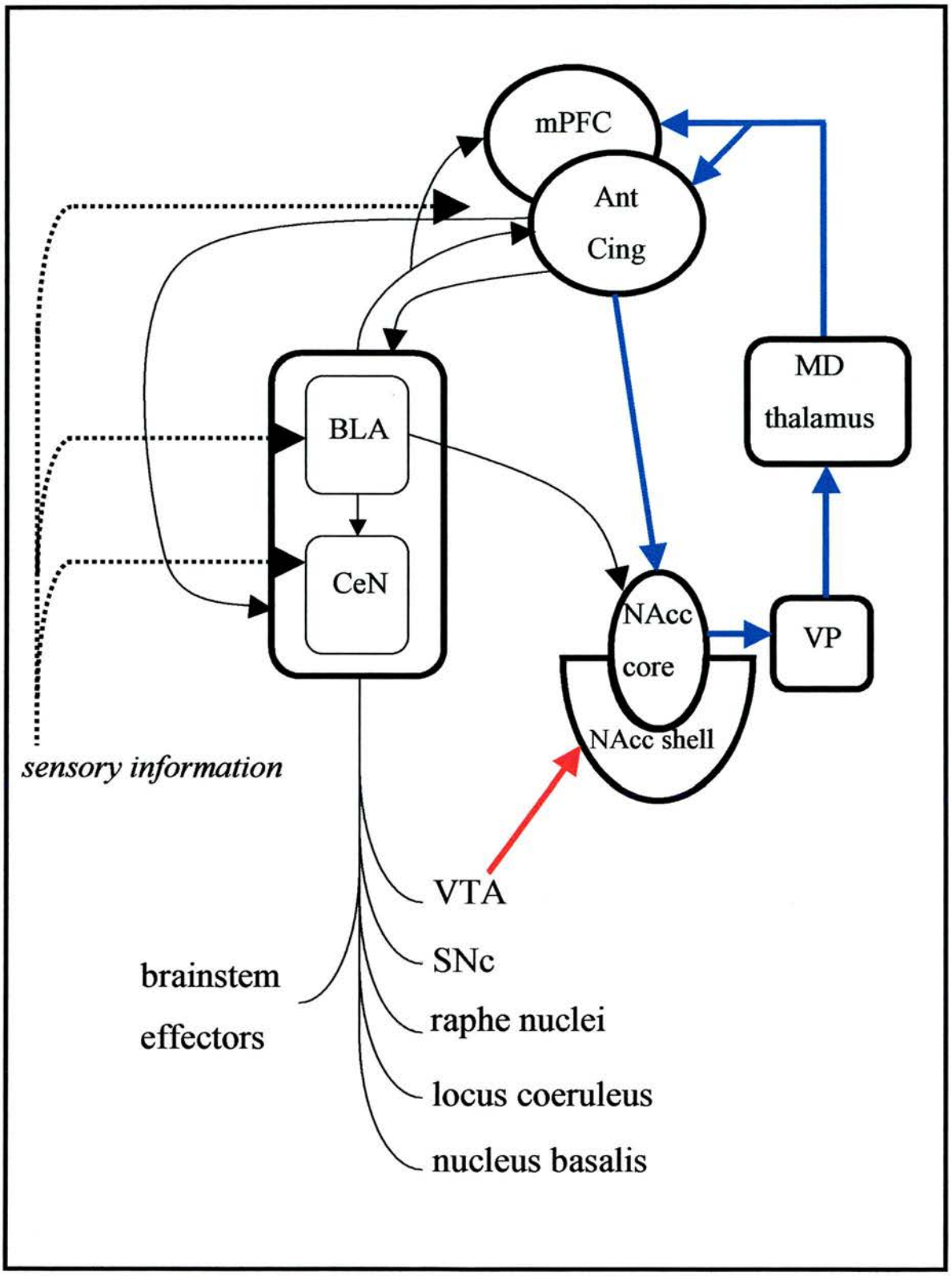


Figure 1: *The cortico-striato-pallido-thalamic or 'limbic' loop, taken and adapted slightly from Parkinson et al (Parkinson, Cardinal et al. 2000). The main loop is shown in bold, and, for clarity, the hippocampal structures are not shown. Abbreviations are as follows: medial prefrontal cortex (mPFC), anterior cingulate (Ant Cing), medial dorsal thalamus (MD thalamus), basolateral nucleus of the amygdala (BLA), central nucleus of the amygdala (CeN), nucleus accumbens (NAcc), ventral pallidum (VP), ventral tegmental area (VTA), substantia nigra pars compacta (SNc).*

The amygdala has long been associated with 'emotional' behaviour. Brown and Schafer (1888) and Kluver and Bucy (1939) showed that bilateral lesions of the temporal lobes, including the amygdala, made monkeys remarkably tame and hypoemotional, apparently because they were no longer able to recognise the emotional or behavioural significance of sensory stimuli and subsequent studies showed that lesions restricted to the amygdala alone could produce similar deficits (Weiskrantz 1956; Aggleton and Passingham 1981). In humans, stimulation of the amygdala produces emotional responses that are usually fearful, though sometimes angry or pleasurable feelings are evoked (McDonald 1998). Damage to the amygdala has also been shown to cause deficits in the perception of emotions in facial expression (Adolphs, Tranel et al. 1994; Young, Aggleton et al. 1995) and impaired learning of, and memory for, emotional events (Bechara, Tranel et al. 1995; Cahill 2000). Most of these emotional responses are dependent on the autonomic, somatic, and endocrine functions of the hypothalamus and brainstem, and stimulation and ablation studies in animals have shown that the amygdala's involvement in biologically-driven behaviours such as arousal, orientation, sleep, eating and drinking, fight or flight responses, reproductive and maternal behaviour occur through its modulation of the activity of these regions (LeDoux 1987; McDonald 1998). However, it is becoming increasingly clear that the amygdala is also important for the processing of positive emotions, and more specifically, for appetitive associative learning. Weiskrantz (1956) observed that amygdalectomized monkeys exhibited impaired conditioned reinforcement learning, and suggested that the effect of the lesion "is to make it difficult for reinforcing stimuli, whether positive or negative, to become established or recognised as such". This view has been reinforced by more recent research, which also indicates that the impairment might largely be due to the amygdala's robust connections with two other brain regions that have also been shown to be particularly involved in conditioned reinforcement learning – the prefrontal cortex (PFC) and the ventral striatum, including the nucleus accumbens (NAcc) (Davis 1992; LeDoux 1992; Rolls 1992). The cerebral cortex massively innervates the amygdala with sensory information, the majority of which comes from the PFC in a highly processed form (McDonald 1998), and it has been shown that both direct and indirect connections between the PFC and the

amygdala are essential for the formation of stimulus-reward associations (Gaffan, Murray et al. 1993). Likewise, the amygdala projects very strongly to the ventral striatum, including the NAcc (Krettek and Price 1978; McDonald 1991; McDonald 1998), and this projection has been shown in the rat to be important for controlling instrumental behaviour that is dependent on stimulus-reinforcement associations (Everitt and Robbins 1992). Moreover, anatomical studies in the rat have shown that there are connections between the PFC and the ventral striatum, suggesting that the PFC is able to interact in a co-ordinated manner with specific amygdalostriatal subcircuits (Groenewegen, Berendse et al. 1990; McDonald 1991; McDonald, Mascagni et al. 1996), though the possible functional significance of this remains to be determined (McDonald 1998). It does, however, appear that in its connections with the PFC, the hypothalamus and brainstem, and the striatum, the amygdala is essential for producing appropriate behavioural responses to biologically relevant sensory stimuli and events, constituting an essential link between those brain regions that process sensory information and those responsible for eliciting emotional, motivational and learned responses (McDonald 1998).

Research into the involvement of the NAcc in reward learning has been hugely influenced by the discovery that the mesolimbic dopamine system, which serves to connect the midbrain ventral tegmental area (VTA) with the ventral striatum (including the NAcc) in the forebrain, is also a potent site for the reinforcing effects of intracranial self-stimulation (Crow 1971; Liebman and Butcher 1973; Lippa, Antelman et al. 1973). This finding was followed by the discovery that many drugs of abuse, including amphetamine, cocaine, heroin, nicotine, cannabis and alcohol, while having very different primary molecular targets, all increase levels of dopamine in the NAcc during self-administration in rats (Hoebel, Monaco et al. 1983; Di Chiara and Imperato 1988; Pettit and Justice 1989; Di Ciano, Coury et al. 1995; Egilmez, Jung et al. 1995; Wise 1996; Ikemoto and Panksepp 1999). Moreover, lesions of the dopamine cell bodies within the VTA impair the intravenous self-administration of cocaine (Roberts and Koob 1982), as do lesions of dopaminergic terminals in the NAcc (Roberts, Koob et al. 1980; Pettit, Ettenberg et al. 1984). Systemic administration of dopamine antagonists, which block dopamine receptors, decrease not only rates of self-stimulation and rates of instrumental responding for

drug reward, but also rates of responding for 'natural' rewards such as food or water (Salamone, Cousins et al. 1997; Wise 1997; Wise 1998; Ikemoto and Panksepp 1999). Such studies have led to the idea that the reinforcing effects of natural rewards such as food and sex might similarly be mediated by the dopamine system (Robbins and Everitt 1996). However, early ideas that increases in dopamine have a direct hedonic impact (Wise 1982) have had to be reassessed in the light of research showing that NAcc dopamine also appears to be involved in aversive events (Salamone 1994; Salamone, Cousins et al. 1997): aversive stimuli such as foot-shock and tail-pinch appear to facilitate the release of dopamine in the NAcc (Abercrombie, Keefe et al. 1989; Bertolucci-D'Angio, Serrano et al. 1990; McCullough, Sokolowski et al. 1993), as do anxiogenic pharmacological manipulations (Bertolucci-D'Angio, Serrano et al. 1990; McCullough and Salamone 1992). The presentation of cues associated with aversive events has also been shown to trigger dopamine release in the NAcc (Wilkinson, Humby et al. 1998; Ikemoto and Panksepp 1999). Various theories have been proposed in an effort to accommodate such contradictory data concerning the role of dopamine. One suggestion is that dopamine innervation of the NAcc might be involved in behavioural activation and the overcoming of response costs rather than being intrinsically rewarding (Salamone, Cousins et al. 1994; Salamone, Cousins et al. 1997). Another is that dopamine is released in response to novel stimuli, whether rewarding or aversive: in-vivo recordings of dopamine neurons (Kosobud, Harris et al. 1994; Mirenowicz and Schultz 1996; Schultz, Dayan et al. 1997) and measurements of dopamine release in NAcc (Fontana, Post et al. 1993; Gratton and Wise 1994; Kiyatkin 1995; Ito, Dalley et al. 2000) consistently show increased dopamine activity to unexpected primary and conditioned appetitive stimuli. Schultz and colleagues have recently proposed that not only do dopamine neurons show markedly increased activity at the presentation of unexpected rewards, but that with training this response transfers to stimuli predictive of those rewards. Moreover, once this transfer has occurred, if an expected reward does not in fact occur, there is a brief reduction in dopaminergic activity at the time that it should have occurred. They suggest that by thus signalling errors in reward prediction dopamine may act as a teaching signal for striatal learning (Schultz, Dayan et al. 1997; Schultz and Dickinson 2000).

The work undertaken for this thesis is divided into two sections. The experiments in the first section (Chpater 2) describe the testing of an appetitive 'blocking' paradigm which was intended to be used to explore and substantiate Schultz et al's (1997) assertion that dopamine signals a reward prediction error. Those in the second section (Chapters 3 and 4) investigate the relative and perhaps dissociable contributions of the basolateral nucleus of the amygdala (BLA) and the central nucleus of the amygdala (CeN) to reward learning through assessing performance on the Schedule Fraction Cue (SFC) task. First, however, some of the issues underlying associative learning are described below in the section on Pavlovian and instrumental conditioning. Then the anatomy of the amygdala is discussed, followed by a review of the role of the BLA and CeN in reward-learning.

1.2 Pavlovian and Instrumental conditioning

1.2.1 Pavlovian conditioning

Pavlovian conditioning is named after I.P Pavlov (1927) who found that if dogs consistently heard a bell rung just before they received food, then they would begin to salivate when they heard the bell without the food needing to be present. Pavlov termed the food the unconditional stimulus (US) and the sound of the bell the conditional stimulus (CS), the salivation to the food the unconditional response (UR) and the salivation to the sound of the bell the conditional response (CR). Further research into this phenomenon revealed that, after repeated pairings, other conditioned stimuli could elicit conditioned responses similar to the unconditioned responses that had initially only been elicited by the unconditioned stimulus. Pavlov himself considered that the CR developed because an association is formed between representations of the CS and the US, and that presentation of the CS therefore elicits a representation of the US, which in turn will elicit a response (an idea termed stimulus substitution theory (Pavlov 1927; Tolman 1934). However, Thorndike's (1911) research into the processes underlying instrumental conditioning (see below) lead others (e.g. Guthrie (1935), Hull (1943)) to propose that Pavlovian conditioning could equally be explained by the formation of a direct association

between a representation in memory of the CS and a component of the response elicited by the US, i.e. the UR (known as stimulus-response (S-R) theory). Importantly, the CS was thought to elicit the response without the animal in any way expecting the US to be presented. There is some evidence to support the S-R theory of Pavlovian conditioning. For example, Aplysia will withdraw its respiratory organ, the gill, if a stimulus is applied to its siphon or mantle shelf, and the strength of this withdrawal reflex is strengthened if stimulation of either siphon or mantle shelf is followed by electric shock to the tail. Carew, Hawkins and Kandel (1983) showed that if, during training, stimulation to the siphon was paired with a tail-shock, whereas stimulation to the mantel shelf was not, then, at test (when shock was not presented), stimulation to the siphon was followed by a stronger withdrawal response than stimulation to the mantle shelf. In stimulus-response terms, presentation of the CS (stimulation of the siphon) directly excited the US response centre (motor neurons responsible for gill withdrawal) and lead to a response (gill withdrawal) that mimicked the one normally elicited by the US (shock to tail). Likewise, in eyeblink conditioning an animal (usually a rabbit) is presented with a tone CS followed by the delivery of a mild shock to the cheek (the US) which causes it to blink (the UR). After a number of pairings the CS, which would not normally cause the animal to blink, will elicit this response (Gibbs, Latham et al. 1978).

However, S-R theory has been unsuccessful in explaining many of the effects found in other Pavlovian conditioning procedures, with perhaps the most notable example being the effect of post-conditioning devaluation of the US. In this procedure, rats are put into a conditioning chamber and presented with a CS (such as a tone) which is followed by the delivery of a food reward into a food hopper. The reward is then devalued by pairing it with lithium chloride (LiCl) injections, which cause nausea, in the home cage. The rats are returned to the conditioning chamber and again presented with the CS, this time not followed by reward, and their tendency to approach the food hopper observed. According to S-R theory, the initial training should result in the formation of an association between the CS and the response of approaching the food hopper. Since the CS is not present during the devaluation of the food reward, the rats should continue to approach the food hopper after its presentation. In fact, they

do not tend to approach the food hopper (Holland and Straub 1979), a result which is entirely consistent with Pavlov's (1927) proposal that Pavlovian conditioning results in the growth of CS-US associations: on the test session the CS will excite a representation of the US that is now undesirable, and the rats will therefore no longer tend to approach the food hopper. Interestingly, though, in Holland and Straub's (1979) experiment, there was some residual tendency to approach the food hopper after devaluation of the reward, which suggests that training resulted in both S-R and CS-US associations being formed. Many other studies have also demonstrated that Pavlovian conditioning fosters the formation of CS-US associations (e.g. Holland (1981; 1990)) and it would also appear that any given CS is able to enter into several CS-US pairings (Dickinson 1980; Mackintosh 1983).

The nature of the US influences both the formation of the CS-US association and the CR. With regard to the former, Konorski (1967) suggested that a US possesses two characteristics, *specific* and *affective*, and that a CS is capable of retrieving information about either, or indeed about both simultaneously. *Specific* characteristics are those that are unique to that stimulus - its sensory properties, duration, intensity, and so forth, whereas *affective* characteristics are held in common with other stimuli and have emotional or motivational qualities that can be appetitive or aversive. For instance, food, water, and sex all have a common appetitive characteristic that means that animals will actively search them out, whereas shock, illness and loud noise all have a common aversive characteristic that means that animals will actively avoid them. Evidence that a CS can become associated with the specific characteristics of the US is provided by tasks involving post-conditioning devaluation of the US, as described above. For example, Holland (1990) conducted a study in which rats learnt to associate a tone with one flavour of sucrose (wintergreen) and white noise with another (peppermint). The wintergreen-flavoured sucrose was then devalued by pairing it with LiCl. When the rats were allowed to consume plain sucrose in the presence of each stimulus, they responded very differently, suggesting that each stimulus was associated with specific information about the flavours of the solutions with which they had been paired. Evidence that a CS can become associated with the affective characteristics of a US is provided by studies that show that the

formation of a CS-US association can sometimes be facilitated by preconditioning the CS with another US of similar affective value (Pearce, Montgomery et al. 1981). Similarly, Ganestan and Pearce (1988) found that blocking, whereby an animal fails to learn about a second CS in the presence of a first CS that already predicts the same US (Kamin 1969) is not disrupted if the US is changed from water at the first stage of training to food at the second (*see below for a more detailed description of blocking*). This suggests that the first CS must have become associated with a representation of the non-specific appetitive characteristics of the US. With regard to the nature of the US influencing the CR, Konorski (1967) proposed that US-elicited responses could be classed as either consummatory or preparatory. A consummatory CR would be performed whenever a CS became associated with a specific characteristic of a US and would normally mimic at least a component of the response to the US. For example, pigeons have been shown to make eating pecks towards a response key that has been paired with food, but drinking pecks towards one that has been paired with water (Moore 1973). A preparatory CR would be performed whenever a CS became associated with an affective characteristic of a US, and would be appropriately appetitive (a general increase in activity, for instance, or approaching the CS) or aversive (a decrease in activity, for instance, or withdrawal from the CS) (Karpicke, Christoph et al. 1977). Again, a single US may elicit several CRs.

So far, Pavlovian conditioning has been shown to result in the growth of connections between the internal representations of the CS and US, but it can also result in the growth of connections between the internal representations of two CSs, even in the absence of a US. For example, in sensory preconditioning an association is first established between CS1 and CS2. CS2 is subsequently paired with, for example, a shock, and CS1 is then found to elicit a fear CR when presented on its own, even though it has never been paired with the shock. It is generally accepted that when CS1 is presented at test, it activates a memory of CS2, which in turn activates a memory of the fear CR (Rizley and Rescorla 1972). In serial conditioning (Holland and Ross 1981) a sequence of stimuli precedes the US and the first stimulus of the sequence will often elicit a CR even though, temporally, it is not contiguous with the US, whilst in second-order conditioning (Pavlov 1927; Mackintosh 1974; Rashotte, Griffin et al. 1977; Gewirtz and

Davis 1997) a CS(1) is first paired with a US and a stable conditioned response established. Then a second stimulus (CS2) is introduced before the first stimulus (CS1), with the US usually given every tenth trial or so. With sufficient training, presentation of CS2 on its own will elicit a conditioned response that is very often (but not always) much the same as that originally elicited by CS1, even though CS2 has never been paired with the US. It could perhaps be expected that learning in serial and second-order conditioning paradigms would happen in the same way as in first-order conditioning, in that pairing of CS2 with CS1 which has already been paired with a US should result in the growth of CS2-CS1 associations. Presenting CS2 on its own should therefore activate a representation of CS2, which would in turn activate a representation of CS1 and elicit a CR. An experiment carried out by Rashotte et al (1977) with pigeons provides evidence for this theory: first, a white keylight (CS1) was paired with food, and then a blue keylight (CS2) was paired with the white keylight (CS1) with no food following. This was followed by a series of extinction trials in which the white keylight was presented alone in order to abolish its association with food. When the blue keylight (CS2) was shown again on its own, the pigeons did not peck at it, suggesting that although it still activated a representation of the white keylight, this representation no longer excited a memory of food. However, an experiment by Rizley and Rescorla (1972) which used a design very similar to that of Rashotte et al (1977) provides evidence against the idea that second-order conditioning allows CS2 to retrieve specific information about CS1: first, a tone (CS1) was paired with a shock US, and then a light (CS2) was paired with the tone but with no shock following. Extinction trials followed in which the tone was followed by no shock. Presentation of the light on its own, however, resulted in very strong conditioned suppression of behaviour. If the light had become associated with the tone, then extinction with the tone should have resulted in a reduction in conditioned suppression during the light. (Holland and Rescorla (1975) obtained a similar effect with rats using appetitive conditioning). Rizley and Rescorla (1972) explained their findings by suggesting that second-order conditioning was due to an association between the light (CS2) and a general representation of the shock (US) which was activated by the presentation of the tone (CS1); the tone therefore aroused a conditioned response of fear which was associated

directly with the light. The contradictory results of these two experiments emphasise that associative learning is considerably more complex and subtle than it was perhaps made out to be during the early days of research, and highlight the problem of distinguishing the formation of associations from their behavioural expression.

In the early days of research, Pavlovian conditioning was thought to occur automatically as long as there was close temporal contiguity between the CS and the US. Temporal contiguity is defined by the interval between the CS and the US, known as the inter-stimulus interval or ISI, and it was found that long ISIs did not generally allow associative learning although they could maintain associations that had already been established. However, temporal contiguity between CS and US has been shown to be neither necessary nor sufficient for Pavlovian conditioning (Hall 1992), and attention has turned towards the possible involvement of other factors. Two of these factors are the role of contingency and the role of unexpectedness of the US and are discussed below:

The role of contingency in associative learning was highlighted in an experiment carried out by Rescorla (1968): rats were divided into four groups and trained to lever-press for reward in one chamber. They were then removed to another chamber where they were all presented with the same number of tone (CS) followed by shock (US) pairings. But the different groups of rats also received additional shocks which were not paired with the tone: Group 1 received the same number of additional unpaired shocks as they did paired shocks, meaning that there was an equal likelihood of their being shocked in the presence and in the absence of the tone. Group 2 received half the number again of additional unpaired shocks as they did paired shocks, and Group 3 received a quarter of the number again of additional unpaired shocks as they did paired shocks; for Groups 2 and 3 there was therefore a greater likelihood of being shocked in the presence rather than the absence of the tone. Group 4 received no additional unpaired shocks at all. Strength of conditioning to the tone-shock pairing was assessed by presenting the tone alone to the rats whilst they were again lever-pressing for reward in the original chamber, and measuring the magnitude of conditioned suppression. It was found that the strength of conditioning differed greatly between the four groups even though all the rats had received the

same number of tone-shock pairings. Group 1, which received an equal number of paired and unpaired shocks showed no conditioning, Groups 2 and 3, which received fewer unpaired shocks than paired shocks, showed some conditioning, whilst Group 4, which received no additional unpaired shocks, showed the greatest conditioning. These results challenged the view that associative learning depends only on the temporal contiguity of the CS to the US by demonstrating that the degree to which the US occurs in the absence as well as in the presence of the CS is also extremely important in determining associative strength. It was originally thought that contingency would replace temporal contiguity in explaining the acquisition of behaviour. However Miller et al (1988) and Wasserman and Miller (1997) have pointed out that an organism must first learn the association between CS and US through temporal contiguity before being in a position to calculate the frequency with which the CS and the US have occurred together. Since learning must occur before contingency can be calculated, it is unlikely that contingency as such is the basis of associative learning.

The realisation that unexpectedness of the US plays an important role in associative learning was largely due to a phenomenon known as *blocking*, which was described by Kamin (1969): if a light and a tone are simultaneously presented to an animal, and are consistently followed by a shock, then, at test, the animal will react to the presentation of the light alone in the same way that it reacts to the compound presentation of both light and tone. If, however, prior to this, the animal had been trained to associate only the tone with the shock, then presentation of the light alone at test will not cause it to react as if it were expecting a shock. This is because at the compound stimulus stage of training the presentation of the light along with the tone does not give any new predictive information to the animal - it already knows to expect a shock after the tone. Kamin's (1969) blocking paradigm also illustrates the role played by contingency in associative learning. For example, in a hypothetical appetitive paradigm, an animal might be presented with 10 pairings of a tone (CSA) followed by reward, and then with 10 pairings of a compound tone (CSA) plus light (CSB) followed by reward. It is then tested with a single presentation of the tone (CSA) not followed by reward, and with a single presentation of the light (CSB) not followed by reward. 20 out of a total of 21 presentations of

the tone (95.4%) are therefore followed by reward, and 10 out of a total of 11 presentations of the light (90.9%). If the animal is merely responding according to the proportion of stimulus-reward pairings, it should become conditioned to the light, though perhaps not quite as strongly as to the tone. But if the animal is responding according to the probability that a reward is preceded by either the tone or the light, then all 20 rewards are preceded by a tone, but only 10 are preceded by a light. The animal therefore has twice the chance of receiving a reward if it responds to the tone rather than to the light, and so it will 'block' conditioning to the light.

The discovery of blocking led to the development of several theories of learning such as those put forward by Rescorla and Wagner (1967), Mackintosh (1975), and Pearce and Hall (1980), all of which stress the importance of surprise. Perhaps the most influential of these is that of Rescorla and Wagner (1967) who proposed that learning the association between CS and US depends on the US being to some extent unexpected or surprising. As the association between the CS and the US grows stronger, the occurrence of the US becomes less surprising and the rate of learning slows down. No further learning takes place when the US is entirely predicted by the CS - learning has reached its asymptote.

1.2.2 Instrumental conditioning

The limitations of Pavlovian-type learning are obvious in that it allows an animal to detect and learn only about predictive relationships between stimuli and not about the consequences of its behaviour. It is, however, very clear that many animals are able to learn that the performance of a certain action is instrumental in producing a certain outcome. Early theorists such as Thorndike (1911) did not believe that such learning reflected any real understanding of the response-reinforcer contingency on the animals' part, but rather that the accidental pairing of a particular stimulus and a particular response led to an association being formed between the two upon delivery of a reward - an animal would perhaps unintentionally press on a lever, and the subsequent delivery of a reward would establish a hypothetical connection between the neural centre responsible for the perception of the stimulus (the lever) that was present immediately

before the execution of the response and the neural centre responsible for the performance of the response itself (the pressing action). This connection would be strengthened over subsequent unintentional and, eventually, intentional pairings. Thorndike believed that all learning consists of the formation of such stimulus-response (S-R) connections, and encapsulated this idea in his 'Law of Effect': "of several responses made to the same situation, those which are accompanied or closely followed by satisfaction to the animal will, other things being equal, be more firmly connected with the situation" ((Thorndike 1911), p.244).

Miller and Konorski (1969) were perhaps the first to formalise the distinction in 1928 between Pavlovian and instrumental conditioning with their demonstration of the conditioning of an action (a dog's flexion of its hind leg) to a signal for food. Because the action was completely unrelated to the response elicited by the food, it could not be explained by Pavlov's (1927) theory of stimulus substitution, whereby the presentation of a CS elicits a representation of the US, and therefore is able to elicit a CR which is the same as (or very similar to) the unconditioned response elicited by the US itself. They therefore argued for a second, Type II, conditioning process, distinct from classical Pavlovian conditioning which they referred to as Type I. (This distinction was supported by Skinner (1932), who pointed out that Pavlovian conditioning could not explain the increased readiness with which a hungry rat would press a lever when that action resulted in a food pellet. Skinner renamed Konorski's Type II conditioning process operant conditioning because he believed that an animal's response operates on the environment to bring about some change that leads to reward). However, the clearest demonstration of this second type of conditioning was provided by Grindley (1932), who trained restrained guinea pigs to turn their heads to either the left or the right and back again when a buzzer sounded in order to receive the opportunity to nibble at a carrot. He then trained them to turn their heads in the opposite direction in order to receive the reward. Although it could be argued that such training could result in the growth of a tone-carrot Pavlovian association, it seems very unlikely that a head turn should develop as a Pavlovian CR, and even more unlikely that such a Pavlovian CR could be reversed.

Grindley (1932), as did Thorndike (1911), Miller and Konorski (1969) and others (e.g. Guthrie (1935), Hull (1943)), attributed the development of instrumental conditioning to the formation of S-R associations. However, as suggested in the preceding section, one serious drawback of the S-R theory of Pavlovian conditioning is that the CS is thought to elicit the response without the animal in any way expecting the US to be presented. This is also the case with S-R theories of instrumental conditioning – animals are expected to have no explicit knowledge of the outcome of their actions. There is some evidence to support this expectation in that instrumental behaviour can become mechanistic or 'habit'-driven. For instance, animals trained to perform an action in order to obtain a food reward have been shown to persist with that action even after the reward has been devalued (Dickinson 1980; Adams 1982; Wolpaw 1997). However, there is also a great deal of evidence to suggest that, contrary to S-R theory, animals do in fact anticipate the outcomes for which they are responding. In one particularly influential study conducted by Colwill and Rescorla (1985), hungry rats were trained to make one response (pressing a lever) to receive one reward (food pellets) and to make another response (pulling a chain) to receive another reward (sucrose solution). An aversion to one of the rewards was then formed by allowing the rats free access to it in their home cage and afterwards injecting them with LiCl. When returned to the conditioning chamber and tested in extinction (i.e. with no reward following any response made) the rats made fewer of the responses that had previously led to the now-devalued reward than of the responses that had previously led to the non-devalued reward. The results of this experiment cannot wholly be explained according to S-R theory since if the animals had acquired a tendency to perform the two responses in the absence of any knowledge of the outcome, then the devaluation of the reward (which occurred away from the conditioning context) should have had no effect at test. They can, though, be explained if the animals have learned about the relationship between the two responses and rewards (R-US associative learning or action-outcome (A-O) learning).

On the other hand, the results of Colwill and Rescorla's (1985) experiment also showed that although the rats showed reduced responding for the devalued reward compared to the non-devalued reward, they nevertheless did continue to respond for it. It would therefore appear that

the rats acquired both S-R associations and R-US associations. It might be that the relative influence of S-R and R-US associations on behaviour is dependent on the extent of training, and there is some evidence to support this possibility: rats have been shown to rely on R-US associations during the early stages of instrumental conditioning, but to then switch to S-R associations as learning becomes "habit"-driven (Adams and Dickinson 1981; Dickinson 1985). But there is also evidence to suggest that instrumental learning need not be either S-R or R-US driven, and that animals are able to integrate the information provided by S-R associations and by R-US associations in order to learn that in the presence of a particular stimulus, a particular response will be followed by a particular outcome (S-(R-US) learning (Tolman 1932; Rescorla 1992). For example, in an experiment carried out by Rescorla (1992), rats first learnt that chain-pulling resulted in a food reward and lever-pressing resulted in a sucrose reward in the presence of stimulus A, but that the reverse was true in the presence of stimulus B, i.e. lever-pressing resulted in the food reward whilst chain-pulling resulted in the sucrose reward. The reinforcer devaluation technique was then used to condition an aversion to the one of the rewards, and the rats then given the opportunity of performing the two responses in the presence of each stimulus in extinction. It was found that the rats performed the response that was not associated with the now-devalued reward in the presence of the appropriate stimulus. If only S-R associations were acquired during the first stage of training, then the devaluation treatment would not have worked. Likewise, if only R-US associations were acquired, the devaluation treatment would have weakened both responses to the same extent in the presence of both stimuli. Rescorla (1992) suggested that his results could be explained by the rats first acquiring the R-US association and then associating the R-US in its entirety with the S.

For Tolman (1932), the essential characteristic of instrumental conditioning was that it results in behaviour that is goal-directed. This idea has been further developed by Balleine, Dickinson and colleagues. In their 1994 paper Dickinson and Balleine (1994) define a goal-directed action as one in which "performance is mediated by the interaction of two representations: (1) a representation of the instrumental contingency between the action and the outcome, and (2) a representation of the outcome as a goal for the agent" (p.1) at the time of

performance. The studies carried out by Colwill and Rescorla (1985) and Rescorla (1992) described above, along with others (e.g. Bolles et al (1980), Balleine (1992)), would appear to provide clear evidence that performance is controlled by the instrumental contingency (though it is of interest that Dickinson and Balleine (1994) acknowledge only free-operant lever-pressing in rats as being controlled by the instrumental action-outcome contingency, stating that the status of other behavioural tasks such as spatially directed locomotion in runways and mazes is ambiguous and may be under the control of Pavlovian relationships between distal stimuli and the reward, see also Hershberger (1986) and Bussey et al (1997)). The outcome devaluation procedure provides evidence that rats are aware that they want the outcomes for which they work – they will work less for devalued food even if the test is conducted in extinction when there is no opportunity to learn a new relationship between the response and the less pleasant outcome (Adams and Dickinson 1981; Adams 1982; Colwill and Rescorla 1985).

Thus far instrumental conditioning has been discussed in terms of primary reinforcement – that is instrumental responding has been for a US that is intrinsically rewarding in itself, such as food or water. However, it is possible for a neutral stimulus to serve as an instrumental reinforcer by virtue of being paired with a primary reinforcer; in this case the initially neutral stimulus is termed a conditioned or secondary reinforcer. For example, a group of hungry rats might be presented with a series of tone CSs followed by delivery of a food reward. When then given the opportunity of pressing a lever which results in the tone being delivered (but not followed by reward), they will do so much more frequently than will a control group of rats which had been subjected to random presentations of the tone CS and reward (Hyde 1976). Although this effect is normally short-lived it can be maintained for long periods of time if the initial CS-US relationship is reverted to intermittently (Everitt and Stacey 1987). Conditioned reinforcers can act as powerful maintainers of instrumental behaviour – animals have been known to make many hundreds of responses in order to obtain tokens which can then be exchanged for food (Kelleher 1958), whilst humans, of course, will work for money (or the awardment of a PhD!).

As with Pavlovian conditioning, the degree of temporal contiguity, in this case between response and reinforcer, is an important factor in determining the effectiveness of instrumental conditioning. Although conditioning is far more effective when the reinforcer follows immediately after the response, it will occur even when the two are separated by quite lengthy intervals (Lattal and Gleeson 1990; Dickinson, Watt et al. 1992). Likewise, contingency between response and reinforcer is also important in determining the effectiveness of instrumental conditioning, as shown in an experiment carried out by Hammond (1980): thirsty rats were given the opportunity to lever-press for water during a session that was divided into 1-second intervals. For one group, every response within a 1-second interval was followed at the end of the interval with a probability of 0.12 by a delivery of water. This was also the case for two other groups, one of which was occasionally given and the other more frequently given 'free' deliveries of water at the end of a 1-second interval during which a response had not in fact been made. It was found that responding was strongest in the group that received no 'free' water, and weakest in the group that received most 'free' water, suggesting that the contingency between response and water was an important determinant of the extent of instrumental conditioning as measured by vigour of responding.

1.2.3 Pavlovian–instrumental interactions

In the preceding two sections Pavlovian and instrumental conditioning have been discussed as if they were independent and mutually exclusive learning mechanisms, and it may be that they are indeed subserved by dissociable neural systems. However, there is a great deal of evidence to suggest that Pavlovian CSs can have a major impact upon instrumental performance, an effect that has been termed Pavlovian-to-instrumental transfer (PIT), and that these two forms of learning are in fact normally recruited in parallel in order to enable an integrated adaptive response to the environment (Konorski 1967; Rescorla and Solomon 1967; Mackintosh 1974). This impact appears to be largely motivational in that Pavlovian stimuli appear to be able to modulate the vigour of instrumental responding (Konorski 1967; Rescorla and Solomon 1967; Dickinson 1994). A key demonstration of this is provided by an experiment carried out by

Lovibond (1983): hungry rabbits were first trained to lift a lever in order to receive a delivery of a sugar solution directly into the mouth. The lever was then removed for a number of Pavlovian conditioning sessions during which a 10 sec stimulus was paired with delivery of the sugar solution. Finally, the lever was restored and the rabbits allowed to respond with the Pavlovian stimulus occasionally being presented. It was found that the rabbits responded more vigorously when the stimulus was present.

It appears that PIT might occur through two mechanisms. First, Pavlovian CSs have been shown to influence instrumental performance through a response-cueing process (Trapold 1972). In an experiment carried out by Colwill and Rescorla (1988) one group of hungry rats were trained to associate a CS with delivery of food pellets whilst another group of hungry rats was trained to associate the same CS with delivery of sucrose solution. Half of the rats in each group were then trained (in separate sessions) to perform one response (lever-pressing) for the food pellet reward and another response (chain-pulling) for the sucrose solution reward, with the remaining rats learning the opposite action-outcome contingency. All the rats were then given the opportunity to perform both instrumental responses in the presence of the CS but with neither reward forthcoming (i.e. in extinction). It was found that the rats performed more vigorously the response which led to the same outcome in the second stage of training as that with which the CS had been associated in the first stage of training. For example, those rats trained to associate the CS with sucrose solution in the first stage and then to lever-press for food pellets and to chain-pull for sucrose solution in the second stage of training tended to chain-pull at test. This result cannot be explained by any motivational influence that the CS might exert, since it would influence both responses to the same degree. Instead, it would appear that, by some as yet unelucidated process, the CS acts selectively to invigorate the response with which it shares an outcome, perhaps by reinstating conditions that are more similar to those in which the instrumental action was trained (Dickinson 1994).

Second, Pavlovian stimuli have been shown to have a more generalised potentiating influence on instrumental performance that depends on the relevance of the outcome to the subject's motivational state: Dickinson and Dawson (1987) trained hungry rats to associate one

stimulus with a food pellet reward and a second stimulus with a sugar solution reward. The hungry rats were also trained to lever-press for the same food pellet reward, in the absence of both stimuli. When tested in extinction, in the occasional presence of one or other of the stimuli, it was found that they responded more vigorously in the presence of the stimulus that they had previously associated with the food pellet reward rather than the stimulus that they had previously associated with the sugar solution reward. However, this preference was reversed when the rats were tested in a state of thirst rather than hunger, suggesting that not only do Pavlovian stimuli have an invigorating effect on performance, but that this effect varies according to the motivational state of the rats – in this case whether they are hungry or thirsty. Moreover, the results of this experiment imply that the relevance of the Pavlovian outcome to the subject's motivational state may have a greater influence on instrumental performance than does reinstatement of the instrumental training condition as discussed above (Dickinson 1994).

1.2.4 Incentive learning

In the preceding section on instrumental conditioning mention was made of Balleine, Dickinson and colleagues' (1994) concept of goal-directed action. They have further expanded this into a theory concerning the motivational control of goal-directed action by primary motivational states such as hunger and thirst. Dickinson and Balleine suggest that this can occur through two processes. The first process involves a Pavlovian association between a contextual or discriminative stimulus and the outcome presented during instrumental training as described above, with the extent of the stimulus' influence on instrumental performance depending on the relevance of the outcome to the subject's motivational state. This process has been termed Pavlovian incentive learning (Dickinson et al (2000). The second process involves an instrumental association between an action and an outcome, which controls the relevance of the outcome to the subject's motivational state, or, in other words, the incentive value of the outcome. It is this second process that Dickinson and Balleine (1994) term instrumental incentive learning. Motivational states therefore do not directly control outcome value, but rather the subject has to learn the incentive value of an outcome whilst in a particular

motivational state, say hunger, by directly experiencing it whilst in that motivational state, i.e. whilst hungry. This is probably best illustrated by an experiment carried out by Balleine (1992): non-hungry rats were trained to lever-press for a food reward (different to the food given at all other times during the experiment). Half the rats were then allowed unrestricted access to their normal food for the next 24 hours whereas the others were placed on a food-restricted schedule. All the rats were then given the opportunity to lever-press in extinction. Surprisingly, all the rats showed an equal level of responding, though it could be expected that those rats that were in a state of food deprivation at the time of testing should respond more vigorously. Balleine (1992) proposed that this is because the rats were tested in extinction and therefore did not have the opportunity to re-experience the food reward whilst hungry – the food reward therefore retained the same lower incentive value that it had held during training, when the rats were not hungry.

Instrumental incentive learning has been shown to modulate instrumental performance in response to changes, either up or down, in the level of food deprivation (Balleine and Dickinson 1991; Balleine 1992) and water deprivation (Lopez, Balleine et al. 1992), as well as to transitions from food to water deprivation (Dickinson and Dawson 1988; Dickinson and Dawson 1989). It has also been shown to modulate instrumental performance in outcome devaluation experiments (Balleine and Dickinson 1991). Both Pavlovian and instrumental incentive learning therefore appear to play a fairly generalised role in the motivational control of goal-directed action.

1.3 Anatomy of the amygdala

The amygdala was first described by Burdach (1819-1822) in the early 19th century as an almond-shaped mass of grey matter in the temporal lobe of the human cerebral cortex and named as such – amygdala is Greek for almond. It lies ventromedial to the striatum and anterior to the ventral portion of the hippocampal formation (McDonald 1998). In the years since its discovery, the amygdala has undergone ever-increasing structural differentiation and it is now recognised as being a complex of nuclei and cortical regions, which themselves are divided into

subunits, the number and classification of which are still being debated (Alheid and Heimer 1988; Pitkanen, Savander et al. 1997; McDonald 1998; Swanson and Petrovich 1998) ***(Figure 2)***. Until quite recently the most widely accepted classification of amygdalar structure was that made by Johnston (1923), based on a comparative analysis of the amygdala in different species. Johnston proposed that the amygdala is divided into a primitive group of nuclei associated with the olfactory system (central, medial and cortical nuclei) and a phylogenetically newer group of nuclei (lateral, basal and accessory nuclei). It has, however, now been recognised that the central and medial nuclei exhibit anatomical and histological characteristics that are distinct from the rest of the cortical group (Alheid 1995), leading McDonald (1998) to propose that there are three main groups: the superficial cortex-like nuclear group, the centromedial nuclear group and the basolateral nuclear group. On the other hand, Swanson and Petrovitch (1998) have argued for a somewhat different classification, suggesting that, cytoarchitectonically, chemoarchitecturally and with respect to their connectivity, cell groups within the amygdala are actually differentiated parts of the traditional cortex, the claustrum and the striatum, and belong to four obvious functional systems – the main olfactory (cortical and basomedial nuclei), accessory olfactory (medial nucleus), autonomic (central nucleus) and frontotemporal cortical (lateral and basolateral nuclei) systems. Much of the amygdala is therefore given over to the olfactory system, but the lateral/basolateral nuclei (LA/BLA) and the central nucleus (CeN) have been particularly implicated in learning and in the control of emotional processes. Although there is some confusion over nomenclature, the basolateral (BLA) complex comprises the lateral nucleus (LA) together with what McDonald (1998) and Pitkänen et al (1997) term the basal nucleus and Swanson and Petrovitch (1998) term the anterior and posterior parts of the basolateral nucleus (BLAa and BLAp). This latter conforms most closely to the nomenclature used by Paxinos and Watson (1998) in their atlas of the rat brain, who refer to the basolateral amygdaloid nucleus, anterior part (BLA) and the basolateral amygdaloid nucleus, posterior part

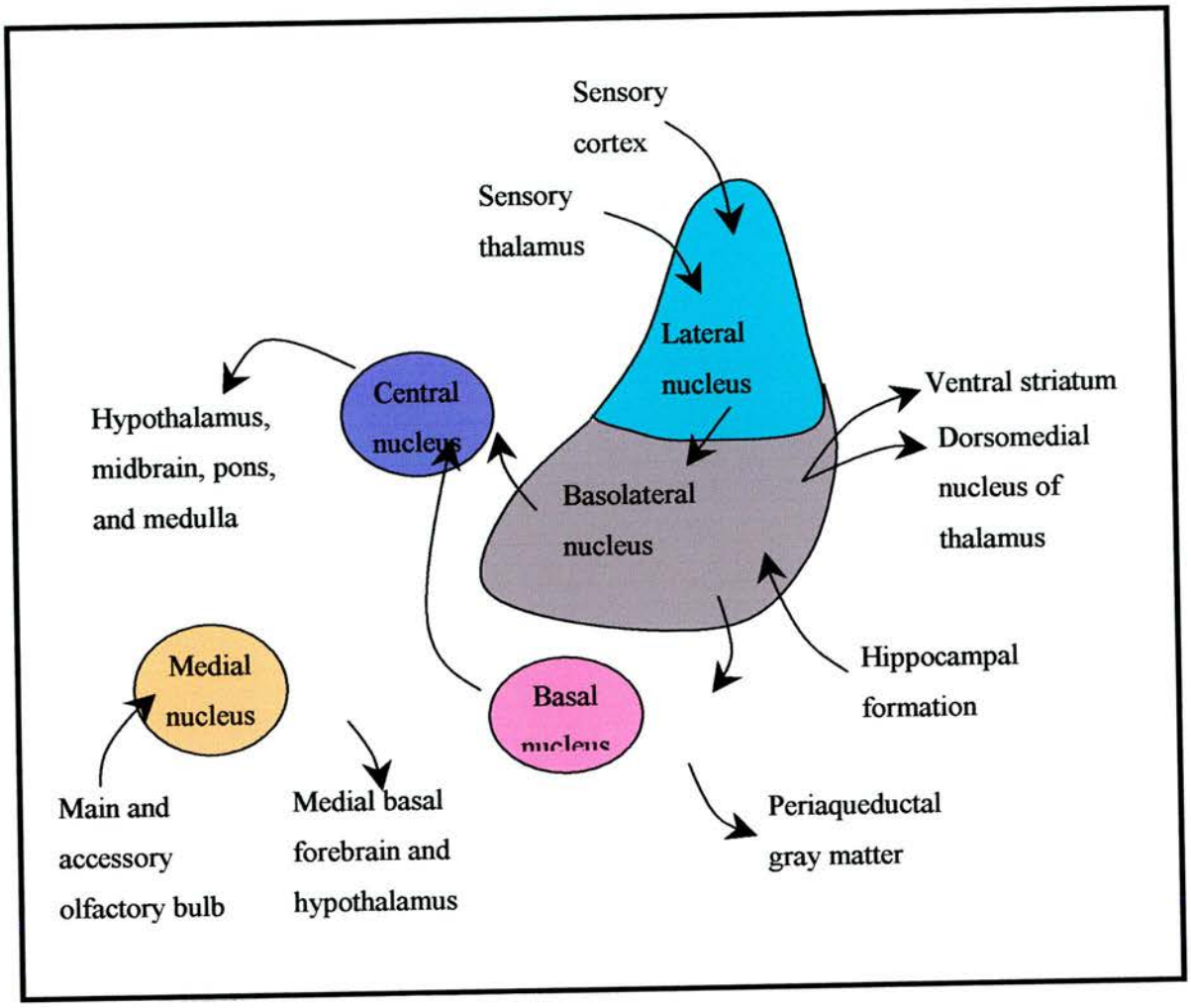


Figure 2: *A much-simplified diagram of the major divisions and connections of the amygdala. Taken from Carlson (Carlson 1998)*

(BLP). (Because Paxinos and Watson's atlas is the stereotaxic / histological reference for this thesis, their nomenclature will be used henceforth.) The BLA complex corresponds to the region originally termed amygdala by Burdach (1819-1822), and which Meynert (1867) claimed to be a ventromedial extension of the claustrum (which he believed to be the deepest layer of cortex). This belief has been borne out by recent research, which has shown that neurons in the BLA complex do in fact share many features in common with cortical neurons (McDonald 1992; Alheid 1995; Swanson and Petrovich 1998).

The BLA complex is more commonly thought of as teardrop rather than almond-shaped, with the LA at the apex and bounded laterally by the external capsule, medially by the CeN and ventromedially by the BLA/BLP (or basal nucleus). The LA is made up of three

distinct subdivisions in the rat - the dorsolateral, ventrolateral and ventromedial subdivisions. Within the LA, the dorsolateral division projects to the other two divisions, but the latter do not send any substantial projections to the dorsolateral division or to each other. At the interdivisional level, therefore, the flow of information is unilateral (Pitkanen, Savander et al. 1997). There is some controversy over the number of subdivisions that constitute the basal nucleus. McDonald (1998) suggests that there are two major subdivisions – the magnocellular basal nucleus and the parvicellular basal nucleus – whereas Pitkänen et al (1997) suggest that there are three: the magnocellular, intermediate and parvicellular divisions. Pitkänen et al (1997) suggest that the parvicellular division gives rise to most of the interdivisional projections within the basal nucleus, projecting to both the magnocellular and intermediate divisions, but that the magnocellular division reciprocates to some extent.

The CeN is phylogenetically older than the BLA complex. As mentioned above, although originally grouped together as "primitive" by Johnston (1923), the central and medial nuclei exhibit anatomical and histological characteristics that are distinct from the rest of the cortical nuclei (Alheid 1995). McDonald (1992) points out that the principal neurons in the lateral portions of the CeN resemble the medium sized spiny neurons of the adjacent striatum rather than the pyramidal cells of the cortex, and describes a very dense band of neurons labelled for gamma-aminobutyric acid (GABA) and for glutamic acid decarboxylase (GAD - the enzyme that converts glutamate to GABA) that extends uninterruptedly through the caudate putamen, the CeN and into the medial amygdalar nucleus. Other regions of the amygdala contain very few and scattered GABA neurons, suggesting that they depend upon glutamate in their extrinsic projections, as do most cortical projection neurons (pyramidal cells). Alheid et al (1995) have advanced the concept of an extended amygdala, suggesting that the central and medial nuclei of the amygdala extend right through the bed nucleus of the stria terminalis to the shell of the NAcc The NAcc shell is thus seen as a mixture of striatal neurons and CeN neurons, sharing with the CeN a variety of histochemical features and connections. Although this concept is still being debated, there is a general recognition that the CeN is striatal in nature (McDonald 1992; Alheid 1995; Pitkanen, Savander et al. 1997; Swanson and Petrovich 1998).

As with the BLA complex, there is some difference of opinion over the number and nomenclature of its subdivisions: both Pitkänen et al (1997) and McDonald (1998) suggest that there are four, which they name the capsular, lateral, intermediate and medial subdivisions. Paxinos and Watson (1998), however, suggest that there are only three, which they name the central amygdaloid nucleus, capsular part (CeC), the central amygdaloid nucleus, lateral division (CeL) and the central amygdaloid nucleus, medial division (CeM). There are heavy intradivisonal projections along the rostrocaudal and mediolateral axes (P: 46) within the subdivisions, and extensive connections between them, with the lateral division projecting to the capsular and medial divisions, the capsular division projecting to medial division, and the medial division projecting back to the capsular division. The capsular and medial divisions receive major inputs from the lateral and basal nuclei and are reciprocally interconnected (Pitkanen, Savander et al. 1997; McDonald 1998).

The amygdala receives sensory information from the sensory cortices of all the sensory modalities – in fact Young (1994) has described the amygdala as a whole as being at the hub of a vast network of cortical connections. As mentioned earlier, much of the amygdala is given over to processing olfactory and gustatory/visceral information, and this arrives at the amygdala at a somewhat earlier stage of cortical processing than does information from the somatosensory, auditory and visual cortices. Most sensory information enters by way of the LA and is then relayed on to other amygdalar nuclei (Price 1987; LeDoux, Cicchetti et al. 1990; Bordi and LeDoux 1992; Mascagni, McDonald et al. 1993; Romanski, Clugnet et al. 1993; Campeau and Davis 1995; Quirk, Repa et al. 1995). However, the BLA/BLP also receives moderate to heavy inputs from the gustatory/visceral and somatosensory cortices and much lighter inputs from the auditory and visual cortices (McDonald 1991; McDonald 1998). Likewise, the CeN receives fairly robust projections from these cortices (Mascagni, McDonald et al. 1993; McDonald 1998). Reciprocal projections from the LA and the BLA/BLP to the sensory cortices are not very widespread, whilst the CeN has none (McDonald 1998).

The amygdala also receives substantial projections from the PRC, for which it is one of the chief forebrain targets, the others being the striatum, hypothalamus and medial thalamus

(McDonald, Mascagni et al. 1996). In the rat the PFC consists of medial, orbital and lateral regions, all of which are themselves divided into subregions. The medial PFC comprises four subregions, arranged from dorsal to ventral – the infralimbic, prelimbic, anterior dorsal cingulate and medial precentral subregions. The orbital PFC also comprises four subregions – the medial, ventral, ventrolateral and lateral orbital cortices, whilst the lateral PFC is made up of the dorsal and ventral agranular insular cortices (McDonald 1998). The LA receives moderate to light input from the PFC, mostly from the infralimbic subregion of the medial cortex, and its reciprocal projections to the PFC are moderate and mostly to infralimbic and prelimbic subregions of the medial cortex, with some input into the ventral agranular insular subregion of the lateral cortex (McDonald, Mascagni et al. 1996). The BLA/BLP receives heavy inputs from medial cortex of the PFC, most substantially from the prelimbic subregion with lighter input from the infralimbic, anterior dorsal cingulate and medial precentral subregions, and also some input from the ventral agranular insular subregion of the lateral cortex. In turn, the BLA/BLPs' projections to the PFC are widespread and dense, innervating mainly the infralimbic subregion, but also the prelimbic, anterior dorsal cingulate and medial precentral subregions of the medial cortex. There are also projections to the ventral and lateral orbital subregions of the orbital cortex, and to the agranular insular subregion of the lateral cortex (McDonald, Mascagni et al. 1996). The CeN receives light input from the PFC, mainly from the infralimbic subregion of the medial cortex, but also from the prelimbic and medial precentral subregions. The lateral CeN also receives a fairly substantial input from the dorsal agranular insular subregion of the lateral PFC. However, there is no reciprocal projection from the CeN to the PFC.

There is also considerable interaction between the amygdala and the striatum. The LA both receives and sends substantial projections from and to the ventral striatum including the NAcc, providing a particularly dense input to the shell region of the NAcc. The BLA/BLP also has reciprocal connections with the ventral striatum, but the projections that it receives are considerably lighter than the ones that it sends: the BLA/BLP massively innervates the striatum and is the major source of amygdaloid inputs to the NAcc. These are topographically organised, with the parvicellular division projecting mainly to the shell region of the NAcc and the

magnocellular division projecting to the core (Krettek and Price 1978; McDonald 1991; McDonald 1998). There is, however, very little connection between the CeN and the striatum. The NAcc, of course, sends projections to the ventral pallidum, which in turn innervates (mediodorsal) thalamus, which has bi-directional connections with PFC, which, in full circle, projects back to the NAcc – the limbic corticostriatal loop. But the amygdala is also directly connected with the thalamus - the BLA both receives and sends substantial projections from and to the thalamus (LeDoux, Farb et al. 1990), whilst the CeN receives substantial projections from the thalamus but reciprocates in a very minor way (Moga, Weis et al. 1995).

It should also be noted that there are close associations between amygdala and hippocampus. The hippocampal formation (composed of the dentate gyrus, hippocampus proper (CA3, CA2 and CA1 fields), the subicular complex and the entorhinal cortex (Amaral and Witter 1989) all have moderate to heavy projections to the LA, which are in turn reciprocated. Likewise, there are widespread and dense interconnections between the BLA/BLP and the hippocampal formation (McDonald, Mascagni et al. 1996; McDonald 1998). The CeN also receives substantial inputs, especially from the subicular complex and entorhinal cortex but does not reciprocate (Cullinan, Herman et al. 1993; McDonald 1998).

Finally, the CeN is characterised by its very extensive bi-directional connections with regions within the midbrain, pons and medulla that govern autonomic and skeletomotor responses including the hypothalamus, the dorsal motor nucleus of the vagus nerve, the nucleus of the solitary tract, the parabrachial nucleus, periaqueductal grey and the pedunculopontine tegmental nucleus (Davis 1992). Moreover, the CeN both projects to and receives inputs from monoaminergic and cholinergic cell groups within the midbrain, pons and medulla, including the (dopaminergic) SNc and VTA, (noradrenergic) locus coeruleus, (serotonergic) raphe nuclei and (cholinergic) nucleus basalis magnocellularis (Price 1987; Amaral, Price et al. 1992; Davis 1992; Gallagher and Holland 1994). In contrast, connections between the LA / BLA/BLP and the autonomic and endocrine domains of the midbrain, pons and medulla are relatively scarce. The LA receives a few projections from the VTA and locus coeruleus, and fairly substantial projections from the raphe nuclei, but does not reciprocate. The BLA/BLP receives light

projections from autonomic domains within the midbrain and pons, including periaqueductal grey and pedunculopontine tegmental nucleus, whilst other projections from the SNc, VTA, dorsal raphe, and locus coeruleus, provide dopaminergic, serotonergic and noradrenergic innervations respectively. Again, these projections are not reciprocated (Pitkanen 2000).

From the above, it can be seen that the amygdala interacts with a wide array of cortical and subcortical structures through which it can influence autonomic, hormonal and motor function. Until fairly recently, the amygdala was considered to be both a structural and a functional unity, and there is still an argument for regarding it as such: dense intra-amygdala connections cut across the divisions proposed by Swanson and Petrovitch (1998) and serve to integrate the activities of the different nuclei. One leading theory is that the BLA is responsible for emotional Pavlovian learning in that it acts as a site of CS-US association for sensory information arriving via the lateral nucleus, and then uses this learned information to control the activity of the CeN (LeDoux, Cicchetti et al. 1990; Davis 1992; Maren and Fanselow 1996; Pitkanen, Savander et al. 1997). In turn, the CeN acts as a "controller of the brainstem" (Kapp, Whalen et al. 1992), using its widespread projections to the midbrain, pons and medulla both to orchestrate the autonomic, neuroendocrine and reflexive components of emotional responses, and also to influence attentional and activational processes through its interactions with ascending arousal systems (LeDoux, Cicchetti et al. 1990; Davis 1992; Kapp, Whalen et al. 1992; Maren and Fanselow 1996; Robledo, Robbins et al. 1996; Pitkanen, Savander et al. 1997). For instance, the LA, BLA and CeN have been shown to be critically involved in different aspects of fear conditioning, and interact to such an extent that they appear to constitute a single functionally unified system (Davis 1992).

However, the BLA does more than just control the CeN – it has independent bi-directional connections with structures including the PFC and the ventral striatum, allowing it to influence complex behaviour (Everitt and Robbins 1992). Moreover, high order projections also arrive directly at the CeN, especially its lateral subdivision, and McDonald (1998) has suggested that the lateral / capsular division of the CeN may provide a gateway of sensory convergence that parallels that provided by the BLA. Although much of the early research focused on the

amygdala as an entirety out of necessity, advances in neuroscience have made it possible to examine the possible roles that different nuclei and subsystems within the amygdala might play in associative learning, and evidence is now beginning to emerge that the CeN may be capable of learning and /or subserving behavioural expression independently of the BLA. Killcross et al (1997), for instance, have dissociated the basolateral and central nuclei of the amygdala with regard to aversive conditioning procedures, and many researchers are now attempting to dissociate them with regard to appetitive associative learning.

Two experiments within this thesis are undertaken with the aim of determining whether the BLA and the CeN are involved in a particular task, the Schedule Fraction Cue (SFC) task, which employs both Pavlovian and instrumental learning, and if so, whether the roles that they play can be dissociated.

1.4 The BLA and CeN and reward learning

As detailed in the previous section, the study of appetitive associative learning has traditionally taken two forms, Pavlovian and instrumental conditioning, and the following review will look at the involvement of the BLA and the CeN in them.

Although the functions of the amygdala have been studied for many years, prior to 1989 most experimentally induced lesions were electrolytic, aspirative, or radiofrequency and destroyed not only amygdala neurons, but also axons passing through the amygdala between the medial forebrain bundle / substantia innominata region and the temporal and insular cortices. The serious implications of this were demonstrated in a study carried out by Dunn and Everitt (1988), who found that lesions of the rat amygdala using N-methyl D-aspartic acid (NMDA), an excitotoxic amino acid that destroys neurons but generally spares fibre bundles running through the area of lesion, had no effect on the acquisition of a conditioned taste aversion, a result that contradicted the findings of previous studies in which electrolytic lesions had been made (Rolls and Rolls 1973; Nachman and Ashe 1974; Aggleton, Petrides et al. 1981). Dunn and Everitt (1988) went on to show that, with electrolytic lesions, impaired acquisition of a conditioned

taste aversion was due to the destruction of axons passing through the amygdala to and from the insular gustatory cortex. The importance of this finding is that, previously, the impairment had been thought to support the idea that the amygdala was an important component in one aspect of emotional behaviour – the modification of motivational responses to current goals in the context of past experience (Rolls and Rolls 1973; Rolls 1990). It is therefore essential that lesions are made with excitotoxic amino acids for valid conclusions to be drawn regarding the localisation to the amygdala of behavioural changes. For this reason, this review concentrates on studies conducted after 1988. It is worth noting, though, that there is a downside in using neurotoxic amino acids rather than electrolytic or radiofrequency lesions. The latter are highly controlled and discrete, whereas the former tends to be more diffuse and harder to control. Also, there is a problem in that different neurotoxic amino acids are used to lesion the amygdala, and these might have different effects.

1.4.1. Basolateral nucleus (BLA)

1.4.1.1. Role of the BLA in Pavlovian conditioning

To date, very few studies have looked at the involvement of the BLA in appetitive Pavlovian conditioning, and most of these have done so only as an initial training phase in instrumental conditioning. For instance, both Cador et al (1989) and Burns et al (1993) used what they describe as Pavlovian conditioning in the first phase of training their rats in the acquisition of a new response with conditioned reinforcement task (see below). This phase entailed the rats learning to associate a compound stimulus of a light coming on and a water dipper being raised with water (Cador, Robbins et al. 1989) or sucrose (Burns, Robbins et al. 1993) reward. Cador et al found that excitotoxic lesions of the BLA made after conditioning had no effect on performance, whereas Burns et al found that lesioning the BLA both prior to and after conditioning impaired performance. It might be that these contradictory results are attributable to Cador et al's lesions leaving the lateral nucleus largely intact whereas those of Burns et al did not, in which case, one could expect that lesioning the BLA including the lateral nucleus would

impair Pavlovian conditioning. But this leads to yet another contradiction, with the results of the 'Pavlovian' conditioned place preference tasks discussed below. However, the design of both Cador et al's and Burns et al's experiments involve the rats having to push open a panel in order to access the liquid reward, and it could be argued that their tasks involve instrumental discrimination learning rather than Pavlovian conditioning.

A very few studies have employed conditioning procedures that are generally accepted as Pavlovian, such as place preference and Pavlovian approach behaviour (often known as autoshaping), again with varying results. Hiroi and White (1991) showed that both electrolytic and neurotoxic lesions of the lateral nucleus of the amygdala attenuated the acquisition and expression of amphetamine-reinforced conditioned place preference, whereas electrolytic lesions of the BLA and the CeN did not. McDonald and White (1993) likewise showed that damage to the lateral nucleus of the amygdala impaired acquisition of food-reinforced conditioned place preference using an 8-arm radial maze task. In contrast, Everitt et al (1991) found that a previously established sucrose-reinforced conditioned place preference was completely abolished by lesioning the BLA. Again, examination of Everitt et al's (1991) lesions suggests that this apparent contradiction might be due to the lateral nucleus of the amygdala being lesioned as well as the basolateral nucleus. These conflicting results highlight a major difficulty in amygdala research – that of, when making lesions, discriminating between adjacent subnuclei which might differ functionally. In many of the papers discussed in this thesis, the lateral nucleus of the amygdala is of necessity included as part of the BLA though there is mounting evidence that they in fact subserve different functions. However, it is accepted, at least by the Robbins and Everitt group, and possibly also by the Holland and Gallagher group, that the BLA is critical for the acquisition of appetitive conditioned place preferences (Everitt and Robbins 1992; Hatfield, Han et al. 1996).

A somewhat more satisfactory set of experiments into the involvement of the BLA in appetitive Pavlovian conditioning has been carried out by Hatfield et al (1996). They first employed a straightforward first-order conditioning procedure in which BLA-lesioned and control rats received light - reward pairings that were intended to endow the light CS with

reinforcement value and found that both groups rapidly acquired first-order conditioning to the light CS. They then wanted to establish whether the light CS had actually acquired conditioned reinforcement value from being associated with the US for both groups. One of the most common ways of assessing whether an event has acquired incentive value in conditioning is to measure its ability to serve as a reinforcer in new learning (Mackintosh 1983). Hatfield et al (1996) therefore went on to use a Pavlovian second-order conditioning procedure in which their rats were given pairings of a tone CS followed by the light CS, but with no reward following. The rate of acquisition of Pavlovian second-order conditioning to the tone would indicate the reinforcing power of the light CS. Hatfield et al found that whilst the control rats were able to acquire second-order conditioning, the BLA-lesioned rats were not.

In their discussion, Hatfield et al (1996) suggested that the fact that the BLA-lesioned rats were able to acquire first-order conditioning to the light CS implied that they did not suffer from a general impairment in learning. They also hypothesised that BLA-lesioned rats were unable to acquire second-order conditioning to the tone CS because, for these rats, the light-food pairings in the initial training phase did not endow the light CS with reinforcement value so that it could act as a conditioned reinforcer in its turn. However, they recognised that it could equally well be argued that BLA lesions do allow the acquisition of reinforcement value by the CS but do not allow Pavlovian second-order associative conditioning. Hatfield et al (1996) therefore explored this possibility in a second experiment reported in the same paper, which utilised another appetitive Pavlovian conditioning procedure, reinforcer devaluation. As explained previously, the reinforcer devaluation procedure has its basis in the idea that the production of a conditioned response depends on the conditioned stimulus gaining access to some kind of internal representation of the unconditioned stimulus. Moreover, the CS is not only able to acquire the value of the US at the time of conditioning, but is able to gain access to post-training changes in the value of the US which will influence subsequent performance of a task (Holland and Straub 1979). In using this procedure, Hatfield et al (1996) argued that, if in BLA-lesioned rats, first-order CSs do not gain access to the value of the US, then it could be expected that they would be insensitive to post-training devaluation of the US. They found that this was indeed the

case. In line with other studies (Dunn and Everitt 1988; Cahill and McGaugh 1990; Hatfield, Graham et al. 1992) Hatfield et al (1996) found that, like normal rats, BLA-lesioned rats acquired an aversion to the reward when it was followed immediately by a lithium chloride injection, but, unlike normal rats, they did not show less conditioned responding to the light CS after devaluation of the US. Overall, Hatfield et al's (1996) results indicate that BLA lesions have no effect on the acquisition and display of first-order conditioned responses and on the acquisition of food aversion, but that they do impair both the acquisition of Pavlovian second-order conditioning and the ability to spontaneously adjust the conditioned response to post-training alterations in the value of the US.

1.4.1.2 Role of the BLA in instrumental conditioning

Several different instrumental paradigms have been used to explore the involvement of the BLA in appetitive conditioning, including stimulus-cued recovery of extinguished responding, acquisition of a new response with conditioned reinforcement and second-order instrumental associative learning. Implicit in most of these experimental designs is that BLA-lesioned rats are as well able as normal rats to make instrumental responses for reward. However, it is worth noting that in their second experiment Cador et al (1989) explicitly set out to determine whether BLA-lesioned rats are able to lever-press for a water reward as efficiently as normal rats and found that this was indeed the case. Likewise, Meil and See (1997) found that BLA-lesioned rats are as well-able to lever-press for self-administered infusions of cocaine as normal rats. It would therefore appear that the BLA is not necessary for the maintenance of instrumental responding per se, and that lesioning this structure does not interfere with the unconditioned effects of rewards.

Having established that this was the case, Meil and See (1997) went on to use stimulus-cued recovery of extinguished responding to look at the effects of lesions of the BLA on appetitive conditioning. In this procedure, rats are first trained to make instrumental responses for a reward in the presence of a stimulus paired with the reward. They then enter an extinction phase, during which responding is no longer followed by reward. Finally, the stimulus

previously paired with the reward is reintroduced and its ability to reinstate extinguished responding is assessed. Meil and See (1997) found that BLA-lesioned rats made fewer lever presses on the first day of extinction compared to control rats, though thereafter there was a significant decrease in responding for both groups. However, when, after 20 days of extinction sessions, the BLA-lesioned and control rats were presented with the stimuli that had previously been associated with infusions of the drug, the BLA-lesioned rats showed no increase in the very small number of responses that they had been making throughout the extinction sessions whereas the control rats showed a significant increase.

This finding, that BLA lesions block the ability of drug-associated stimuli to reinstate extinguished responding, suggests that, for the BLA-lesioned rats, the conditioned stimulus has not acquired any reinforcement value, and therefore cannot act as a conditioned reinforcer in its turn. Conditioned reinforcers are previously neutral stimuli that have gained motivational salience by their association with primary rewards. A stringent criterion for assessing a stimulus's ability to act as a conditioned reinforcer is to test its capacity to reinforce the acquisition of a new response in the absence of the primary reward (Everitt and Robbins 1992). For instance, rats are first trained to associate an arbitrary stimulus with reward (Pavlovian conditioning). They are then presented with two levers; pressing on one of the levers is followed by presentation of the stimulus (but no reward) whilst pressing on the other lever has no consequences. The ability of the stimulus to act as a conditioned reinforcer is therefore assessed by measuring the extent of pressing on the reinforced lever compared with the non-reinforced lever. Normal rats respond much more frequently on the lever producing the conditioned reinforcer than on the control lever, but this differential responding is much reduced in BLA-lesioned rats (Cador, Robbins et al. 1989, Burns, Robbins et al. 1993).

A very similar procedure to the acquisition of a new response with conditioned reinforcement procedure is second-order instrumental associative learning. Rats are first trained to associate an arbitrary stimulus with reward, and then given the opportunity to work for the presentation of that stimulus, with the reward being presented only at the end of the session (e.g. sex reward) or occasionally throughout the session (e.g. drug reward). Everitt et al (1987) found

that it is only the presentation of the stimulus that keeps the rats working – without it they stop. Everitt and Robbins (1992) suggest that this procedure has the advantage over first-order instrumental conditioning when the reward is such that its presentation will profoundly disrupt the measurement of ongoing behavioural responses. They also suggest that second-order schedules of reinforcement allow "the investigation of stimulus-reward associations in a more integrated and so less abstract behavioural context than does the acquisition of a new response with conditioned reinforcement procedure" (p.406), presumably because the latter is conducted in extinction. In a seminal study, Everitt et al (1989) showed that BLA lesions attenuated responding for sexual reinforcement under a second order schedule of reinforcement. Interestingly, the lesions also caused rats to be insensitive to the omission of the conditioned stimulus that was important to maintaining responding in control subjects. Again, this suggests that the conditioned stimulus has not acquired any reinforcement value, and therefore cannot act as a conditioned reinforcer in its turn. Rats with excitotoxic lesions of the BLA also fail to acquire a second order schedule of reinforcement maintained by intravenous cocaine (Whitelaw, Markou et al. 1996).

The results of the instrumental conditioning studies described above suggest that although lesions of the BLA have no effect on first-order conditioning, they do impair the association between arbitrary stimuli and primary reward and its subsequent influence over behaviour. However, as Hatfield et al (1996) argued with regard to Pavlovian conditioning, it might be that this impairment reflects disruption in processes underlying operant learning rather than disruption of the ability of the CS to acquire reinforcement value. Malkova et al (1997) went some way to addressing this problem by using an instrumental form of the reinforcer devaluation procedure that Hatfield et al (1996) employed to answer the same question with regard to Pavlovian second-order conditioning. Monkeys were first taught to visually discriminate rewarded from unrewarded items. The reward was either a peanut or a fruit snack. One of the rewards was subsequently devalued by allowing the monkeys to feed on it to satiation, and the monkeys then asked to choose between pairs of items, one of which was peanut-rewarded and one fruit snack-rewarded. Malkova et al found that although both normal

and amygdalectomized monkeys were able to learn the visual discrimination for the primary reinforcement task equally well, normal monkeys preferentially chose the item associated with the non-devalued reward whereas amygdalectomized monkeys were just as likely to choose either item. Malkova et al concluded that for the amygdalectomized monkeys, the conditioned stimuli were not able to access the value of the reward after its devaluation. They suggested that the amygdalectomized monkeys failed to associate the stimuli items with the hedonic value of the food reward, and, after devaluation of the reward, were unable to adapt their instrumental responses to the altered value of the reinforcer.

Although Malkova et al (1997) lesioned the entire amygdala in their monkeys, their results support Hatfield et al's (1996) findings, discussed above, that the (basolateral) amygdala plays a necessary role in reinforcer devaluation; the reinforcer devaluation effect therefore applies to instrumental conditioning as well as to Pavlovian conditioning paradigms. Malkova et al's experimental design is also interesting in that they used food rewards to bait all items during the test phase whereas most devaluation procedures do not (Hatfield, Han et al. 1996). Malkova et al argued that although particular stimulus items might become directly associated with a devalued food, this would only occur after the monkeys had chosen, and that the same items were only used once in the session. They went on to argue that for an animal to respond appropriately to the devaluation procedure, a stimulus item must become associated in memory with the value of one particular foodstuff so that the animal can selectively avoid the item baited with the devalued food reward whilst choosing the item baited with the non-devalued food reward. Malkova et al suggested therefore that the amygdala must be necessary for associating stimuli with the value of one particular food as opposed to the value of another particular food, but not for associating stimuli with food reward as opposed to no food reward.

The evidence discussed above suggests that the BLA is critical for the acquisition of positive incentive value by formerly neutral stimuli in Pavlovian and instrumental conditioning, and for the ability of conditioned stimuli to gain access to the current motivational significance of unconditioned stimuli. Such information may then be used to support Pavlovian second-order conditioning (Hatfield, Han et al. 1996) and the translation of conditioned associations into

instrumental action, as with acquisition of a new response with conditioned reinforcement (Cador, Robbins et al. 1989; Burns, Robbins et al. 1993), second-order instrumental associative learning (Everitt, Cador et al. 1989; Whitelaw, Markou et al. 1996), and also reinforcer devaluation in both Pavlovian (Hatfield, Han et al. 1996) and instrumental (Malkova, Gaffan et al. 1997) paradigms.

There is evidence to suggest that this translation of conditioned appetitive associations into instrumental action might be mediated by connections between the BLA and the ventral striatum. The BLA directly and massively innervates the ventral striatum, including the NAcc (Kelley, Domesick et al. 1982; Russchen and Price 1984; Kelley and Delfs 1991; McDonald 1991; McDonald 1991; Groenewegen, Wright et al. 1996; Heimer, Alheid et al. 1997), an area which has long been implicated in primary reward. Moreover, the termination of amygdaloid neurons in the ventral striatum is closely related to the termination of the mesolimbic dopaminergic projection from the VTA (Yim and Mogenson 1982; Yim and Mogenson 1983; Heimer, Alheid et al. 1997), a neurotransmitter system that is increasingly thought to be involved in reward learning and expectation. For instance, as described above, it would appear that the BLA is critical to the acquisition and expression of conditioned place preference, but there is evidence to suggest that dopaminergic innervation of the ventral striatum is also important in mediating this behaviour: dopamine agonists support the acquisition of a conditioned place preference both when given systemically and when infused directly into the ventral striatum (Phillips and Fibiger 1987), whilst dopamine depletion from the ventral striatum blocks the acquisition of a conditioned place preference (White and Carr 1985). Besides lesioning the BLA, Everitt et al (1991) also lesioned the ventral striatum in some rats, and in others made 'disconnection' lesions of the BLA and the ventral striatum (including the NAcc) by unilaterally lesioning both structures in the same animal but on opposite sides of the brain. They theorised that if the BLA and the ventral striatum are indeed functionally interrelated, then asymmetric lesions should result in behavioural effects that closely resemble those produced by bilateral lesions of either structure. They found that this was indeed the case - bilateral lesions of the BLA and of the ventral striatum abolished conditioned place preference,

as did the 'disconnection' lesions. Likewise, as discussed above, it would appear that the BLA is involved in the acquisition of a new response with conditioned reinforcement. It has been shown that psychomotor stimulant drugs such as d-amphetamine selectively increase responding with conditioned reinforcement (Robbins 1976; Beninger, Hanson et al. 1980), and that these potentiative effects are dependent upon increased dopaminergic activity in the ventral striatum (Taylor and Robbins 1986; Robbins, Cador et al. 1989; Wolterink, Phillips et al. 1993). Both Cador et al (1989) and Burns et al (1993) found that infusions of d-amphetamine into the NAcc continued to potentiate responding on the lever producing the conditioned reinforcer, albeit from a much lower baseline, even though the BLA had been lesioned. It would appear, therefore, that the BLA and the ventral striatum interact under the conditions of this experimental paradigm to mediate the control over behaviour by a conditioned reinforcer – the BLA appears to be important for the association between arbitrary stimuli and primary reward and its subsequent influence over behaviour is dependent upon interactions with the ventral striatum where modulation by dopamine release can occur.

Interestingly, it is not only the ventral striatal dopaminergic system that appears to be involved in processes whereby formerly neutral stimuli acquire positive incentive value in Pavlovian and instrumental conditioning through their predictive association with primary goals and thereby come to control instrumental behaviour – recent evidence indicates that dopaminergic mechanisms within the amygdaloid complex itself might also be involved. As the culmination to a series of papers, Hitchcott et al (1998) have shown that infusion of 7-OH-DPAT, a dopamine receptor antagonist, into the BLA (but sparing the lateral nucleus) has no effect upon the expression of Pavlovian conditioned approach behaviour, but selectively abolishes the ability of the reward-associated stimulus to support the acquisition of a new response. These findings are consistent with idea that the BLA is involved in the association between arbitrary stimuli and primary reward and its subsequent influence over behaviour, but the underlying mechanisms are somewhat unclear. However, there is some evidence to suggest that the mesoamygdaloid dopamine projection exerts an inhibitory influence over the mesoaccumbens dopamine system in that microinjection of dopaminergic agonists into the

amygdala is known to inhibit dopaminergic activity in the NAcc (Louilot, Simon et al. 1985), though whether this influence is exerted via the connections between BLA and the ventral striatum, or via descending pathways back to the dopaminergic cell bodies is not yet known.

To conclude, evidence discussed above shows that circuitry including the BLA is critical for the acquisition of positive incentive value by formerly neutral stimuli in Pavlovian and instrumental conditioning, and for the ability of conditioned stimuli to gain access to the current motivational significance of unconditioned stimuli.

1.4.2 Central nucleus of the amygdala (CeN)

1.4.2.1 Role of the CeN in Pavlovian conditioning

As detailed above, the CeN projects to a wide array of sites in the midbrain, pons and medulla, some of which are the origin of outputs that directly control autonomic and behavioural responses, and others of which are ascending systems that innervate the forebrain. As might be expected given its connectional anatomy, lesions of the CeN impair a number of somatic and autonomic conditioned responses such as conditioned freezing, fear-potentiated startle and conditioned decrease in heart rate (bradycardia) (Gentile, Jarrell et al. 1986; LeDoux, Iwata et al. 1988; Davis 1992; Kapp, Whalen et al. 1992; Maren and Fanselow 1996; Powell, Chachich et al. 1997; Fendt and Fanselow 1999). It is of interest, though, that both the unconditioned startle reflex and unconditioned bradycardia are unaffected by CeN damage (Davis 1986) and it would appear that these are dependent on the CeN only in that it is through the CeN that the BLA obtains access to brain sites involved in visceral and motor control processes, such as the periaqueductal grey, caudal pontine reticular nucleus and dorsal motor nucleus of the vagus (Kapp, Whalen et al. 1992).

However, the CeN is not just involved in aversive conditioning and the expression of fear-related behaviours. CeN lesions abolish the vagally mediated conditioned insulin response that environmental cues associated with food will normally elicit whilst once again leaving the unconditioned response intact (Roozendaal, Oldenburger et al. 1990). CeN lesions also abolish

the acquisition of conditioned orienting responses towards cues associated with the delivery of food, though the spontaneous orienting response (e.g. rearing towards a novel light stimulus when it is initially presented) is not affected. Interestingly, the physical characteristics of the CS determine the exact nature of the response: a visual CS, for instance, will elicit a rearing response, whereas an auditory CS will elicit abrupt movements but no rearing (Gallagher, Graham et al. 1990; Hatfield, Han et al. 1996). This differentiation depends on integration of sensorimotor function (Gallagher and Holland 1992), and appears to involve CeN projections to the SNc, which in turn sends a dopaminergic projection to the dorsal striatum (Holland 1977; Gonzales and Chesselet 1990; Han, McMahan et al. 1997). Supporting evidence is provided by the finding that damage to the nigrostriatal dopaminergic neurons impairs spontaneous orienting to sensory cues (Carli, Evenden et al. 1985; Carli, Jones et al. 1989; Gallagher and Holland 1992).

Similarly, the CeN has been shown to be involved in Pavlovian approach behaviour or autoshaping (Bussey, Everitt et al. 1997), the acquisition of which is dependent also on the integrity of the NAcc core and its dopaminergic innervation (Parkinson, Olmstead et al. 1999; Parkinson, Cardinal et al. 2000). Unlike the BLA, the CeN does not project directly to the NAcc (Zahm and Brog 1992; Zahm, Jensen et al. 1999) but it does project to the VTA (Krettek and Price 1978; Price and Amaral 1981; Amaral, Price et al. 1992; Fudge and Haber 2000), and it might be that it is able to regulate the VTA's dopaminergic projection to the NAcc core (Everitt, Parkinson et al. 1999; Everitt, Cardinal et al. 2000; Parkinson, Cardinal et al. 2000). Other findings are consistent with this hypothesis. For instance, dopaminergic lesions of the CeN or infusions of dopamine receptor antagonists into the amygdala have marked effects on extracellular dopamine levels in the NAcc (Louilot, Simon et al. 1985) whilst dopaminergic lesions of the NAcc result in impairments in autoshaping, much like the effects of excitotoxic NAcc core (Parkinson, Olmstead et al. 1999) and excitotoxic CeN lesions (Everitt, Cardinal et al. 2000). Moreover, there is evidence that dopaminergic mechanisms within the amygdala itself might be involved in Pavlovian approach behaviour - Hitchcott and Phillips (1998) found that post-training infusions of a dopamine D3 receptor antagonist into the CeN enhanced acquisition

of Pavlovian approach behaviour whereas infusions into the BLA did not. All in all, the above findings suggest that a distributed corticostriatal network underlies Pavlovian approach behaviour. It might be that Pavlovian approach behaviour is a more extreme manifestation of the orienting response, but whether this is the case or not, the involvement of the CeN in the above forms of aversive and appetitive Pavlovian conditioning appears to lie in its access to output systems governing autonomic and behavioural responses and to ascending systems that innervate the forebrain.

On the other hand, CeN lesions do not appear to affect several other forms of Pavlovian appetitive conditioning. Not only are CeN-lesioned rats able to learn the association between a stimulus and reward as measured by 'food-cup behaviour' (Gallagher, Graham et al. 1990; Hatfield, Han et al. 1996), but Hatfield et al (1996) demonstrated that they are also able to go on to acquire second-order conditioning as robustly as normal rats, and are, moreover, equally sensitive to reinforcer devaluation. This suggests that, for CeN-lesioned rats, the CS is not only able to acquire the value of the US at the time of conditioning, but that it is also able to gain access to post-training changes in the value of the US, thus allowing the rats to make appropriate adjustments in the performance of a task. Hatfield et al's (1996) findings are supported by the results of an earlier experiment carried out by Gallagher and Holland (1992) in which CeN-lesioned and normal rats were first trained to associate a light CS paired with food, and then, later, when fully satiated, tested for food consumption in the presence of the light CS (CS-potentiated feeding). Both CeN-lesioned and normal rats showed an increase in consumption in the presence of the light, suggesting that both groups were equally influenced by the motivational value acquired by a CS through association with a reward.

As mentioned above, CeN lesions abolish the conditioned orienting response whilst leaving the spontaneous orienting response intact (Gallagher, Graham et al. 1990). This has been shown to be the case in both first-order and second-order Pavlovian conditioning: rats show normal acquisition of both first- and second-order conditioned responses but no acquisition of orienting responses to either the first- or second-order CS (Hatfield, Han et al. 1996). The orienting response can be said to be the outward expression of attention towards a

stimulus, and can be elicited in a bottom-up manner due to the novelty or salience of a stimulus, or modulated from the top-down by learned significance; Holland, Gallagher and colleagues have suggested, therefore, that the CeN is involved in the regulation of attentional processing of stimuli in associative learning (Gallagher 2000). However, recent research suggests that the CeN is also critically involved in another behaviourally and functionally separable aspect of attention, namely the allocation of attentional processing resources to stimuli – in other words, the CeN appears to regulate the *associability* of stimuli (Gallagher and Holland 1992; Gallagher and Holland 1994; Holland and Gallagher 1999). As discussed in more detail in the section on Pavlovian conditioning above, associability is a learning theory concept used to determine how much processing is devoted to a stimulus (Rescorla and Wagner 1972; Pearce and Hall 1980) and the associability of a stimulus can be increased by, for instance, a shift in its consistent predictive relationship with a given outcome to a less predictive relationship. Holland and Gallagher (1993; 1993) have shown that a shift in the predictive relationship of a light to a tone enhances attentional processing and therefore conditioning in normal rats, but not in CeN-lesioned rats.

The CeN projection to cholinergic neurons in the nucleus basalis (nBM) and the substantia innominata (SI), and their cholinergic innervation of posterior parietal cortex (PPC), have also been shown to be important in the mediation of shifts in attention, with immunotoxic lesions of cholinergic neurons in the nBM and in the substantia innominata (SI) and immunotoxic lesions of the cholinergic innervation of PPC producing impairments very similar to those seen with CeN lesions (Chiba, Bucci et al. 1995; Bucci, Holland et al. 1998; Han, Holland et al. 1999). Importantly, none of these lesions had any effect on performance when training procedures did not encourage increased attentional processing, suggesting that this system as a whole is important in the regulation of shifts in attention that are brought about by changes in the predictive relationship between cue and outcome (Gallagher 2000).

1.4.2.2 Role of the CeN in instrumental conditioning

To date, little research has been done into the involvement of the CeN in instrumental conditioning, but CeN lesions, like BLA lesions, do not impair first-order instrumental responding. Moreover, unlike BLA lesions, lesions of the CeN do not appear to significantly impair the way in which stimuli endowed with positive affect can support goal-directed instrumental behaviour (Everitt, Parkinson et al. 1999). This is in accord with the results of the Pavlovian second-order conditioning experiments mentioned above (Gallagher, Graham et al. 1990; Hatfield, Han et al. 1996), providing support for the idea that CN lesioned rats acquire normally the motivational value associated with cues during appetitive learning. Interestingly, although CeN lesions do not impair the acquisition of a new response with conditioned reinforcement, they do abolish its potentiation by increased levels of dopamine in the NAcc, in this case within the shell region (Robledo, Robbins et al. 1996). The CeN's projections to VTA would therefore seem to regulate the mesolimbic dopamine system in a dissociable manner, with dopaminergic innervation of the NAcc core mediating effects on Pavlovian approach behaviour, and dopaminergic innervation of the shell mediating the effects of dopamine and both direct and indirect dopamine agonists on instrumental behaviour (Everitt, Parkinson et al. 1999; Parkinson, Olmstead et al. 1999).

Overall, the CeN would appear to be involved in the regulation of arousal and attention during learning, and also in the associative learning of emotional responses when these are mediated by the hypothalamic, midbrain and brainstem regions to which it projects.

1.4.2.3 Summary

Traditionally, the prevailing model of information transfer within the amygdala has held the LA/BLA to be the primary site for the convergence and association of conditioned and unconditioned stimuli, at least with regard to aversive conditioning. The LA/BLA is then able to access and integrate the neural substrates of autonomic, neuroendocrine and reflexive behavioural responses through its projections to the CeN. The flow of information was therefore

thought to be lateral-to-central, and the CeN to be merely an output nucleus subordinate to the LA/BLA (LeDoux, Iwata et al. 1988; Clugnet and LeDoux 1990; LeDoux, Cicchetti et al. 1990; Davis 1992; Fendt and Fanselow 1999). However, evidence from the appetitive conditioning studies discussed above suggest that the functions of the CeN might be more complex than traditionally thought, transcending output, attentional and arousal processes. This should not be altogether surprising: as noted earlier, in the anatomy section, the CeN receives direct projections from higher-order cortices as well as through the LA/BLA. Moreover, an increasing number of diverse afferents to the CeN are being found, whilst its connections with the neurochemically defined ascending reticular activating systems (cholinergic, dopaminergic, serotonergic and noradrenergic) bring the entire forebrain under the modulatory control of the CeN (McDonald 1998). As Killcross et al (1997) revealed for aversive conditioning, the studies discussed above show that double dissociations of function exist between the BLA and the CeN in appetitive conditioning. For instance, although some forms of Pavlovian conditioned responses such as conditioned freezing, fear-potentiated startle and conditioned bradycardia are abolished by both BLA and CeN lesions (Gentile, Jarrell et al. 1986; LeDoux, Iwata et al. 1988; Davis 1992; Kapp, Whalen et al. 1992; Maren and Fanselow 1996; Powell, Chachich et al. 1997; Fendt and Fanselow 1999), other forms such as Pavlovian approach behaviour and conditioned orienting are sensitive only to CeN lesions whilst Pavlovian place preference is sensitive only to BLA lesions (Hatfield, Han et al. 1996; Parkinson, Robbins et al. 2000; Holland, Hatfield et al. 2001). And, although neither BLA nor CeN lesions impair first-order Pavlovian conditioning to a stimulus paired with reward, BLA lesions do impair second-order Pavlovian conditioning and also reinforcer devaluation. This suggests that whilst CeN-lesioned rats are able to re-evaluate affective value in the neural representations of rewards and thus make appropriate adjustments in the performance of a task, BLA-lesioned rats are not (Gallagher, Graham et al. 1990; Hatfield, Han et al. 1996). Likewise, although both BLA- and CeN-lesioned rats are able to make first-order instrumental responses for reward (Cador, Robbins et al. 1989; Everitt, Parkinson et al. 1999), BLA-lesioned rats are impaired in both second-order instrumental conditioning (Everitt, Cador et al. 1989; Whitelaw, Markou et al.

1996) and in the acquisition of a new response with conditioned reinforcement (Cador, Robbins et al. 1989; Burns, Robbins et al. 1993), again suggesting that the BLA (but not the CeN) is necessary for a CS to acquire affective value. Moreover, dopaminergic mechanisms within the BLA and the CeN also influence Pavlovian and instrumental conditioning in a dissociable way: Hitchcott and Phillips (1998) found that the expression of Pavlovian approach behaviour to a reward-associated CS was not affected by infusions of a dopamine D3 receptor agonist into the BLA, but was attenuated by infusions into the CeN, whereas the ability of the reward-associated CS to support the acquisition of a new instrumental response (acquisition of a new response with conditioned reinforcement) was abolished selectively by infusions into the BLA but not by infusions into the CeN.

The existence of such dissociations rather argues against the idea that associations are formed and stored exclusively within the LA/BLA but sent to the CeN for subsequent response production. Whilst it is not yet clear whether the CeN actually participates in the associative mechanisms underlying conditioning (Gallagher, Graham et al. 1990; Morris, Frey et al. 1999), it does receive the appropriate neuronal afferents. Also, other structures apart from the BLA are known to participate in both appetitive and aversive Pavlovian conditioning – for example, the anterior cingulate cortex, the NAcc core and the hippocampus (Bussey, Everitt et al. 1997; Maren, Aharonov et al. 1997; Cahill, Weinberger et al. 1999; Parkinson, Willoughby et al. 2000). If the CeN does indeed participate in the associative mechanisms underlying conditioning, it would seem likely that it supports simpler Pavlovian CS-UR associations since it is not required for a CS to gain access to the current value of the specific US with which it is paired. This latter would necessitate CS-US associations, for which the BLA is required. Support for this comes from an electrophysiological study into appetitive conditioning in rats carried out by Muramoto et al (1993), who found that neurons in the BLA are more likely to acquire patterns of activity to a CS that are similar to those generated initially to the US than are CeN neurons. BLA neurons are also more likely to be sensitive to the affective nature of the US, showing different patterns of activity to appetitive and aversive USs and their corresponding CSs.

It would therefore appear that separate subsystems within the amygdala make use of different associative mechanisms, modulating behaviour in distinct ways: the BLA subsystem is concerned with changes in the incentive value of cues in Pavlovian appetitive conditioning whereas the CeN subsystem is concerned with arousal and attentional processing. Normally, of course, these subsystems operate together, possibly as part of a larger limbic corticostriatal circuit. Experiment A in chapter 3 of this thesis looks at the involvement of the BLA in an appetitive conditioning task, the Schedule Fraction Cue (SFC) task, in which cues signal how close a rat is coming to achieve reward, whilst Experiment B uses the same task to see whether the performance of BLA- and CeN-lesioned rats can be dissociated.

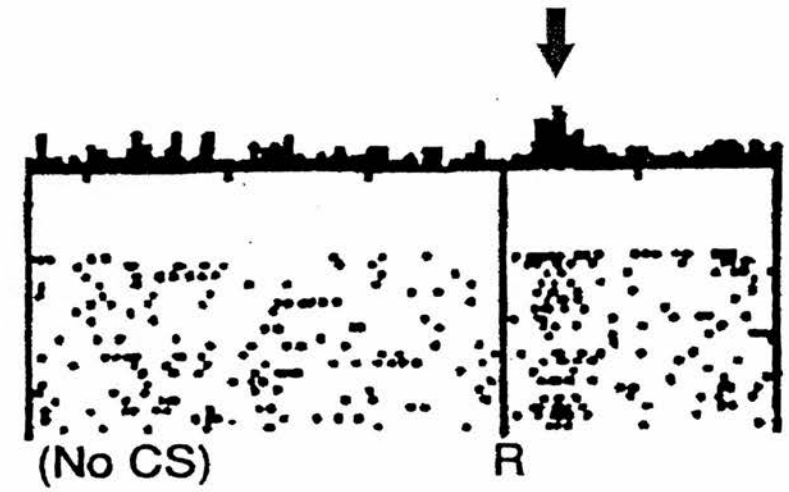
Chapter 2 - Reward Expectation and the Blocking Task

2.1 Introduction

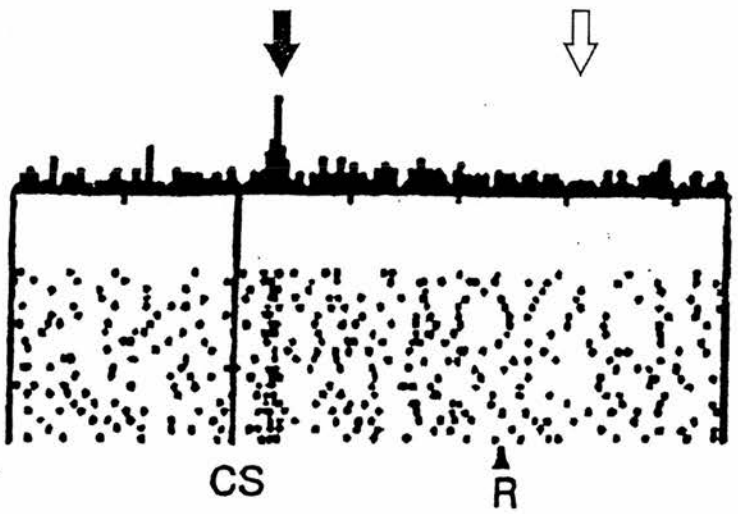
As intimated in the general introduction, dopamine would appear to play a role in reward learning. Research carried out by Wolfram Schultz and his colleagues (Schultz, Apicella et al. 1992; Schultz, Apicella et al. 1993; Schultz, Apicella et al. 1993; Schultz 1997; Schultz, Dayan et al. 1997) has focused on how neural activity recorded at the dopamine cell body in the VTA or in the SNc may be conveying information about reward rather than on the effect that dopamine may be having in the NAcc. Their work suggests that dopamine neurons, rather than responding unconditionally to reward or reward-predicting stimuli, are involved in signalling deviations from the predictions of future appetitive events. Schultz et al (Schultz, Apicella et al. 1993; Schultz, Apicella et al. 1993) recorded from individual dopamine neurons in the VTA and in the SNc of monkeys learning that a given stimulus is followed by a reward. They found that at the onset of conditioning there is an increase in electrical activity when the animal receives an 'unexpected' reward, but that as the animal learns that a stimulus consistently precedes the reward, and therefore predicts it, this increase in electrical activity shifts forward from the presentation of the reward to the presentation of the stimulus. If, after the animal has learned to associate the tone with the reward, it does not receive the expected reward after hearing the tone, there is still an increase in electrical activity after the tone, but also a marked decrease in electrical activity at precisely the time the reward should have occurred ***(Figure 3)***.

Figure 3:
This figure is taken from Wolfram Schultz et al (Schultz, Dayan et al. 1997) and shows changes in dopamine neurons' output code for an error in the prediction of appetitive events. The top figure shows reward occurring in the absence of prediction (no CS), with increased dopaminergic activity at the time of reward presentation. The middle figure shows reward occurring after it has been predicted by the CS, with increased dopaminergic activity at the time of CS presentation, but not at the time of reward presentation. The bottom figure shows extinction of learning, with the CS predicting the reward, but no reward occurring. There is increased dopaminergic activity at the time of presentation of the CS, and depressed dopaminergic activity at the time that the reward should have occurred.

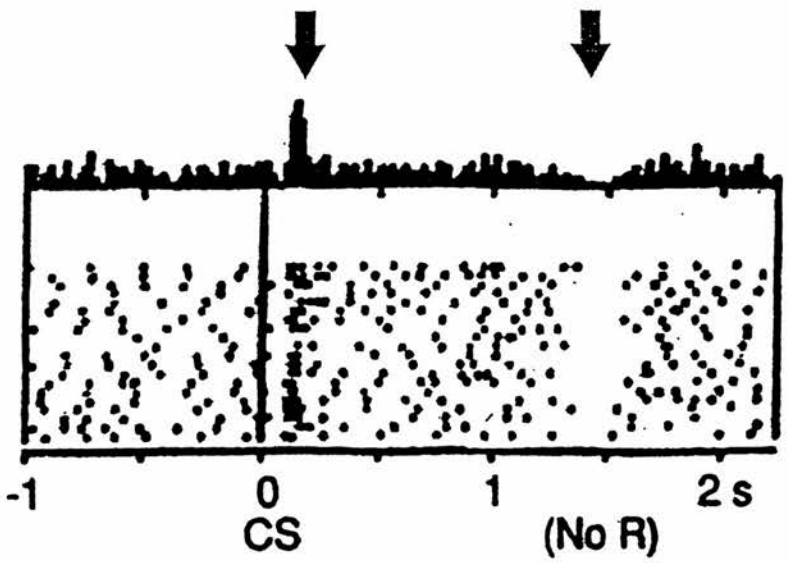
Schultz et al's data



learning



after learning



extinction of learning

This suggests that the expected time of reward must be encoded in the fluctuating activities of the dopamine neurons. Schultz et al (1997) suggest that this shift in dopaminergic activity 'strongly resembles the transfer of an animal's appetitive behavioural reaction from the US to the CS' (p.1594). They claim that 'the activity of dopamine neurons codes for a deviation or error between the actual reward received and predictions of the time and magnitude of the reward' (p.1594). This claim is based on Rescorla and Wagner's (1972) theory that learning is driven by the difference between expected and actual outcome.

But does this shift in dopaminergic activity really reflect the psychological expectation of reward, or is it merely due to the temporal contiguity of stimulus and reward? One way in which this question could be addressed is by recording the activity of dopamine neurons during an appetitive blocking paradigm. As explained in greater detail previously, blocking differentiates between temporal contiguity (CS-US pairing) and contingency (causal/predictive relationship between CS and US): if conditioning depends only on a CS and a US being paired then presentation of the compound CS-BCS in the second stage of training should result in effective conditioning to the BCS. Since conditioning to the BCS does not occur, the subject must be taking some other calculation into consideration, incorporating the conditional probabilities of the US occurring when the CS is present as well as when the CS is absent. In an appetitive blocking paradigm, the probability that a CS will lead to reward is far higher than the probability that a BCS will lead to reward. If, therefore, dopamine neurons are encoding expectancy of reward rather than temporal contiguity in shifting their activity from the presentation of a reward to the stimulus predicting its delivery, then it could be expected that they will not do so when the BCS is presented alone at test.

There is some evidence that this will indeed be the case. Montague et al (1996) have constructed a neural network model (given in **Appendix A**) to explain how dopamine neurons predict rewards, incorporating the neurophysiological data obtained by Schultz and his colleagues with what is known from learning theory about how stimulus-reward associations are learnt. Such models are important because if they do successfully predict the real behaviour of dopamine neurons in simple situations such as the presentation of a stimulus which is then

followed by a reward, then they can be used to hypothesise what may be happening at the neuronal level in more complex situations such as in a blocking paradigm. In their paper, Schultz et al (1997) illustrate how Montague et al's (1996) model can predict the delivery of a future reward from a single sensory cue, and claim that it matches the activity of real dopamine neurons during learning: initially there is no response whatsoever to the presentation of the cue, but there is a strong response to the delivery of the reward. After several presentations of the cue followed by the reward, this response shifts forward to the presentation of the cue and there is no response to the reward. If the reward is not delivered in one of the learning trials, then there is a large negative fluctuation in the model's output, which, the authors suggest, mimics the depression seen in dopaminergic activity when an expected reward is not delivered. This shift in activity from the time of presentation of the reward to the time of presentation of the stimulus is also clearly illustrated in the first implementation of Montague et al's model carried out by the author and Eric Bowman (see **Appendix A: *Figure 32, Implementation 1***). If this implementation of the model does indeed mimic the neurophysiological data obtained by Schultz et al (1997) it also serves to demonstrate that there is temporal contiguity between stimulus and reward. Schultz et al (1997) have used Montague et al's (1996) model to make several predictions about the activity of dopaminergic neurons in different learning situations, and these have been explored by further implementations of Montague et al's model carried out by the author and Eric Bowman (see **Appendix A: *Figure 32, Implementations 2-4***). These further implementations of the model suggest that the dopaminergic response is based on contingency, in that dopamine neurons should respond to a CS but not to a simultaneous BCS **(Appendix A: *Figure 32, Implementation 2*)**, but also suggest that dopamine neurons have access to a representation of the second conditioned stimulus in serial conditioning **(Appendix A: *Figure 32, Implementations 3 and 4*)**. This latter finding has implications for the blocking paradigm in which animals are purported to ignore (i.e. not become conditioned to) the existence of a second stimulus after learning that a first stimulus leads to reward. Thus, even though behavioural blocking may occur, there might also be a neural representation of the

blocked stimulus. It would be of great interest to see if this is indeed the case in biological neurons.

The immediate aim of this experiment was the design and behavioural testing of such a blocking paradigm. However, there are two other considerations to be taken into account in doing so. First, Schultz et al's (1997) data were obtained from different neurons at different stages of conditioning, giving the impression of an abrupt switch from activity occurring at presentation of the reward to activity occurring at presentation of the stimulus. They did not really show the dynamics of activity during the learning process, but only the beginning and end points. It is important to follow the whole learning process from activity at reward and its shift forward to activity at stimulus within a single neuron, and preferably over a single recording session. This means designing a paradigm that will allow appetitive blocking to occur in as short a time as possible. Second, Schultz et al's (1997) data were obtained from experiments involving discriminative conditioning. It is important to engage Pavlovian conditioning in order to access what is believed to be the involuntary nature of learning within the dopamine system. Licking is an almost automatic behaviour in the rat, and so it was decided to use how often the rat licked a spigot in order to obtain a saccharin solution reward as the measure of conditioning. As discussed previously, conditioned stimuli will elicit conditioned responses similar to the unconditioned responses that had initially only been elicited by the unconditional stimulus. In the blocking paradigm, therefore, there should be a gradual increase in licking at the time of presentation of the CS as the rat learns the association between the CS and the reward, but not at the time of presentation of the BCS. This increase in licking at the time of presentation of the CS will hopefully be correlated with Schultz et al's reported shift in dopaminergic activity from the presentation of a reward to the presentation of a stimulus predicting it. (In the blocking paradigm, licking will not, of course, 'shift' from presentation of the reward to the preceding stimulus since the rat will lick to consume the reward). It will then be possible to record from individual neurons in the VTA of naive rats whilst they are taking part in the paradigm, allowing behaviour and neuronal activity to be correlated on a trial by trial basis. This will enable us to pinpoint exactly when learning is occurring.

2.2. Experiment A

2.2.1. General methods

2.2.1.1. Subjects

8 adult male Hooded Lister rats (Charles River, UK) weighing 500-700 gm were housed in pairs and maintained on a 12 hour light/dark cycle. Testing took place between 12 noon and 4.00pm during the light cycle, and on weekdays only. Food was freely available, but access to water during weekdays was restricted to one hour a day, between 4.00pm and 5.00pm. At the weekend, water was freely available. The rats were weighed every other day to ensure that they did not fall beneath 85% of their free-feeding weight. The guidelines laid out in the "Principles of laboratory animal care" (N.I.H. Publication no. 86-23, revised 1985) and the requirements of the U.K. Animals (Scientific Procedures) Act 1986 were adhered to throughout the experiment.

2.2.1.2 Apparatus

The apparatus was a commercial operant conditioning system (MED Associates, USA). Experimental sessions were conducted in front-opening modular operant test chambers (working area: 24cmW, x 30.5L x 29D), which were housed within sound attenuated cubicles (inside dimensions: 66cmW, x 50.8L x 50.8D). The front panel of each sound attenuated cubicle had a darkened viewing window. Each operant test chamber was fitted with a spigot for liquid delivery, which was positioned in the centre of the left wall 8cm above the grid floor. This spigot was of the same type as that of the water bottle used in the home cage. Liquid was pumped to the spigot by a syringe pump fitted with a 50ml glass syringe (Rocket, UK) which was situated outside the operant test chamber. An electronic contact lickometer recorded the number of licks made by the rat to a time resolution of 5 msec. Each operant test chamber had a house light situated on the leftside end wall 27cm above the grid floor and a tone generator (4.5kHz, 60dB) situated on the left wall 10cm above the spigot. Ambient lighting was provided

by light-emitting diodes in the two end holes of a five-hole nosepoke array situated on the right wall. Med-PC software, run on a PC, was used for data acquisition and experimental control.

2.2.1.3 Procedure

Pretraining

The rats were first habituated to the taste of saccharin by being given free access to (0.25%) saccharin solution instead of water for 24 hours. This concentration of saccharin was used because Clark et al (1990) have shown it to be the most desirable to rats. The rats were then put on a water-restriction schedule, being allowed access to water only for one hour a day between 4 and 5pm. The rats were allowed to habituate to this schedule for 2 to 3 days before being run in the experiment.

Training and testing

This experiment was divided into three stages: 1) presentation of a conditioned stimulus (CS) alone, 2) presentation of a compound CS and 'blocked' conditioned stimulus (CS+BCS), and 3) presentation of unrewarded probes to test the rats' responses to the CS and to the BCS. It was predicted that this would lead to blocking - namely, that the rats would show increased licking after presentation of the CS probe in expectation of the reward, but not after presentation of the BCS probe since reward would not be expected.

Stage 1

The first stage took place over five days, with each daily experimental session lasting for an hour. Rats (already accustomed to licking a spigot in order to obtain water in the home cage) were trained to lick the spigot after a 1-second presentation of a light or tone CS in order to receive a reward of 0.1ml of 0.25% saccharin solution. For odd-numbered rats, the CS was the light, and for even-numbered rats the CS was the tone. Licking of the spigot was polled 1 second after presentation of the CS: if the rat licked the spigot before 1 second after the presentation of the CS, this was recorded but had no consequence other than the reward not being immediately delivered, but if the rat licked the spigot after 1 second and within 3 seconds of presentation of the CS, a drop of saccharin solution was pumped to the spigot and dispensed

over a further 2 second period. Once the rat had finished licking the reward and had not licked the spigot for 1 second, the CS was then presented again after an inter-trial interval of between 5 and 15 seconds, thereby repeating the cycle. If the rat did not lick the spigot within 3 seconds after presentation of the CS, it was not rewarded with a drop of saccharin solution, and the CS was again presented after the same inter-trial interval ***(Figure 4).*** The period during which the CS was presented was termed Time1, and the period during which the reward was presented (or not, in the case of the probes – see Stage 3) was termed Time2.

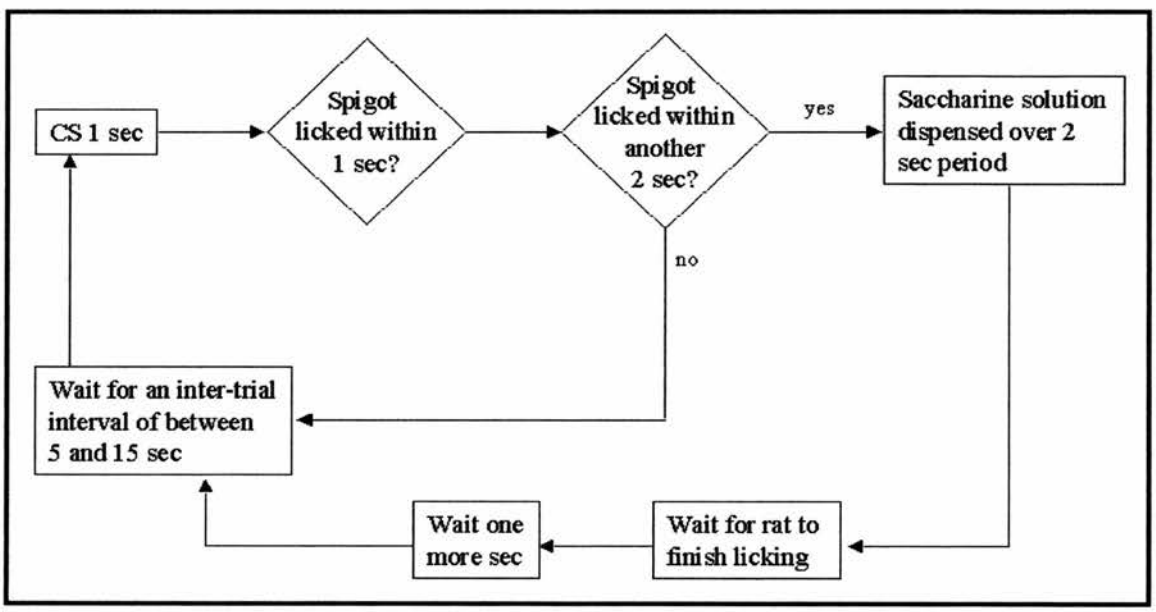


Figure 4: *Flowchart of the experimental procedure*

The computer recorded the number of licks made by the rat during the inter-trial interval (excluding the 1 second "no licking" period after the reward) before the CS onset, i.e. the baseline lick rate, the number of licks during presentation of the CS (Time1), the number of licks in the 1 second interval between the CS and the reward during which licking did not immediately lead to delivery of the reward, and the number of licks during the reward itself (Time2). The computer also recorded the reaction times of each rat, i.e. how long it took to lick after presentation of the CS and after presentation of the saccharin solution reward. These data provided the on-line display, but the behavioural data were analysed *post hoc*: see the Analysis section below.

Stage 2

In order to establish blocking the rats were presented simultaneously with the preconditioned stimulus (the CS) and with a second 'blocked' stimulus (the BCS) at Time1, followed by the reward at Time2. The BCS was the tone for odd-numbered rats, and the light for even-numbered rats. The second stage also took place over five days, with each daily experimental session lasting for an hour.

Stage 3

Finally, during a single hour-long experimental session, the rats were presented with randomised presentations of both the preconditioned CS and the 'blocked' stimulus (BCS) individually at Time1, with no reward following at Time2. These unrewarded presentations of the CS and the BCS were termed probes. It was expected that the rats would lick the spigot after a presentation of the CS probe in expectation of the reward, but that they would not lick after a presentation of the BCS probe because they would not associate this with the reward.

2.2.2 Analysis of data

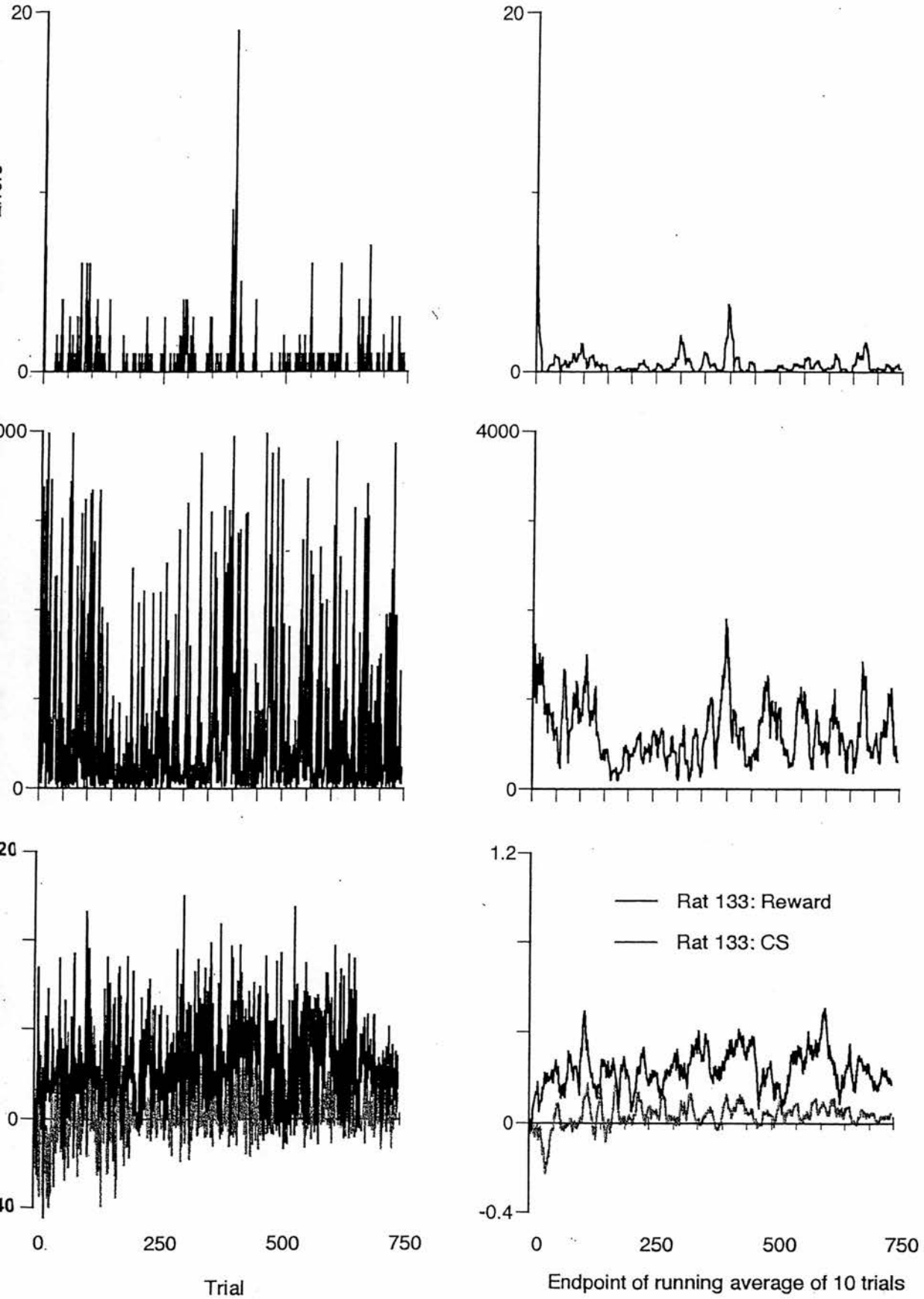
Behavioural data from each rat were smoothed by using a running average over 10 trials as follows: for the first 9 trials, trial 1 was referenced to itself, then trials 1 and 2 were averaged and referenced to trial 2, and then trials 1, 2, and 3 were averaged and referenced to trial 3 etc. until trials 1, 2, 3, 4, 5, 6, 7, 8 and 9 were averaged and referenced to trial 9. This meant that there was necessarily an edge effect with the first 9 trials, which decreased as certainty of information increased with the number of trials included in the smoothing process. Thereafter, trials 1 to 10 were averaged and referenced to trial 10, and then trials 2 to 11 were averaged and referenced to trial 11 etc. until the last trial was reached. In effect, a window of 10 trials was moved along the trial data, one trial at a time. This smoothed data was then averaged over all 8 rats. The results and discussion sections will deal with these averaged data, but raw and smoothed data from a randomly chosen single rat, 133, ***(Figure 5)*** are also presented in order to allow visual comparison. There is necessarily a trade-off between the use of a running average

to smooth data, and the accuracy with which the precise time course of learning can be tracked. This is particularly pertinent to the error rate measure, but also to the Reaction Time measure, since both decrease very rapidly at the very beginning of training. Percentage licking contact time, error rate and reaction time were used as measures of performance. The percentage licking contact time measure was determined by dividing a given epoch (the inter-trial interval (baseline) / presentation of the CS (Time1) / presentation of the reward (Time2)) by the 5 msec sampling rate to give the total number of possible times during that epoch in which the computer could have indicated that the rat was licking. The number of times that the computer indicated that the rat was actually licking during the epoch was then divided by the total number of possible times that it could have licked during that epoch and multiplied by 100 to give the percentage licking contact time. The baseline (inter-trial interval) percentage licking contact time rate was subtracted from the percentage licking contact time during presentation of the stimulus (Time1) and from the percentage licking contact time during the reward period itself (Time2); this subtraction was done on a trial-by-trial basis and then averaged across rats as described above. The mean percentage licking contact time for the CS probe was calculated by averaging across every presentation of the CS probe at Time2 throughout the testing session for each rat, and then averaging across all eight rats. The mean percentage licking contact time for the BCS probe was calculated in the same way.

Figure 5

This figure presents data from rat 133 for Stage 1 of Experiment 1. Raw data are shown in the left column, and smoothed data are in the right column. The horizontal axis for the raw data graphs shows the trial number, whereas for the smoothed data graphs it shows the end point of the running average of 10 trials. The top row shows the number of errors per trial made by rat 133, i.e. the number of CS presentations made before the animal licked in order to obtain the reward. The middle row shows the reaction time for rat 133, i.e. how quickly it licked the spigot after presentation of the CS. The bottom row shows the percentage licking contact time during the reward and the CS. The raw data are extremely noisy, but smoothing allows trends in the individual animal to be revealed. It can be seen from the top and middle right hand graphs that error rate and reaction time both decrease over trials. The bottom graph shows that the lick rate during presentation of the CS increases with training, as it does during the reward.

Stage 1: Raw and smoothed data from rat 133



2.2.3 Results

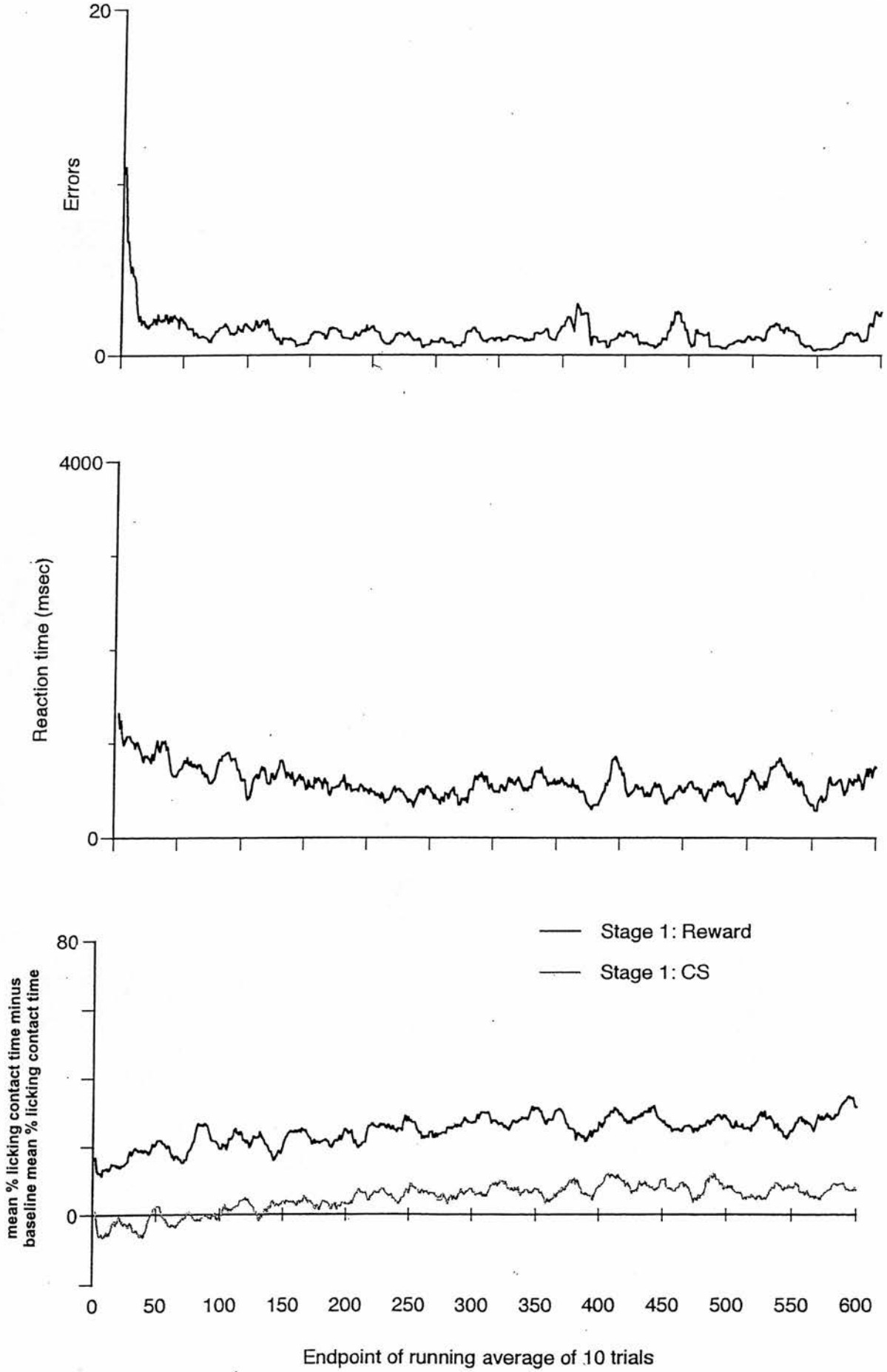
During Stage 1 the rats were presented with the CS followed by the reward. It can be seen from ***Figure 6, top graph*** that the number of errors made per trial (i.e. the number of CS presentations before the animal licked in order to obtain the reward) decreased very rapidly, stabilising at approximately 2 per trial after about 70 trials. Reaction time (i.e. how quickly the rat licked the spigot after presentation of the CS) decreased more steadily over trials, to approximately 500 msec after 200 trials ***(Figure 6, middle graph)***. As described above, the baseline (or intertrial interval) percentage licking contact time was subtracted from the percentage licking contact time for both the presentation of the CS at Time1 and the presentation of the reward at Time2 ***(Figure 6, bottom graph)***. It can be seen that the percentage licking contact time during the presentation of the CS gradually increased over trials, going above zero (which represents the baseline rate) after approximately 100 trials. The percentage licking contact time during the presentation of the reward also increased steadily over trials, and was consistently higher than the percentage licking contact time during the presentation of the CS. During Stage 2 the rats were presented simultaneously with both the preconditioned CS and a 'blocked' stimulus (BCS) at Time1. It was found that the rats' response with regard to error rate continued to improve slightly during this stage whilst their reaction time remained more or less the same. Percentage licking contact time during the CS+BCS at Time1 and during the reward at Time2 was maintained from the first stage ***(Figure 7, top, middle and bottom graphs)***.

Figures 6 and 7

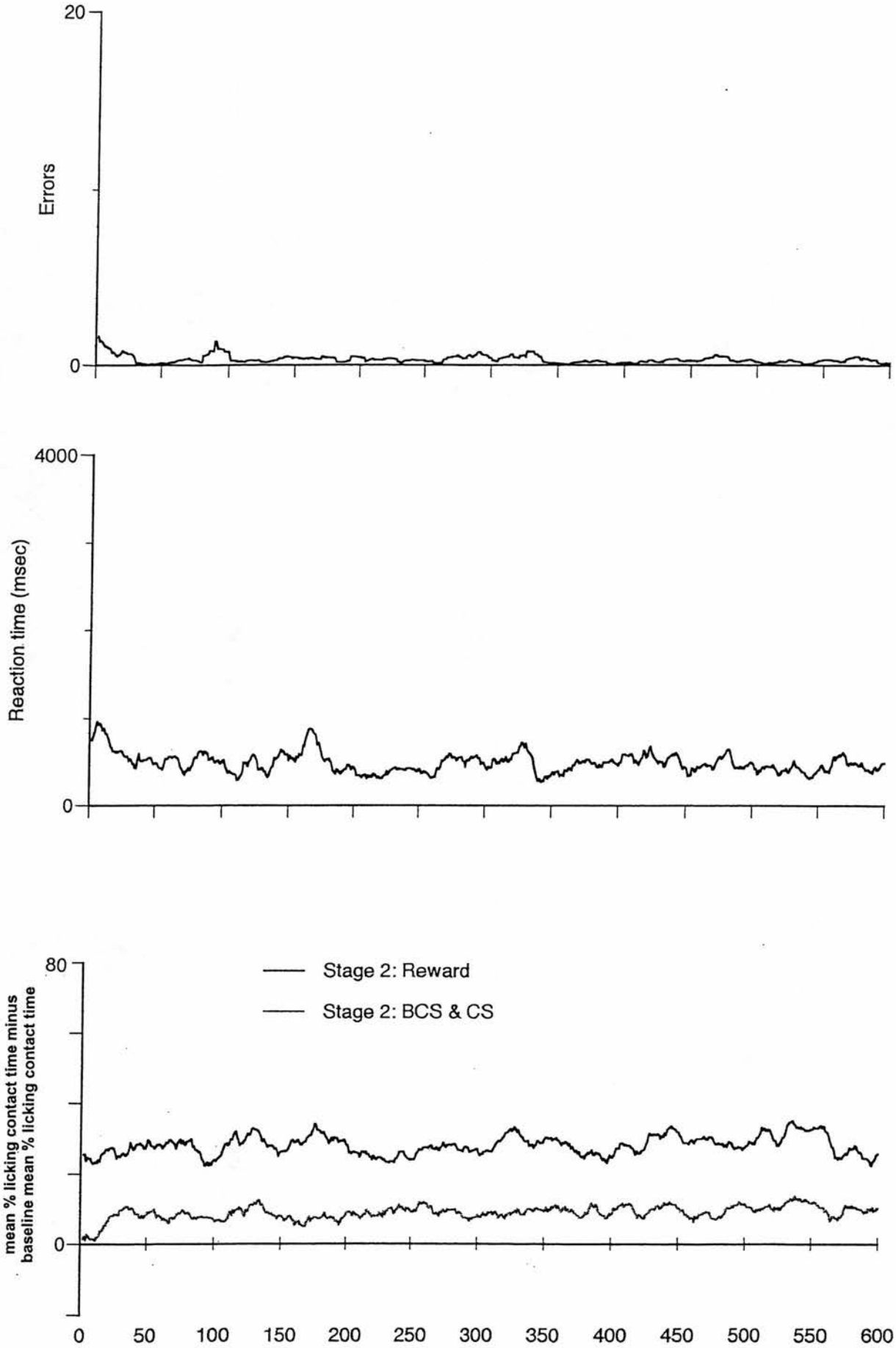
The horizontal axes show the end point of the running average of 10 trials after smoothing. The top graph for each figure shows the average number of errors made by the rats, i.e. the average number of CS presentations made before the animals licked in order to obtain the reward. The middle graph shows the average reaction time for the rats, i.e. how quickly they licked the spigot after presentation of the CS. The bottom graph for each figure shows the mean percentage licking contact time during the CS and the reward; this was calculated on a trial-by-trial basis for each epoch and then averaged across all the rats.

Figure 6: *Averaged smoothed data from all 8 rats for Stage 1 of Experiment 1 during which the rats were presented with the CS followed by the reward.*
Figure 7: *Averaged smoothed data from all 8 rats for Stage 2 of Experiment 1 during which the rats were presented with both the preconditioned CS and the 'blocked' BCS followed by reward*

Stage 1: Smoothed data from all rats



Stage 2: Smoothed data from all rats



Concurrent presentation of the BCS did not, therefore, disrupt the conditioning effects elicited by presentation of the CS alone.

Stage 3 showed that, unfortunately, blocking was not achieved. It can be seen from the bar chart below ***(Figure 8)*** that the mean percentage licking contact time for the BCS probe is in fact slightly higher than for the CS probe, though subjection of the two means to t-test indicated that this difference is not significant ($t = -1.16$; $df = 7$; $p > 0.05$).

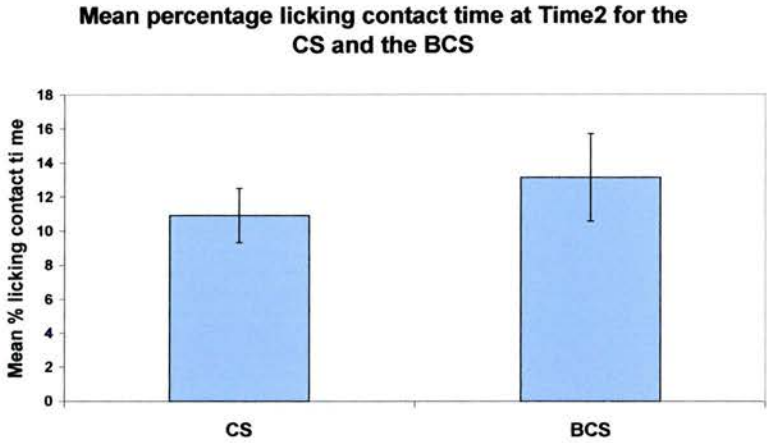


Figure 8: *Mean percentage licking contact time for the CS probe and the BCS probe at at Time2 (when the reward should have occurred)*

2.2.4 Discussion

During Stage 1, the rats showed an increase in percentage licking contact time to above zero during the presentation of the CS, suggesting that they did indeed learn the association between stimulus and reward. This increase in percentage licking contact time therefore could be seen as reflecting the transfer of the rat's appetitive behavioural reaction from the reward to the preceding stimulus as predicted by learning theory. Conversely, since the rats also showed an increase in percentage licking contact time during the presentation of the reward, it could be argued that the rats did not in fact learn the association between the CS and the reward and exhibited only a generalised increase in percentage licking contact time as they learned that the spigot dispensed saccharin solution. However, since the rats had to wait a second after

presentation of the CS before licking would lead to reward, and also had to wait for a second after the reward was finished before the 5-15 second inter-trial interval began (as described in the Training and Testing section above), this seems somewhat unlikely. The fact that percentage licking contact time during presentation of the BCS decreased dramatically in the second experiment also supports the argument that the rat is paying attention to the CS. The increase in percentage licking contact time at the time of the reward could also be explained as resulting from increased efficiency on the rats' part as they learnt to limit their licking to the times that the CS and the reward were presented, thereby decreasing percentage licking contact time during the inter-trial interval (which forms the baseline). Despite the fact that the rats appeared to have learnt the association between the CS and the reward during Stage 1, blocking was not achieved during Stage 3. There are at least three possible reasons why this might be the case:

1) During Stage 1, no reward was given if the rat did not lick the spigot after presentation of the CS; this would have weakened the formation of the CS-reward contingency, making it more difficult for the rat to learn the initial association between stimulus and reward. Nevertheless learning occurred as training progressed through Stage 1 and into Stage 2, as evidenced by the fewer errors made over time. However, more CSs overall would have been rewarded during Stage 2 than during Stage 1. Since a BCS was presented at the same time as every CS in Stage 2, it might be that the weak Stage 1 CS-reward contingency was over-ridden by a possible stronger (and undesirable) second stage BCS-reward contingency as well as a stronger CS-reward contingency. It might even be that the rat perceived the simultaneous presentation of the CS and the BCS in Stage 2 as a compound stimulus that provided a more salient prediction of reward, with both the CS and the BCS components having equal associative strength at test. Either possibility would, of course, mean that the 'blocked' CS was not, in fact, blocked at all.

2) At test, the rats were presented with randomised presentations of both the CS and the BCS individually, with no reward following. Essentially, extinction of licking was used as the measure of learning, with the assumption that extinction would be slower for the preconditioned CSs if blocking had indeed worked. This assumption was based on the theory that the extinction

of a behaviour involves 'unlearning' it - it would therefore take the rat longer to 'unlearn' an association between the CS and the reward than to 'unlearn' an association between the BCS and the reward that (ideally) would not have been 'learnt' by them in the first place. It could, however, be argued that 'extinction' is not really the 'unlearning' of a behaviour, but the learning of a new behaviour, an argument that is supported by the fact that animals very rapidly re-acquire an extinguished behaviour once the original parameters are reinstated. If extinction does involve the learning of a new behaviour, then presumably the rats would learn equally well that neither the CS nor the BCS are followed by reward in Stage 3. The use of extinction as a measure of learning is therefore rather problematical.

3) The blocking paradigm was originally designed for use with aversive conditioning (Kamin 1969) and has very rarely been used with appetitive conditioning. It could be argued that whether the paradigm uses aversive or appetitive conditioning is irrelevant as long as the conditioning stimulus fully predicts the outcome in the first and second stages of training. However, this is not the case - blocking has been shown to work better with aversive rather than appetitive conditioning (Holman and Mackintosh 1981) so factors other than the predictability of the stimulus must influence performance. One obvious factor is the salience of the CS-outcome association. In aversive paradigms, the CS is associated with a negative outcome, such as a shock, which cannot be avoided. The animal's attention is therefore likely to be fully focused on the predicting CS. In an appetitive paradigm there is no aversive outcome, and therefore, perhaps, less necessity for the animal's attention to be fully focused on the predicting CS – the animal's attention will depend on how much it 'wants' the reward, how satiated or tired it is etc. It could also be argued that appetitive conditioning is analogous to an animal's foraging behaviour - trying to locate in time and space whereabouts a reward is to be found - and attention is therefore less likely to be focused on any one event. It might even be that appetitive and aversive conditioning occur by different neural pathways, and that the strong, prototypical blocking effect can be found only with aversive conditioning. Blocking has also been shown to work better within classical rather than instrumental conditioning paradigms (Holman and Mackintosh 1981); this is most probably due to the fact that errors can be made

during instrumental conditioning, thus leading to a weakening of the CS-outcome contingency. Appetitive paradigms often involve an instrumental conditioning component, however small - for instance, unless a rewarding drug is administered intravenously, the animal may or may not collect the reward. In the present study licking, an almost automatic behaviour in the rat, was used as the behavioural measure in an effort to come as close as possible to appetitive classical conditioning, but whether this is in fact achieved is a moot point.

Overall, this experiment showed that the rats did appear to learn the association between the CS and the reward as evidenced by the increase in percentage licking contact time to above zero during the presentation of the CS. The experiment also showed that learning could be achieved within the first 200 trials, but the entire procedure took 11 days in all: 5 days for both Stage 1 and Stage 2 training, followed by the test day. Since the ultimate aim is to record the entire learning process from a single neuron in an individual animal, this was thought to be far too long - it would be difficult to 'hold' the same neuron for that length of time. Blocking, however, was not achieved using this particular paradigm, for several possible reasons that are discussed above. It was therefore decided to run a second experiment in which a new version of the Blocking paradigm was used.

2.3 Experiment B

In the new version of the Blocking paradigm all the stages of blocking were presented within one session, i.e. CS followed by reward, CS plus BCS followed by reward, CS not followed by reward (i.e. the CS probe) and BCS not followed by reward (i.e. the BCS probe). The stimuli were presented in blocks of 15 trials in the following sequence:

	Time1		**Time2**
6 trials:	CS	followed by	reward
6 trials:	CS+BCS	followed by	reward
1 trial:	CS probe1	followed by	NO reward
1 trial:	BCS probe	followed by	NO reward
1 trial:	CS probe2	followed by	NO reward

In all, 12 CSs and 6 BCSs were followed by reward, and 2 CSs and 1 BCS were followed by no reward (i.e. the proportion of CSs followed by reward (12/14) was equal to the proportion of

BCSs followed by reward (6/7)). This means that if the rats were merely responding to the temporal contiguity of stimulus and reward, then they should become as well conditioned to the BCS as they do to the CS. But the probability of a BCS being followed by a reward is 6 out of 15 trials, whereas that of a CS being followed by a reward is 12 out of 15 trials, which means that if the rats become conditioned to the best predictor of reward (contingency), they should differentiate between the CS and the BCS. The effectiveness of the new version of the paradigm in achieving blocking was explored through hypotheses pertaining to the rats' behaviour at Time2, when the reward is made available (or not, in the case of the probes) (planned comparisons 1-3), and to how the rats react to the presentation of the stimuli at Time1 (planned comparisons 4-8). These are given below (the planned comparison means are in italics and enclosed by brackets).

Time2

It was expected that the rats would show an increase in mean percentage licking contact time when the reward was delivered at Time2 after presentation of the CS. Much of this increase would be due to the rats consuming the reward, but it was thought likely that, if the rats had made the association between the CS and the reward, some of the increase could be attributed to anticipatory licking. Likewise, it was expected that the rats would show an increase in mean percentage licking contact time when the reward was delivered at Time2 after presentation of the CS+BCS, and that this increase would contain both consummatory and anticipatory elements. However, since it was hoped that the rats had made the association between only the CS component of the combined CS+BCS stimulus and the reward, and not between the BCS component and the reward, it was hypothesised that:

1. after the presentation of a CS probe1 at Time1, the rats will respond at Time2 as if it were a CS followed by reward by showing an increase in mean percentage licking contact time compared to Baseline *(CS probe1 Baseline and CS probe1 Time2).* (This increase in mean percentage licking contact time would not, however, be as great as after a CS followed by reward since it would not include consummatory licking of the reward.)

2. after the presentation of a BCS probe at Time1, the rats will **not** respond at Time2 as if they expected the presentation of a reward, and will **not** therefore show an increase in mean percentage licking contact time compared to Baseline *(BCS probe Baseline and BCS probe Time2).*

3. after the presentation of a CS probe2 at Time1, the rats will respond at Time2 as if it were a CS followed by reward by showing an increase in mean percentage licking contact time compared to Baseline *(CS probe2 Baseline and CS probe2 Time2).* As with CS probe1, this increase in mean percentage licking contact time would not be as great as after a CS followed by reward since it would be anticipatory only.

Time1

It was expected that, if the rats had learned the association between the reward and the preceding CS, then they would show an increase in mean percentage licking contact time at the time of presentation of the CS (Time1) as well as at the presentation of the reward (Time2). Likewise, if the rats had learned the association between the reward and the CS+BCS, then they would show an increase in mean percentage licking contact time at the time of presentation of the CS+BCS. However, since it was hoped that the rats had made the association between only the CS component of the combined CS+BCS stimulus and the reward, and not between the BCS component and the reward, it was expected that the rats would show an increase in mean percentage licking contact time at the time of presentation of the CS probes, since they would expect them to be followed by reward, but that they would not show an increase in mean percentage licking contact time at the time of presentation of the BCS probe since there would be no expectation that it would be followed by reward. It was therefore hypothesised that:

4. the rats will respond to the presentation of a CS preceding a reward by showing an increase in mean percentage licking contact time compared to Baseline, suggesting that they have learned the association between the CS and the reward *(CS Baseline and CS Time1).*

5. the rats will respond to the presentation of a CS+BCS preceding a reward by showing an increase in mean percentage licking contact time compared to Baseline *(CS+BCS Baseline and CS+BCS Time1).*

6. the rats will respond to the presentation of the CS probe1 at Time1 as if it were a CS and they expected a reward to follow by showing an increase in mean percentage licking contact time compared to Baseline *(CS probe1 Baseline and CS probe1 Time1).*

7. the rats will **not** respond to the presentation of the BCS probe at Time1 by showing an increase in mean percentage licking contact time compared to Baseline *(BCS probe Baseline and BCS probe Time1.*

8. the rats will respond to the presentation of the CS probe2 at Time1 as if it were a CS and they expected a reward to follow by showing an increase in mean percentage licking contact time compared to Baseline *(CS probe2 Baseline and CS probe2 Time1).*

One of the main problems in Experiment 1 was seen as being the possible weakening of the CS-reward contingency during Stage 1, when no reward was given if the rat did not lick the spigot after presentation of the CS. By Stage 2 the rat has learned the association between the CS and the reward, and so more (B)CSs are followed by reward, which in turn strengthens the association between the (B)CS and the reward. It was hoped that the interleaving of stimuli in this new version would reduce this potential effect. The new version of the experiment also had the added advantage that the probe trials were followed by rewarded trials, meaning that testing for blocking was not dependent on measuring the extinction of learning.

2.3.1 General methods

Four adult male Hooded Lister rats (Charles River, U.K.) of equivalent age and weight as those employed in the first experiment were used. Their housing and maintenance was as described in the General methods section for the first experiment, and they underwent the same pretraining. The apparatus was identical to that used in the first experiment. The protocol was as described in Stage 1 of the previous experiment, though the different stimuli (i.e. CS, CS+BCS, CS probe and BCS probe) were presented in blocks of 15 trials as described above, and the reward given after each correct trial was doubled to 0.2ml of saccharin solution in order to increase motivation in the rats. For the odd numbered rats, the CS was the light and the BCS was the

tone, and for the even numbered rats vice versa. Testing took place over three days, with each daily experimental session lasting for an hour.

2.3.2 Analysis of data

Percentage licking contact time was used as the measure of conditioning, but in this experiment the baseline (inter-trial interval) percentage licking contact time rate was not subtracted from the percentage licking contact time during presentation of the stimulus (Time1) and from the percentage licking contact time during the reward period itself (Time2) as in the previous experiment. This was in order to allow the visual comparison of baseline data for the CS, BCS, and probes. For each rat the percentage licking contact time was averaged across every presentation of the CS at Time1 throughout the testing sessions, and likewise for every presentation of the CS+BCS, of CS probe1, of the BCS probe and of CS probe2 to give five means in all. The percentage licking contact time was also averaged across every presentation of the reward (or absence of reward in the case of the probes) at Time2, again giving five means. In addition, the percentage licking contact time was averaged across all the inter-trial intervals preceding each type of stimulus, whether CS, CS+BCS etc. in order to obtain a baseline for each. The means were then averaged across all four rats according to type to give the mean percentage licking contact time for the CS Baseline, CS Time1 and CS Time2, for the CS+BCS Baseline, CS+BCS Time1 and CS+BCS Time2, for the CS probe1 Baseline, CS probe1 Time1 and CS probe1 Time2, for the BCS probe Baseline, BCS probe Time1 and BCS probe Time2, and , for the CS probe2 Baseline, CS probe2 Time1 and CS probe2 Time2. A bar chart showing these means is given ***(Figure 9)***, and various hypotheses (given below in the results section) were explored by carrying out planned comparisons on pairs of the means. Since this experiment was a pilot experiment, and therefore employed only four rats, it was decided not to use the Bonferroni correction since this would increase the probability of a Type 2 error, defined as the probability of accepting the null hypothesis when it is, in fact, true, by making it more difficult to detect actual differences between the means (Keppel, Saufley et al. 1992). This should be borne in mind when considering the results.

2.3.3 Results (of planned comparisons)

1) The planned comparison between *CS probe1 Baseline and CS probe1 Time2* was significant (t=-3.65; df=3; $p<0.05$) suggesting that after the presentation of a CS probe1 at Time1, the rats did respond at Time2 as if they expected the presentation of a reward.
2) The planned comparison between *BCS probe Baseline and BCS probe Time2* was not significant (t=-0.99; df=3; $p>0.05$) suggesting that after the presentation of a BCS probe at Time1, the rats did not respond at Time2 as if they expected the presentation of a reward.
3) The planned comparison between *CS probe2 Baseline and CS probe2 Time2* was not significant (t=-1.11; df=3; $p>0.05$) suggesting that after the presentation of a BCS probe at Time1, the rats did not respond at Time2 as if they expected the presentation of a reward.
4) The planned comparison between *CS Baseline and CS Time1* was not significant (t=-2.52; df=3; $p>0.05$), suggesting that the rats did not respond to the presentation of the CS preceding the reward and therefore have not learned the association between the CS and the reward
5) The planned comparison between *CS+BCS Baseline and CS+BCS Time1* was not significant (t=-3.16; df=3; $p>0.05$), suggesting that the rats did not respond to the presentation of the CS+BCS preceding the reward and therefore have not learned the association between the CS and the reward
6) The planned comparison between *CS probe1 Baseline and CS probe1 Time1* was significant (t=-5.31; df=3; $p<0.05$) suggesting that the rats did respond to the presentation of the CS probe1 at Time1 as if it were a CS and they expected a reward to follow.
7) The planned comparison between *BCS probe Baseline and BCS probe Time1* was significant (t=3.25; df=3; $p<0.05$) suggesting that the rats did in fact respond to the presentation of the BCS probe at Time1, i.e. that blocking did not occur.
8) The planned comparison between *CS probe2 Baseline and CS probe2 Time1* was not significant (t=0.93; df=3; $p>0.05$) suggesting that the rats did not respond to the

presentation of the CS probe2 at Time1 as if it were a CS and they expected a reward to follow.

2.3.4 Discussion

The immediate aim of these experiments was to design and behaviourally test an appetitive blocking paradigm which used licking, an almost automatic behaviour in the rat, as the measure of conditioning. Since blocking differentiates between temporal contiguity (CS-US pairing) and contingency (predictive relationship between CS and US), the paradigm could then be used to address the question of whether the shift in dopaminergic activity from the onset of a given reward to the onset of the earliest stimulus predicting it seen by Schultz et al (Schultz, Apicella et al. 1992; Schultz, Apicella et al. 1993; Schultz, Apicella et al. 1993; Schultz 1997; Schultz, Dayan et al. 1997) really reflects the psychological expectation of reward or is merely due to the temporal contiguity of stimulus and reward. The blocking paradigm employed in the first experiment was conventional in design, but failed to achieve blocking: mean percentage licking contact time was the same for the BCS probe as for the CS probe at Time2, showing that reward was equally expected by the rats after both. It was thought that perhaps the fact that no reward was given if the rat did not lick the spigot after presentation of the CS during Stage 1 might have weakened the formation of the CS-reward contingency, making it more difficult for the rat to learn the initial association between stimulus and reward. In an effort to prevent this possible weakening, in this experiment all the stages of blocking were presented within one session, i.e. CS followed by reward, CS plus BCS followed by reward, CS not followed by reward (i.e. the CS probe) and BCS not followed by reward (i.e. the BCS probe). Whether or not this new version of the blocking paradigm achieved blocking is a moot point, as discussed below.

It can be seen from the bar chart ***(Figure 9)*** that, as would be expected, the rats did respond to the delivery of the reward at Time2 after presentation of the CS and of the CS+BCS with an increase in mean percentage licking contact time. It can also be seen from the bar chart

Figure 12: Mean percentage licking contact time at Baseline, Time1 and Time2 for the CS, CS+BCS, CS probe1, BCS probe and CS probe2

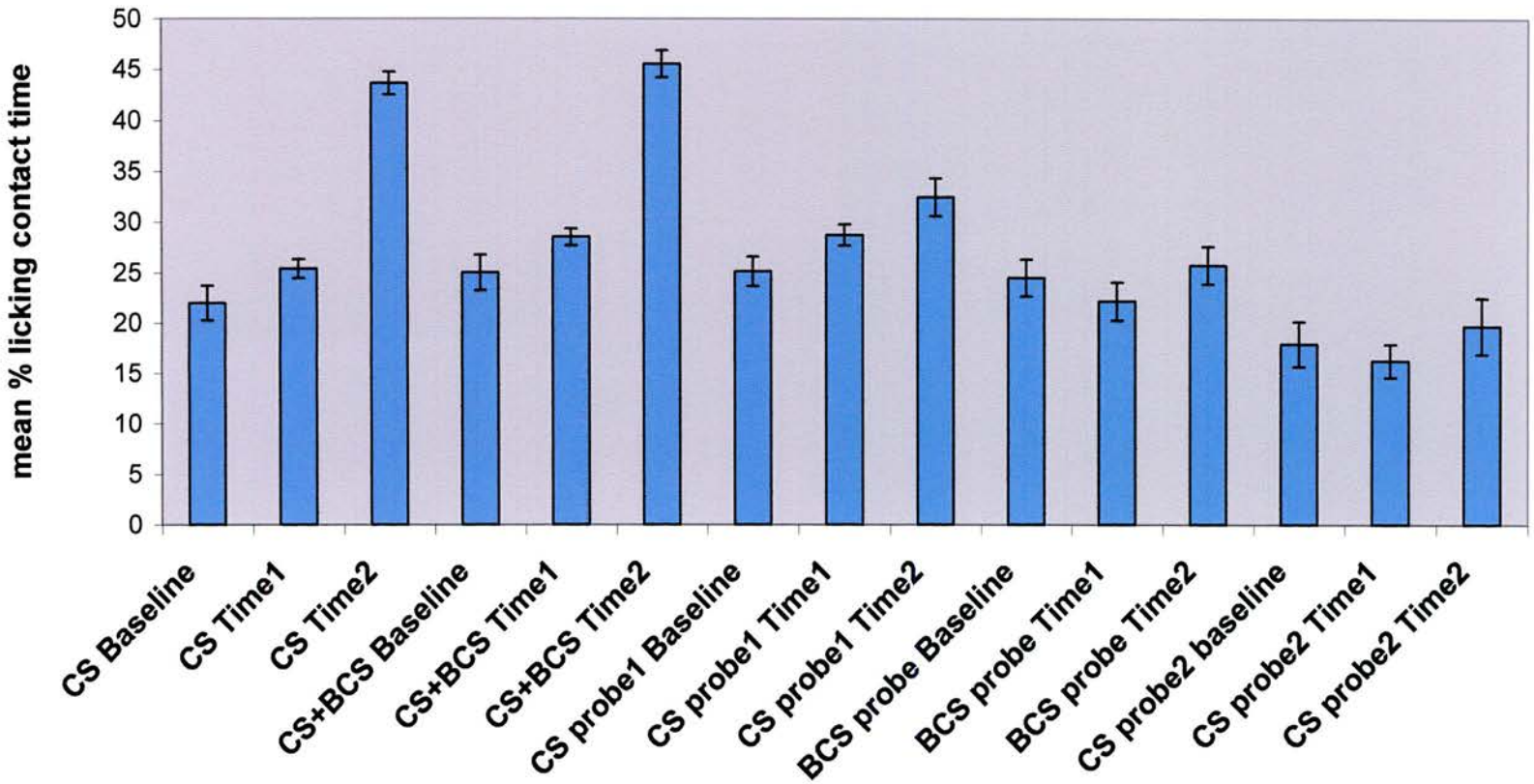


Figure 9: *Mean percentage licking contact time for the CS, CS+BCS, CS probe1, BCS probe and CS probe2 at Baseline, Time1 and Time2*

that there was an increase in mean percentage licking contact time at Time2 after presentation of the CS probe1, suggesting that the rats did expect delivery of a reward; this is supported by the result of the planned comparison between *CS probe1 Baseline and CS probe1 Time2,* which shows a significant difference between the two means. The bar chart also shows that, compared to baseline, there was only a very small increase in mean percentage licking contact time at Time2 after presentation of the BCS probe, suggesting that the rats had not made any association between the BCS component of the combined CS+BCS and the subsequent reward, and therefore did not expect a reward to follow the probe. This interpretation is supported by the result of the planned comparison between *BCS probe Baseline and BCS probe Time2,* which shows no significant difference between the means. Combined, these results suggest that blocking has been achieved and that this version of the blocking paradigm is successful in demonstrating that stimulus-reward contingency has greater control over behaviour than does stimulus-reward contiguity. Unfortunately, this happy conclusion is thrown into doubt by the result of the planned comparison between *CS probe2 Baseline and CS probe2 Time2*, which, in contrast to the planned comparison between *CS probe1 Baseline and CS probe1 Time2*, is not significant. This suggests that the rats did not expect a reward to follow presentation of CS probe2, and that they must therefore have discriminated between the two CS probes in some way. Since the CS probes were identical, it would seem highly likely that this discrimination must have been based on when exactly they occurred in the paradigm: CS probe1 occurred before the BCS probe, whereas CS probe2 followed it. It is possible that the rats responded to CS probe1 as if it were a CS, but that the intervening BCS probe somehow caused them to become aware of the CS probe2 as a separate stimulus that was never followed by reward.

The results of the planned comparisons for Time1, when the CSs, CS+BCSs and probes are presented, are also hard to interpret. As discussed in the introduction, conditioned stimuli elicit conditioned responses similar to the unconditioned responses that initially had only been elicited by the unconditional stimulus. In the blocking paradigm, therefore, there should be an increase in mean percentage licking contact time at the time of presentation of the CS and of the CS+BCS as a consequence of the rats having learnt the association between the CS and the

reward. This should also be the case for the CS probes, but not for the BCS probe since the rats should not have made any association between the BCS and reward (or, indeed, lack of reward). However, in this experiment, though the bar chart shows an increase in mean percentage licking contact time at presentation of the CS and of the CS+BCS, the results of the planned comparisons between *CS Baseline and CS Time1* (p=0.086) and between *CS+BCS Baseline and CS+BCS Time1* (p=0.051) are not significant. This suggests that the rats did not in fact learn the association between the preceding CS and the reward, and that their increased licking at CS Time2 and CS+BCS Time2 was purely in response to the reward itself, and did not incorporate any element of expectant licking. It should perhaps be borne in mind, however, that only 4 rats were used in this experiment, which might very well affect significance levels, and also that p values that are not significant but are less than or equal to 0.1 are often taken as indicating a trend towards significance. On the other hand, it was suggested in the discussion to the previous experiment that blocking might not have been achieved because of the possible weakening of the CS–reward contingency during Stage 1, when no reward was given if the rat did not lick the spigot after presentation of the CS. It was hoped that the interleaving of stimuli in this new version of the blocking paradigm would reduce this potential effect. It could be argued, however, that the rats are just as likely to learn that the CS is followed by reward 6 times out of 8, that the compound CS+BCS is followed by reward 6 times out of 6 and that the BCS (i.e. as a probe) is never followed by reward. If this were the case, the CS+BCS–reward contingency would be stronger than the CS–reward contingency, which would perhaps explain why the planned comparison between *CS+BCS Baseline and CS+BCS Time1*comes closer to significance than the planned comparison between *CS Baseline and CS Time1*. Moreover, it is possible that the rats find the CS+BCS more salient than the CS alone, and therefore come closer to forming an association between the CS+BCS and the subsequent reward than between the CS and the subsequent reward).

Moreover, in contrast to the results of the planned comparisons between *CS Baseline and CS Time1* and between *CS+BCS Baseline and CS+BCS Time1,* the planned comparison between *CS probe1 Baseline and CS probe1 Time1* is significant. Though this is consistent with

the fact that the rats also showed an increase in mean percentage licking contact time at CS probe1 Time2, it is difficult to explain given that the rats did not appear to learn the association between the preceding CS (whether alone or in compound with the BCS) and the reward. The results of the planned comparisons between *BCS probe Baseline and BCS probe Time1* and between *CS probe2 Baseline and CS probe2 Time1* are equally difficult to interpret. Although the planned comparison between *BCS probe Baseline and BCS probe Time2* is not significant, suggesting that the rats did not make any association between the BCS component of the combined CS+BCS and the subsequent reward, and therefore that blocking had been achieved, the result of the planned comparison between *BCS probe Baseline and BCS probe Time1* is significant. Moreover, the bar chart reveals that this significant result is due to the rats showing a decrease in mean percentage licking contact time when compared to the baseline. This suggests that blocking did not occur, but that the rats in fact learned that there was an association between the BCS probe and the lack of reward. It might be that conditioned inhibition has occurred in that the rats have learned that the BCS signals the omission of the reward, but this seem unlikely since this property would have been evident when the BCS was presented in conjunction with a CS, i.e. as the CS+BCS – mean percentage licking contact time would presumably have been less at Time2 for the compound CS+BCS trials than for the CS only trials. The bar chart shows that this is not the case. However, this does not preclude the BCS probe from acting as a conditioned inhibitor if, as suggested above, the rats did indeed treat the compound CS+BCS as a stimulus in its own right, so that the CS is followed by reward 6 times out of 8, the compound CS+BCS is followed by reward 6 times out of 6 and the BCS (as a probe) is never followed by reward. Although the result of the planned comparison between *CS probe2 Baseline and CS probe2 Time1* is not significant, the fact that the bar chart reveals that the rats again show a decrease in mean percentage licking contact time at CS probe2 Time1 when compared to the baseline supports this interpretation: as discussed above, the rats discriminated between the two identical CS probes, presumably by when exactly they occurred in the paradigm, and it seems possible that the rats become aware of the CS probe2 as a separate stimulus that was never followed by reward. This possibility should, however, be considered

carefully, given that the results of the planned comparisons between *CS Baseline and CS Time1* and between *CS+BCS Baseline and CS+BCS Time1* are not significant – it is difficult to explain why the rats were able to learn that the BCS probe (and possibly also CS probe2) signalled absence of reward when they were not able to learn that the CS preceded reward.

To summarise, the results of the planned comparisons between *CS probe1 Baseline and CS probe1 Time2* and between *BCS probe Baseline and BCS probe Time2* provide evidence to support the idea that blocking has been achieved, and that this version of the Blocking paradigm is successful in demonstrating that stimulus–reward contingency has greater control over behaviour than does stimulus–reward contiguity. However, the fact that the result of the planned comparison between *BCS probe Baseline and BCS probe Time1* was significant suggests that blocking did not in fact occur, whilst the information provided by the bar chart, showing a decrease in mean percentage licking contact time for the BCS probe at Time1 when compared to baseline, points towards the BCS probe acting as a conditioned inhibitor. It might be that the cyclical nature of this new version of the paradigm, in which the probe trials occur regularly after every twelve trials, encouraged the rats to respond to the BCS probe and CS probe2 as conditioned inhibitors that predict the absence of reward; they responded to CS probe1 as if they expected a reward to follow since it followed on immediately from rewarded presentations of the CS and of the CS+BCS, but the absence of reward then alerted them to the arrival of the other two probe trials. Although this possibility must be treated with caution given that the rats did not appear to learn the association between the CS and the reward, it would seem sensible to refine the paradigm by randomly interspersing the probe trials amongst the presentations of the CS and the CS+BCS followed by reward, and perhaps by using fewer of them. It might even be sensible to randomise presentations of the CS and CS+BCS.

Given the ambiguous and conflicting nature of the results obtained in the second experiment, the new version of the paradigm was simulated using an implementation of Montague et al's (1996) model in an effort to hypothesise what might happen at the neuronal level, and by implication, the behavioural level ***(Figure 10)***. In the implementation the stimuli are presented sequentially between timesteps 12 and 17 as follows: the CS is presented for the

first 6 trials with the reward following at timestep 20, the CS+BCS is presented for the next 6 trials with the reward following at timestep 20, CS probe1 is presented for 1 trial with no reward following at timestep 20, BCS probe is presented for 1 trial with no reward following at timestep 20 and CS probe2 is presented for 1 trial with no reward following at timestep 20. The sensory responses in the model to the onset and offset of each type of stimulus are gradual (which probably more closely resembles what happens in real neurons) but it can be seen that, for the first cycle of the sequence, the first two or three trials during which the CS is presented results in activity only when the reward is presented at timestep 20. But with the next few trials (still CS), this activity shifts forward slightly in time, though not quite to the beginning of the stimulus presentation. When the 6 trials of CS+BCS are then presented, activity still occurs when the reward is presented at timestep 20, but it is less than for the CS trials, and there is also activity at the time of presentation of the (compound) stimulus. When CS probe1 is presented for the first time, activity occurs at the time of presentation of the stimulus but there is inhibition at the time that the reward should have occurred. When the BCS probe is presented there is likewise activity at the time of presentation of the stimulus, but there is no activity at the time that the reward should have occurred. Presentation of the CS probe2 results in an identical pattern of activity as for CS probe1.

The second cycle of the sequence is somewhat different from the first. The CS trials result in some activity at timestep 20 when the reward is presented, but more activity at the time of presentation of the stimulus. The CS+BCS trials, however, result only in activity at the time of presentation of the (compound) stimulus. The presentation of CS probe1 and CS probe2 continue to result in activity at the time of presentation of the stimulus, and inhibition at the time that the reward should have occurred. Presentation of the BCS probe, however, shows no activity at either the time of presentation of the stimulus or at the time that the reward should have occurred. This pattern of results is constant for all subsequent cycles.

The fact that the CS trials result in some activity at the time of presentation of the reward but more activity at the time of presentation of the stimulus suggests that the model does not fully learn that the CS predicts the reward. This is in accordance with the results from the

second experiment, wherein the rats showed an increase in mean percentage licking contact time at the time of presentation of the reward (Time2), but did not show an increase at the time of presentation of the CS (Time1). The fact that the CS+BCS trials result only in activity at the time of presentation of the (compound) stimulus and not at the time of presentation of the reward suggests that the model has learnt that the CS+BCS stimulus is the best predictor of reward. This is again in accordance with the results from the second experiment, at least to some degree – the increase in mean percentage licking contact time at the time of presentation of the CS+BCS just missed significance, and is larger than the increase in mean percentage licking contact time at the time of presentation of the CS – and provides some support for the suggestion that the compound CS+BCS is perceived as a separate stimulus by the rats.

However, the patterns of activity shown by the two CS probes in the model are very different from those shown by the CS probes in the second experiment. In the model both CS probes show identical patterns, with activity at the time of presentation of the stimulus and inhibition at the time that the reward should have occurred. This is very much what would be expected if the model has learnt that the CS predicts the reward, implying that this is indeed the case, though to a lesser extent than with the CS+BCS. But in the second experiment only CS probe1 showed an increase in mean percentage licking contact time at the time of presentation of the reward (Time2) and at the time of presentation of the CS probe1 (Time1), suggesting that the rats did expect delivery of a reward. CS probe2 did not show an increase in mean percentage licking contact time at the time of presentation of the reward (Time2), and in fact showed a (non-significant) decrease in mean percentage licking contact time at the time of presentation of the CS probe2 (Time1). It was tentatively suggested that the rats might have perceived CS probe2 as a conditioned inhibitor that predicted the absence of reward.

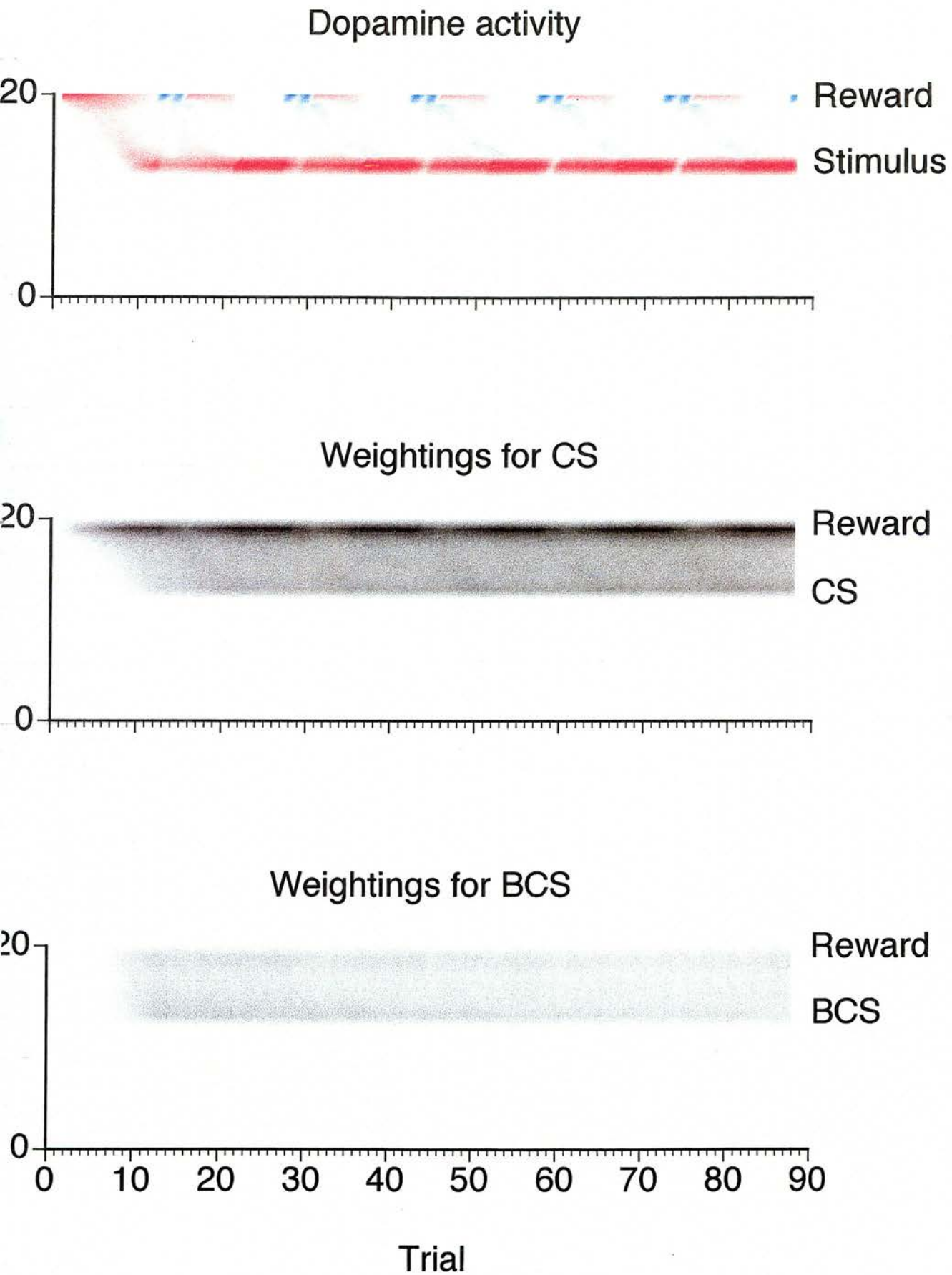
The pattern of activity shown by the BCS probe in the model is also very different from that shown by the BCS probe in the second experiment. In the model presentation of the BCS probe results in **no** (*not* inhibited) activity at either the time of presentation of the stimulus or the time that the reward should have occurred – in other words, the model completely ignores the BCS probe. This would certainly suggest that blocking has occurred. In the second

experiment, however, although the fact that there is no significant increase in mean percentage licking contact time at the time of presentation of the reward (Time2) suggests that blocking has occurred, the fact that there is a significant *decrease* in mean percentage licking contact time at the time of presentation of the BCS probe (Time1) suggests that the BCS probe may be acting as a conditioned inhibitor. All in all, it would appear from ***Figure 10*** that this implementation of the new version of the paradigm results in blocking. The fact that the CS trials result in some activity at the time of presentation of the reward but more activity at the time of presentation of the stimulus may be due to the cyclical nature of the paradigm, as explained above – the presentation of the probe trials (which are not followed by reward) immediately before the presentation of the 6 CS trials may prevent the model from fully learning that the CS predicts the reward, or, rather, may cause the model to learn that the CS is not the best predictor of reward since it is followed by reward only 6 times out of 8. However, the implementation does not wholly concur with the behavioural data, and it may be that the constraints of the artificial network do not allow it to express some of the complexities present in actual behaviour – such as whether the CS+BCS is perceived as two stimuli (CS and BCS) or as one compound stimulus, or whether the BCS probe is perceived as a conditioned inhibitor or not. Given these ambiguities, it would be of great interest to record from electrodes planted in the VTA of naive rats taking part in the new version of the paradigm. These experiments have shown that it is possible to achieve learning in individual animals over a brief training session, hopefully making it possible to record from one neuron during the entire process. It would be interesting to discover whether the dopamine neurons will comply with the behavioural data described above or with the model. Unfortunately, however, technical problems meant that this line of research had to be abandoned.

Figure 10: new 'cyclical' version of paradigm)

This implementation of Montague et al's model(Montague, Dayan et al. 1996) was suggested by the author and carried out by Eric Bowman, School of Psychology, University of St Andrews. The x axis represents the number of timesteps (20) making up a trial and the y axis represents the number of trials which took place. How dense the red is indicates the degree of dopamine activity occurring relative to baseline, and how dense the blue is indicates the amount of inhibition of dopamine activity occurring relative to baseline.

Implementation 5



Chapter 3 - The Schedule Fraction Cue Task

Experiment A

3.1. Introduction

The Schedule Fraction Cue (SFC) task is a modification of an appetitive conditioning task originally designed by Bowman et al (1996) and used previously by Bowman and Brown (1998) in which cues are used to signal how close rats are coming to achieving reward. Within the task rats are required to carry out a series of three different work schedules, randomly presented. Each work schedule requires the rats to complete a given number of correct responses in order to obtain a reward of constant value: work schedule 3 is signalled by a bright cue light and requires the rat to make three correct responses, work schedule 2 is signalled by a dim cue light and requires the rat to make two correct responses and work schedule 1 is signalled by no cue light and requires the rat to make only one correct response. A correct response consists of the rat maintaining a nosepoke until a tone is sounded, and then moving across to the food hopper flap and opening it. A food pellet is deposited in the food hopper only after the last correct response is made within each work schedule.

Besides being made aware of the 'cost' of the reward in terms of the number of correct responses required to obtain it at the outset of any particular work schedule, the rats are also offered additional information about the task which is non-essential to the outcome: the intensity of the cue light changes with each correct response within a work schedule, thus keeping the rat informed of how close it is to achieving the reward. For example, work schedule 3 is signalled by the presentation of a bright cue light; after the rat has completed the first correct response the cue light changes to dim, and then after it has completed the second correct response the cue light changes to no light. Since the cues change before each next step within a work schedule, they could be regarded as purely discriminative stimuli that indicate the availability of reward, i.e. that a bright cue light signals no reward, a dim cue light signals no

reward, and no cue light signals reward. However, the intention is that they should also indicate reward proximity.

Much of the interest in the SFC task lies, therefore, in discovering how much, if any, of the available information the rats will utilise in working to obtain reward. It might be that they do not make use of the information provided by the cue lights at all, but shuttle between the nosepoke hole and the food hopper flap being rewarded on average on three responses out of six. If this were the case, performance could be expected to be constant for all and throughout each of the different work schedules since the rats would not know which correct response would be followed by a reward. Alternatively, the rats might make use of the cues purely as discriminative stimuli that indicate whether a reward is available on that correct response or not, in which case performance could be expected to differ according to whether a reward was expected or not. A third possibility is that the rats will make full use of all the information available to them, and use the cues as an indication of reward proximity. If this were the case, performance could perhaps be expected to differ not only as to whether a reward was expected or not, but as to how much more work was needed to obtain the reward, for instance performance might differ between the first (unrewarded) correct response of the work schedule requiring three correct responses and the second (also unrewarded) correct response. Previous employment of the SFC task suggests that normal rats do in fact make full use of the information provided by the cues. Brown et al (1996) and Bowman and Brown (1998) found that reaction time (the time taken for the rat to withdraw from the nosepoke hole after the tone has sounded) and movement time (the time taken by the rat to move across to the food hopper flap and open it) both decreased as the rats worked through each work schedule towards reward, whilst performance accuracy (the percentage of correct responses) improved. Analysis of the behavioural data revealed that these improvements in reaction time and accuracy were not merely due to the rats using the cues as discriminative stimuli, but resulted from their using the cues to discern the cost of the reward, and how far they had progressed towards achieving it. Bowman and Brown (1998) therefore concluded that *"reaction times can be used in the rat as a measure of the trial by trial change in motivation induced by the cues"* (p.444).

Up to now the SFC task has been used to investigate motivational processes in the ventral striatum, and in particular the NAcc In its present form, the task has been used by Brown et al (1996) to dissociate the effect of systemic amphetamine on motor readiness versus motivation. Brown et al argued that if the effect of amphetamine was to increase motivation, then it could be expected to improve performance on rewarded correct responses only and not on unrewarded correct responses, but that if the effect of amphetamine was to increase motor readiness, then performance would be improved on all correct responses. They found that the latter was the case, suggesting that amphetamine affects motoric rather than motivational processes. Bowman and Brown (1998) went on to use the SFC task to investigate the effects of excitotoxic lesions of the rat NAcc on the perception of reward cost. Wise (1982) had suggested that the NAcc is responsible for the hedonic efficacy of rewards, an hypothesis he based on the apparent anhedonia produced in rats by dopaminergic blockade with neuroleptics. Other researchers, however, have suggested that this apparent anhedonia could result from the animals not being "willing to work as hard" for reward (Neill 1982), and that the NAcc might not be involved in the assessment of primary reward value as such, but rather in the assessment of the effort required to obtain that reward. Cousins and Salamone (1994) for instance, showed that, unlike normal rats, rats with dopamine depletion in the NAcc prefer to eat less rewarding lab chow rather than lever press for more rewarding sucrose pellets if both foods are concurrently available, but that they will work for the sucrose pellets at a rate consistent with that of normal rats if the lab chow is not available. Bowman and Brown (1998) therefore hypothesised that, with the SFC task, lesions of the NAcc would result in rats being less willing to work for reward as the cost of the reward increased from one to two to three correct responses, and that this reluctance would manifest itself in decreased accuracy and increased reaction and movement times. They found, however, that lesions of the NAcc had no effect on performance of the task – the lesioned rats were as well able as the normal controls to use the cues to discern the cost of the reward in each work schedule, and to relay this information to the motor system, thus determining reaction time. Bowman and Brown (1998) therefore proposed that the NAcc is not essential to interpreting cost-of-reward cues and that alternative pathways must carry this

information to motor structures. They also observed that Shidara et al (1998), who recorded extracellular activity in the NAcc of rhesus macaque monkeys that were performing a task analogous to the SFC task and found neurons that became active after the onset of a cue indicting progress to reward, concluded that these neurons carried only 52% of the information necessary to encoding progress to reward. Brown and Bowman (1998) further suggested that the amygdala might be involved in the interpreting of cost-of-reward cues, given its role in stimulus-reward association and its connectivity with the NAcc.

The SFC task has been used to study perception of reward cost, but the mechanisms underlying its learning and performance are somewhat unclear. The SFC task incorporates elements from both Pavlovian and instrumental conditioning procedures. It could be argued that the initial nosepoke is a Pavlovian conditioned approach response to the light CS, but the fact that the rats are required to hold it for a variable foreperiod suggests that instrumental conditioning must also be involved. Likewise, it is probable that Pavlovian association occurs between the three different light intensities which each signal an individual outcome, i.e. bright = no reward, dim = no reward, and none = reward, but this has been shown to have an impact on instrumental performance. As described in the introduction, other procedures in which Pavlovian cues influence instrumental responding are acquisition of a new response with conditioned reinforcement, second-order instrumental associative learning and Pavlovian-to-instrumental transfer. The SFC task resembles all of these procedures in that the CS is assumed to have a motivational influence over instrumental responding, but it differs in many respects also. In the SFC task, instrumental training takes place first, with the rats learning how to push open the food hopper flat for reward, and then to nose-poke, followed by opening the food hopper flap for reward. Only once instrumental performance is stable are the schedule fraction cue lights (CSs) introduced, one at a time. Moreover, unlike the CSs employed in the test phases of the other procedures, the SFC task cue lights carry information about the task that may or may not be utilised by the rat. Also, unlike the acquisition of a new response with conditioned reinforcement and Pavlovian-to-instrumental transfer procedures, the test phase of the SFC task is not carried out in extinction – the reward continues to be presented upon completion of the

requisite number of correct responses. Perhaps, in this respect, the SFC task most closely resembles some second-order instrumental associative learning procedures in that with the latter a certain number of presentations of the CS (now a conditioned reinforcer) have to be earned before a reward is presented. However, all the procedures have in common the fact that the CS is not necessary to performance of the task, but does influence responding in normal rats.

It is, of course, this last factor that is crucial to the efficaciousness of the SFC task to assess perception of reward cost. It was noted earlier that the rats do not necessarily have to utilise the information supplied by the schedule fraction cues, but could instead just shuttle backwards and forwards between the nose-poke hole and the food hopper, receiving reward on average on three out of six correct responses. However, previous employment of the task suggests that the performance of normal rats is influenced by the schedule fraction cues, which in turn suggests that the schedule fraction cues must have acquired incentive value. As discussed earlier, the results of several different Pavlovian and instrumental conditioning studies suggest that the BLA is critical for the acquisition of positive incentive value by formerly neutral stimuli. There is therefore reason to suppose that lesioning the BLA will have an effect on performance of the SFC task.

3.2 General methods

3.2.1 Materials and methods

3.2.1.1 Animals

Naïve adult male Lister hooded rats were used in this experiment. They were pair-housed and maintained in a temperature-controlled room with a 12 hour light/dark cycle (lights on at 07.00 h). Training and testing took place in the afternoon, between 13.00 and 17.00 h, with each rat being run in the same order and at approximately the same time each day. Prior to training the rats had free access to lab chow and water, but at the onset of training they were put on a restricted diet of 17-20 g a day (including what was earned in the experimental sessions),

receiving their food immediately after the training session. The rats were weighed every other day to ensure that they did not fall beneath 85% of their free-feeding weight. The guidelines laid out in the "Principles of laboratory animal care" (N.I.H. Publication no. 86-23, revised 1985) and the requirements of the U.K. Animals (Scientific Procedures) Act 1986 were adhered to throughout the experiments.

3.2.1.2 Apparatus

The rats were trained in 9-hole operant boxes, which are controlled by the "Spider" computer system (Paul Fray Ltd., Cambridge). The 9 nose-poke holes are arranged in a horizontal array on the back wall of each operant box, 1.5cm above the grid floor. Each nose-poke hole is 1.5cm square, and has a light bulb at the rear that can be illuminated at 3 different brightness levels (1.5W, 1W and off), and a photocell at the front to monitor responses. The 4 nose-poke holes to either side of the central nose-poke hole were capped with transparent covers for this experiment. On the front wall of the operant chamber is a food hopper accessed by a hinged flap, which, when opened, activates a microswitch, and causes a light within the hopper to illumine and dustless precision pellets (45mg, BioServ, New Jersey) to be delivered into the hopper by a silent operation computer-controlled automatic pellet dispenser situated outside the chamber. A 3W house light is situated on the ceiling of the operant box, as is a speaker connected to a tone generator controlled by the computer. The operant chambers are housed individually in soundproof boxes with ventilation fans providing a constant low level of background noise.

3.2.1.3 Experimental procedure

Training took place in several stages over several weeks. First, the rats learned to open the hopper flap to retrieve a food pellet. The central and peripheral nose-poke holes were left dark, but the overhead house light was lit. When the rats pushed open the hopper flap, thereby activating the microswitch, the hopper light came on and a food pellet dropped into the hopper.

This initial stage of training lasted 2-3 days, by the end of which time the rats were retrieving approximately 200 pellets during the half-hour session.

The rats were then moved onto the second stage of training, during which they learned to nose-poke into the central nose-poke hole before turning around and crossing over to the hopper to retrieve a food pellet. First the hopper light would come on, and then, once the rats had pushed opened the hopper flap, the central nose-poke light would come on. The rats would then withdraw from the hopper in order to investigate the nose-poke hole, causing the hopper light to be extinguished. Once the rats had nose-poked into the central nose-poke hole, thereby breaking the photocell beam, the central nose-poke light would be extinguished, a brief (100ms) tone would sound, the hopper light would re-illumine and a pellet would be dispensed into the hopper. The rats would then return to the hopper to collect the food pellet, and the cycle would start again. This second stage of training lasted 7-10 days, until each rat was consistently earning 150 pellets per session.

Once the rats had mastered this stage, they were required to sustain the nose-poke into the central nose-poke hole for an unpredictable variable foreperiod (100, 200, 300, 400 or 500 msec) until the tone sounded and they could withdraw their snouts and collect the food pellet. If a rat withdrew its snout before the tone, this was deemed an anticipatory error, the tone would not sound, no pellet would be delivered and the overhead house light and the central nose-poke light would be extinguished for 1.5 sec, during which further responses would be ineffective (the "time-out" period). The overhead house light and the central nose-poke light would then re-illumine and the rat would be able to work for reward again. This third stage of training again lasted 7-10 days , until each rat was consistently earning 150 pellets a session.

For the final stage of training, the schedule fraction cues were introduced. Once the rats had pushed opened the hopper flap, the central nose-poke light would illumine. At the same time the lights in the 8 peripheral nose-poke holes would illumine at 1 of 3 different brightness levels (1.5W, 1W and off) and each of these brightness levels signalled the start of 1 of 3 different work schedules. For half the rats, bright cue lights signalled the start of work schedule 1, dim cue lights signalled the start of work schedule 2 and no cue lights signalled the start of

work schedule 3, whilst for the remaining rats this pattern was reversed. Work schedule 1 required just 1 nose-poke sustained for the correct foreperiod, i.e. until the tone sounded, followed by an opening of the hopper flap (this sequence of actions constituted a correct response) in order to obtain the food pellet reward. Work schedule 2 required 2 correct responses in order to obtain the reward, and work schedule 3 required 3 correct responses. The individual responses required within each work schedule constituted the schedule fractions – the only response made in work schedule 1 was referred to as schedule fraction (SF) 1/1, the first response made in work schedule 2 was referred to as SF 1/2 and the second response as SF 2/2, and the first response made in work schedule 3 was referred to as SF 1/3, the second response as SF 2/3, and the third response as SF 3/3. There were therefore 6 schedule fractions in all. As well as signalling the onset of each work schedule, the cue lights also changed for each response within the work schedule. So, for work schedule 3, the first response (schedule fraction 1/3) would be signalled by no light, the second response (SF 2/3) would be signalled by dim lights and the third response (SF 3/3) by bright lights (and vice versa), and for work schedule 2, the first response (SF 1/2) would be signalled by dim lights and the second response (SF 2/2) would be signalled by bright lights (again, and vice versa).

As in the previous stage of training, snout withdrawal from the centre nose-poke hole before the tone had sounded was deemed an anticipatory error, and the rat was timed-out for 1.5 sec. Likewise, withdrawal within 100 msec of the tone sounding was also deemed an anticipatory error, since the rat must have initiated the movement before the tone, and the rat was again timed-out. In the case of a time-out, the house-light and the centre nose-poke hole light would extinguish but the peripheral cue lights would remain on. After 1.5 seconds the house-light and the centre nose-poke hole light would re-illumine and the rat was required to open the hopper flap in order to start working for reward again. It would then have to make another attempt at that same schedule fraction, and with the same foreperiod, i.e. if the rat had made an anticipatory error whilst making the second response for work schedule 3, which required three correct responses in all, it had to make 2 more correct responses in order to obtain reward. If the rat took longer than 2 sec to turn around, cross the grid floor, and push open the

hopper flap, this was deemed a late error, but there was no ensuing penalty. Correct responses were therefore those in which the rat nose-poked, withdrew its snout at least 100 msec after the tone had sounded (reaction time), and crossed the grid floor to push open the hopper flap within 2 sec (movement time). After making a correct response, and perhaps consuming a reward, the rat was free to initiate the next response in its own time. The time period between opening the food hopper flap at the end of one response and nose-poking at the start of the next was termed the post response pause. Each session continued for 30 min or until the rat had achieved 120 correct trials, with the 3 different work schedules being presented pseudo-randomly but in such a way that each of the 6 schedule fractions were presented 20 times in all (4 times at each of the 5 foreperiods). Training continued until performance was stable, which was after about 2 months. Data were then collected over 17 daily sessions (the preoperative data), after which the rats underwent surgery. The rats were allowed to recover for a week, and data were then collected over a further 6 daily sessions (the postoperative data).

3.2.1.4 Surgery

The rats were anaesthetised with 1ml/kg "Sagatal" (Pentobarbitone Sodium B.P., 60mg/ml; Rhône-Mérieux, Harlow UK) and given an injection of an anti-inflammatory long-term analgesic, "Rimadyl" (Carprofen, 50mg/ml; Pfizer, Sandwich UK). They were then secured in a Kopf stereotaxic frame using atraumatic earbars. After the scalp had been cut open and the skull exposed, bilateral holes were drilled through the skull at the co-ordinates AP -2.3mm and ML +/-5mm with respect to bregma and DV -8.8mm from the skull surface, with the skull level. Injections were then made using a stereotaxic-mounted 1.0μl SGE syringe (Scientific Glass Engineering, Milton Keynes UK) as follows: The lesion group received injections of 0.2μl of 0.12M NMDA in phosphate buffer (pH adjusted to 7.4 using 2M NaOH), made manually over 125 sec at a rate of 0.02μl every 5 sec. The needles were left *in situ* for a further 2 min to allow diffusion from the tip before being slowly withdrawn. The control group underwent the same procedure, but was injected with 0.2μl of the phosphate buffer vehicle only. At the end of

surgery the scalp wounds were closed using sterilised michel clips and the rats were placed in a warm environment to recover.

3.2.1.5 Histological procedures

At the end of behavioural testing the rats were given an overdose of "Dolethal" (pentobarbitone sodium, 200mg/ml; Univet, Bicester UK) and then, when deeply anaesthetised, transcardially perfused with saline for 2 min, followed by 4% paraformaldehyde in phosphate buffer for 10 min at 10 ml/min. The brains were then removed from the skull and post-fixed in 4% paraformaldehyde. After a few hours, the brains were transferred into 20% phosphate-buffered sucrose solution to await further processing. Series of four 50μ thick brain sections were then cut using a freezing microtome. The first section in every series was mounted onto gelatine-coated slides, and, when thoroughly dry, stained with cresyl violet for nissl substance. Nissl substance is composed of DNA and RNA and other proteins in the cell nucleus, and is present in all cells of the CNS including neurons and glia. Cresyl violet stains nissl substance a purple/blue colour and other cytoplasmic constituents a paler blue colour. The technique was carried out as follows:

First, cresyl violet staining solution was prepared by dissolving 0.5g of cresyl fast violet acetate (aqueous) in 475ml of distilled water and 25ml of glacial acetic acid using an ultrasonic bath. Its pH was then checked and adjusted if necessary to 3.5 using sodium acetate solution. The slides onto which the sections had been mounted were placed in a formalin bath for a minimum of 30 min in order to ensure that the sections were thoroughly fixed in place, and then paced into a xylene bath for 2 min in order to de-fat them. The sections were then re-hydrated by placing the slides in baths of first 100% and then 50% alcohol for a few minutes before being placed in tap water for a further few minutes. The slides were then placed in a bath of the cresyl violet staining solution for approximately 2 min before being rinsed under running tap water for 5 min and differentiated in 50% and then 100% alcohol. Finally, the slides were cleared in xylene and cover-slipped using the xylene-based mountant DPX.

The sections were examined using a Leitz "Diaplan" light microscope fitted with a Sony DXC-3000P video camera and connected to a high-resolution monitor, and the extent of excitotoxic damage determined according to the level of neuronal loss and associated gliosis throughout the region of interest. For each rat, those sections which corresponded to the photomicrographs and schematic representations at given stereotaxic coordinates in the Paxinos and Watson (1998) atlas were then selected and schematic representations of the lesions at each of these six coordinates were made. The stereotaxic coordinates were -1.40, -2.12, -2.56, -3.14, -3.60 and -4.16 mm relative to bregma and spanned the entire region of interest for both the BLA and the CeN. Photographic representations of some of the lesions were also made using a Pixera camera (PVC 100C) connected to a Power Macintosh 7300/200 using the Pixera VCS 1.2 program.

3.2.2 Analysis of data

The raw data consists of the performance of each rat for each response made during a session. All the rats had to achieve the same number of correct trials (120) within a session; these were made up of equal numbers of presentations of the 6 different schedule fractions (20 x 6), and within these, equal numbers of the 5 different foreperiods (4 x 5). An AWK program was used to convert these raw data into an excel table for each stage of the experiment. In this experiment there were 2 stages: the preoperative stage, which consisted of 17 daily sessions, and the postoperative stage which consisted of 6 daily sessions. Four different measures of performance were used:

1) Mean percentage of correct responses: a correct response is registered when the rat sustains a nosepoke for the required foreperiod and then moves across to the food hopper flap and opens it within 2 sec of the tone sounding. The mean percentage of correct responses is the percentage of correct responses compared to the total number of responses made within a session, averaged over all the sessions within a stage.

2) Mean reaction time (csec): reaction time is the time between tone onset and withdrawal of the rat's snout from the nosepoke hole. The minimum reaction time is 100 msec, and the maximum is 2000 msec. Mean reaction time is averaged across every session and over all the sessions within a stage.

3) Mean movement time (csec): movement time is the time between the rat having withdrawn its snout from the nosepoke hole and it pushing open the food hopper flap. In other words, it is the time taken to cross the grid floor from the nosepoke hole to the food hopper flap and opening it. Mean movement time is averaged across every session and over all the sessions within a stage.

4) Mean post response pause (csec): this is the time between the rat having pushed open the food hopper flap and it pushing its snout into the nosepoke hole for the next response, in other words it is the latency to resume working after making a response. After a complete correct work schedule, the post response pause will include the time taken to consume the reward. Mean post response pause is averaged across every session and over all the sessions within a stage.

The data from the preoperative and postoperative stages for these 4 measures were subjected to several different processes. First the data was depicted graphically and described. It was then subjected to two different analyses: analysis of variance (ANOVA) and Planned Comparisons. Finally, for the main performance measure of mean percentage of correct responses, the extent of the lesions was mapped and compared.

3.2.2.1 Analysis of preoperative performance

The different intensities of cue light in the SFC task are intended to give the rats some way of measuring how close they are to receiving the reward, or, in other words, they indicate 'progress to reward'. But do the different intensities of cue light have any effect on performance, and if so, can the effect on performance be attributed to the rats actually interpreting the cues as indicating progress to reward? Do the different intensities of light have a motivational effect on performance? Given that the SFC task is a relatively novel one, it was thought helpful to first

analyse the effect of the different intensities of cue light and of foreperiod on preoperative performance before going on to look at any possible lesion effects. The data from all the rats was therefore combined, irrespective of the experimental group to which they would later be assigned; this was done to increase the sample size and therefore power of the analysis.

First, graphs of mean values for each of the various measures were drawn up, showing performance on each of the 6 levels of schedule fraction (X axis) for each of the 5 different foreperiods.

ANOVA was then used to analyse the preoperative performance of the rats on each of the measures. ANOVA tests for significant differences between means (for groups or variables) by comparing variances. The total variance is divided into the component that is due to true random error (i.e. the within-group variance) and the components that are due to differences between the means and these are then tested for statistical significance using the F test, which determines whether the ratio of the variance estimates is significantly greater than 1. If so, the null hypothesis, which postulates that there is no difference between the means, can be rejected. Certain restrictive assumptions about the data underlie ANOVA, the most important being, first, the assumption of normality, which requires that the data points are distributed around means according to the normal distribution, and second, the assumption of homogeneity of variance, which requires that the variance of the data points within each of the groups is the same. However, the F test is fairly robust against violations of both assumptions (see Lindman (1974). Further restrictive assumptions govern the use of within-subjects ANOVA, the most important of which is the assumption of homogeneity of covariance (or sphericity). This requires that the correlations among the scores at the various levels of the within-subjects factor should be homogeneous. If this assumption is violated, the true type 1 error rate (i.e. the probability of rejecting the null hypothesis when it is true) may be inflated. Homogeneity of covariance can be tested for using the Mauchly sphericity test, and various approximations applied if the assumption is violated. These approximations vary in their degree of conservativeness, from, for example, the very conservative Greenhouse-Geisser test through the less conservative Huynh-Feldt test to the more liberal Lowerbound test, but all are based on reducing the degrees of

freedom of the numerator and the denominator of the F ratio by multiplying the degrees of freedom by a factor, epsilon, estimated from the sums and means of the variances and covariances. The closer epsilon is to 1.0 the more homogenous are the variances of the differences.

Within-subjects ANOVA is used to determine whether a significant relationship lies between any or all of the factors within an experiment and the dependent measure. First, the effect of each individual factor on the dependent measure is examined by comparing the dependent measure scores at each level of that factor whilst ignoring the existence of the other factors. If there is a significant difference between the scores of the dependent measure at any of the levels, then that factor is said to have a main effect (Kinnear and Gray 1999). Then the possibility that the relationship between one factor and the dependent measure is different for and dependent on each level of another factor is investigated; if this is found to be the case, then a two-way interaction is said to exist. Likewise, if the two-way interaction between two factors changes as the levels of a third factor change, a three-way interaction is said to exist. Significant interaction effects necessarily take precedence over main effects in interpreting data. However, with both interaction effects and main effects, significance only indicates that the data reflects a 'real' relationship, and not a chance pattern of behaviour and it does not indicate the importance of the relationship. Effect size is an indication of how dramatically an independent variable influences a dependent variable (Heiman 1999) and is calculated by computing the proportion of total variance in the dependent scores that is associated with or related to changes in the independent variable. In ANOVA, effect sizes are calculated for each main effect and interaction by dividing the sum of squares for the effect by the total sum of squares in the ANOVA. This can easily be converted into a percentage value by multiplying by 100. Likewise, the presence of a significant F indicates only that two or more conditions differ significantly, but not which ones. Normally, post-hoc tests such as the Scheffe test or the Tukey HSD test, which compare all possible pairs of conditions in order to determine which ones differ significantly from each other, would therefore be used. Unfortunately, in within-subjects designs it is inadvisable to carry out post-hoc tests, because with such a large number of combinations it

would be very difficult to obtain a difference sufficiently large to be significant. But it is possible to look for simple main effects instead; a simple main effect is the effect of one independent variable at one level of a second independent variable. Simple main effects are computed by carrying out a one-way ANOVA upon the data at only one level of the other factor, but it is necessary to apply a correction to the F value by dividing the mean square effect from the one-way ANOVA (the restricted ANOVA) by the mean square error for the interaction from the main analysis. The degrees of freedom associated with the interaction from the main analysis are then used to determine whether the corrected F value is significant or not.

The effect of the different intensities of cue light and different lengths of foreperiod on preoperative performance for each of the measures was analysed using 2-way within-subjects ANOVA, with the 6 different schedule fractions forming the levels of the factor of schedule fraction and the 5 different lengths of foreperiod forming the levels of the factor of foreperiod. Homogeneity of covariance was tested using the Mauchly sphericity test (results given in **Appendix B)** and the Huynh-Feldt approximation applied to correct for any violations; this particular approximation was used since it was thought that the small number of subjects was best served by a mildly conservative correction procedure. Effect sizes were calculated for every significant main effect and interaction in order to assess the impact of each factor on performance. Simple main effects were also calculated since all the ANOVAs were within-subjects, thus making the use of post-hoc tests inadvisable (results given in **Appendix C**).

Following the example of Bowman and Brown (1998) various hypotheses as to how the rats could interpret the cue lights were explored by performing planned (pairwise) comparisons on pairs of conditions from the main effect of progress to reward. In carrying out these pairwise comparisons, the risk is run of committing one or more type 1 errors, defined as the probability of rejecting the null hypothesis when it is, in fact, true. If each pairwise comparison is considered separately and independently from the other pairwise comparisons being carried out during analysis of the experiment, then the type 1 error is more specifically known as a per comparison type 1 error (Keppel, Saufley et al. 1992) but it also possible to consider each pairwise comparison as belonging to a 'set' comprising all the pairwise

comparisons being carried out during analysis of the experiment. In this case, the type 1 error is better described as a familywise type 1 error. Familywise type 1 error refers to the probability of committing type 1 errors over a set of statistical tests (Keppel, Saufley et al. 1992) and occurs whenever a type 1 error is made on any of the pairwise comparisons within the set. As Keppel (1992) points out, where two pairwise comparisons are conducted, familywise type 1 error can occur as a type 1 error on the first test only, as a type 1 error on the second test only, or as a type 1 error on both tests, and as the number of pairwise comparisons within a set increases, so does the number of possible type 1 errors. Familywise type 1 error is approximately equal to the sum of the separate per comparison probabilities. Since the probability of making a type 1 error is determined by the significance level chosen for the experiment, it is possible to control familywise type 1 error by lowering the significance level for each comparison by using a correction such as the Bonferroni test. However, as Keppel (1992) points out, controlling familywise error in such a way makes it more difficult to detect actual differences between the means, thus increasing the probability of a type 2 error. Controlling for both type 1 and type 2 errors is therefore problematical. In discussing this problem, Keppel (1992) suggests that most researchers ignore the theoretical increase in familywise type 1 error and reject the null hypothesis at the usual per comparison probability level with respect to planned comparisons, provided that their number is reasonably small. He states further that some experts suggest that the number of planned comparisons should be one less than the number of treatment conditions. Since the number of pairwise comparisons carried out in this experiment is six, the same as the number of treatment conditions (levels of schedule fraction), it was decided not to use the Bonferroni test.

The main hypothesis is that the different schedule fractions as cued by light intensity will have an effect on performance of the task. Comparisons (paired samples t-tests) were planned between:

1/3 and 2/3, 2/3 and 3/3, 1/2 and 2/2

However, should the results of the above indicate that the rats' performance is indeed affected by the different intensities of cue light, it would still be necessary to carry out further analyses

before concluding that the difference in performance is due to the rats interpreting the different intensities as indicating 'progress to reward'. The first requirement is that there should be a difference in performance between rewarded and unrewarded schedule fractions. Possible comparisons (paired samples t-tests) are given below, but only those in bold type were carried out:

1/3 and 3/3	**2/3 and 3/3**	1/2 and 3/3
1/3 and 2/2	2/3 and 2/2	**1/2 and 2/2**
1/3 and 1/1	2/3 and 1/1	1/2 and 1/1

The second requirement is that the rats should use the cue lights as a means of ascertaining how close they are to achieving the reward, rather than as an indication of the availability of reward. If the former is the case then there will be a difference in performance between all the unrewarded schedule fractions (1/3, 2/3 and 1/2), but if the latter is the case, then performance should be the same for all the unrewarded schedule fractions. Comparisons (paired samples t-tests) were planned between:

1/3 and 2/3, 1/3 and 1/2, 2/3 and 1/2

A comparison was planned to explore another interesting possibility. Schedule fractions 2/3 and 1/2 are both unrewarded and are both signalled by a dim light. It could be expected that performance would therefore be equivalent for both. However, a difference in performance could reflect an awareness of 'cognitive distance from the goal'. In other words, performance at a given schedule fraction may be affected by a rat's level of motivation, which in turn could relate to whether it has already done some of the work required to achieve the goal, or has still all the work to do. An analogy would be to compare the performance of a man running a five mile stretch with his performance running a two mile stretch – barring differences in physical tiredness, would there be differences in motivation and therefore performance at an equivalent stage in both, i.e. after three miles of the five mile stretch compared with the beginning of the two mile stretch? A comparison (paired samples t test) was therefore planned between **2/3 and 1/2**. There were therefore six contrast pairs in all: **1/3 and 2/3, 2/3 and 3/3, 1/2 and 2/2, 1/3 and 3/3, 1/3 and 1/2, 2/3 and 1/2**. The results of the planned comparisons are presented in tabulated form for ease of reference.

3.2.2.2 Analysis of postoperative performance

The main question was whether there was a difference in performance on the SFC task between the BLA-lesioned group and the Sham-lesioned group. First, graphs of mean values comparing postoperative performance for the two groups on each of the various performance measures were drawn up and described. In order to allow better visualisation of the results, separate graphs are given for each foreperiod, giving five in all for each measure. Next, three sets of three-factor mixed ANOVAs were carried out on the Postoperative data for each measure, with Group (either BLA-lesioned and Sham-lesioned rats (first set of ANOVAs) or preoperative Sham-lesioned rats and postoperative Sham-lesioned rats (second set of ANOVAs) or preoperative BLA-lesioned rats and postoperative BLA lesioned rats (third set of ANOVAs)) as the between subjects factor, and schedule fraction (6 levels) and foreperiod (5 levels) as the within-subject factors. The first set of ANOVAs, Postoperative Sham – Postoperative Lesion, was carried out in order to discover whether excitotoxic lesions of the BLA as opposed to sham lesions had any effect on performance of the SFC task. The other two sets of ANOVAs, Preoperative Sham – Postoperative Sham and Preoperative Lesion – Postoperative Lesion, were intended to provide additional support to whatever the outcome of the first set was. For instance, if a significant difference in performance were found between the lesioned and sham rats, a lack of significant difference in the pre- and postoperative performance of the sham rats would support the argument that the significant difference in performance between the lesioned and sham rats was due to the lesion. Alternatively, a significant difference in the pre- and postoperative performance of the sham rats would suggest that any difference in performance between the lesioned and sham rats was due to causes other than the lesion itself. Likewise, a significant difference in the pre- and postoperative performance of the lesioned rats would, if no significant difference were found in the pre- and postoperative performance of the sham rats, support the suggestion that postoperative differences in performance between lesioned and sham rats are due to the effects of the lesion. Homogeneity of covariance was tested using the Mauchly sphericity test **(Appendix B)** and the Huynh-Feldt approximation applied to correct

for any violations. Effect sizes were calculated for every significant main effect and interaction in order to assess the impact of each factor on performance. Simple main effects **(Appendix C)** were also calculated since all the ANOVAs were within-subjects, thus making the use of post-hoc tests inadvisable. The ANOVA results and effect sizes are given in tabulated form for ease of reference. The planned comparisons on pairs of conditions from the main effect of progress to reward described above in relation to the preoperative data were then performed on the postoperative data in order to explore the various hypotheses as to how the rats could interpret the cue lights. Again, the results of the planned comparisons are presented in tabulated form for ease of reference.

3.2.3 Determination of lesion extent

As detailed above in the histology section, schematic representations of the lesions at each of six stereotaxic coordinates (-1.40, -2.12, -2.56, -3.14, -3.60 and -4.16 mm from bregma) spanning the entire region of interest for both the BLA and the CeN were made for each rat. From these it can be seen that some of the rats sustained complete lesions of the BLA / CeN whereas other rats sustained smaller lesions. In many cases adjoining structures were also damaged. Since it could be argued that grouping rats with incomplete lesions with rats that have sustained complete lesions might obscure potential lesion effects, for both experiments the percentage volume of lesion in each rat was estimated from the schematic representations as follows, making it possible to compare size of lesion with performance:

The two hemispheres were first assessed independently, with each hemispheric brain structure within a given schematic representation being allocated an individual score depending on the extent of lesioning within that structure. A score of 1 was given if ~25% of the structure had been lesioned, a score of 2 if ~50% of the structure had been lesioned, a score of 3 if ~75% had been lesioned and a score of ~4 if 100% had been lesioned. The scores for each structure within a given schematic representation were then combined across the hemispheres, meaning that the highest possible score was therefore 8 for a structure that had been completely lesioned in both hemispheres. Not every structure was present in each of the six schematic

representations, and so the number of times a given structure occurred was totalled. This total was then multiplied by 8 to give the maximum possible damage score for each structure throughout the six schematic representations. The maximum possible damage scores for each structure were then added together to give the absolute maximum lesion volume. The actual lesion scores for each structure for each individual rat were then totalled over the six schematic representations, and these added together to give the actual total lesion volume. The percentage volume of lesion in each rat was then worked out by dividing the actual total lesion volume by the absolute maximum lesion volume and multiplying by 100. Two separate percentage volumes of lesion were calculated for each rat – the percentage volume of lesion within the desired structures only, and the total percentage volume of lesion, i.e. within the desired structures and structures adjacent to them. For the BLA-lesioned rats, the desired lesion structures were the lateral amygdaloid nucleus, dorsolateral part (LaDL), lateral amygdaloid nucleus, ventromedial part (LaVM), lateral amygdaloid nucleus, ventrolateral part (LaVL), basolateral amygdaloid nucleus, anterior part (BLA) and basolateral amygdaloid nucleus, posterior part (BLP) whilst the adjacent structures were claustrum (Cl), dorsal endopiriform nucleus (Den), lateral stripe of the striatum (LSS), ventral endopiriform nucleus (VEn), basomedial amygdaloid nucleus, anterior part (BMA), intercalated amygdaloid nucleus, main part (IM), bed nucleus of the stria terminalis, intra-amygdaloid division (BSTIA), basomedial amygdaloid nucleus, posterior part (BMP), basolateral amygdaloid nucleus, ventral part (BLV), anterior cortical amygdaloid nucleus (ACo), posterolateral cortical amygdaloid nucleus (PLCo), cortex-amygdala transition zone (CxA), and piriform cortex (Pir). For the CeN-lesioned rats, the desired lesion structures were central amygdaloid nucleus, capsular part (CeC), central amygdaloid nucleus, lateral division (CeL) and central amygdaloid nucleus, medial division (CeM) whilst the adjacent structures were claustrum (Cl), dorsal endopiriform nucleus (Den), amygdalostriatal transition area (AStr), lateral stripe of the striatum (LSS), ventral endopiriform nucleus (VEn), basomedial amygdaloid nucleus, anterior part (BMA) anterior cortical amygdaloid nucleus (ACo), cortex-amygdala transition zone (CxA), piriform cortex (Pir), interstitial nucleus of the posterior limb of the anterior commissure (IPAC), lateral amygdaloid

nucleus, dorsolateral part (LaDL), substantia innominata (SI), intra-amygdaloid intra-medullary gray (IMG), basolateral amygdaloid nucleus, anterior part (BLA), intercalated amygdaloid nucleus, main part (IM), medial amygdaloid nucleus, anterodorsal part (MeAD), medial amygdaloid nucleus, anteroventral part (MeAV), bed nucleus of the accessory olfactory tract (BAOT), bed nucleus of the stria terminalis, intra-amygdaloid division (BSTIA), intercalated nucleus of the amygdala (I), basomedial amygdaloid nucleus, posterior part (BMP), basolateral amygdaloid nucleus, ventral part (BLV), medial amygdaloid nucleus, posteroventral part (MePV), posterolateral cortical amygdaloid nucleus (PLCo), lateral amygdaloid nucleus, ventromedial part (LaVM), lateral amygdaloid nucleus, ventrolateral part (LaVL), medial amygdaloid nucleus, posterodorsal part (MePD), basolateral amygdaloid nucleus, posterior part (BLP), posteromedial cortical amygdaloid nucleus (PMCo) and amygdalohippocampal area, anterolateral part (AHiAL).

Once the percentage volume of lesion had been obtained for each rat, it was possible to compare size of lesion with performance - this comparison was purely descriptive because only 4 rats were eventually included in the lesion. Separate graphs were drawn showing the postoperative performance of each of the 4 BLA-lesioned rats alongside the averaged postoperative performance of all of the Sham-lesioned rats for each of the 5 foreperiods on the main measure of mean percentage of correct responses. It was assumed that any data point from the BLA-lesioned rats lying outwith the range of +/-2 standard deviations of the averaged sham-lesioned rat data indicated a possible lesion effect. Formal correlational analysis, comparing extent of lesion with performance, was considered, but given the small number of rats in each group, decided against.

3.3 Results

3.3.1 Histological analysis

12 animals in all were assigned to the BLA lesion group and are given below. However, only 4 of the 12 rats were deemed to have reasonable bilateral lesions of the BLA and are given in bold:

98/299	98/307	98/317	98/325
98/302	98/310	98/321	98/332
98/303	98/311	98/324	**98/333**

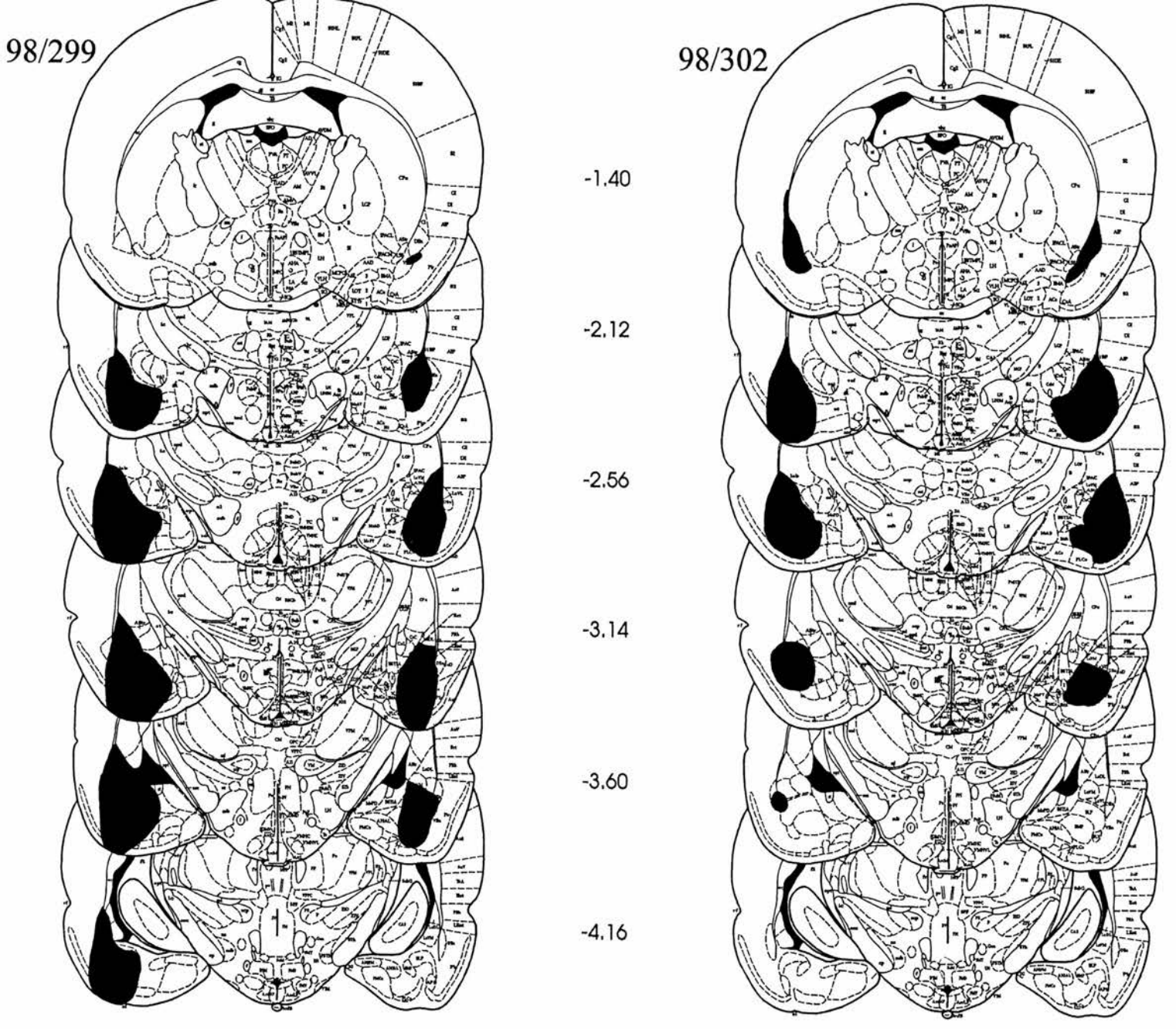
7 animals were assigned to the Sham lesion group. These were rats:

98/294	98/298	98/320	98/329
98/295	98/306	98/328	

The brains from the BLA lesion group and the Sham lesion group were subjected to histological procedures as described in the Histological procedures section. Schematic representations of the lesions sustained by each rat included in the lesion group were drawn and are given in ***Figure 11***, and also photomicrographs of each lesion at -2.56mm with respect to bregma ***(Figure 12)***. Photomicrographs of normal BLA and CeN are shown in ***Figure 13*** for comparative purposes. Overall, in all the rats, the lesions were too large, taking out not only BLA in the anterior part of the brain, but also the dorsal and ventral endopiriform nuclei (DEn and VEn) down to piriform cortex, the intercalated nucleus of the amygdala (IM), and parts of the anterior basomedial amygdaloid nucleus (BMA). Further back in the brain, the lesions took out the posterior and ventral parts of the basolateral amygdaloid nuclei (BLP and BLV) and the posterior part of the basomedial nucleus of the amygdala (BMP) as well as BLA. Only two of the four rats included in the lesion group (98/299 and 98/303) had substantial lesioning in the posterior part of the BLA, i.e. from approximately -3.60 mm from bregma, taking out the dorsolateral, ventrolateral, and ventromedial nuclei (LaDL, LaVL and LaVM) of the amygdala, the dorsal endopiriform nucleus (DEn), the posterior and ventral basolateral nuclei (BLP and BLV), the posterior part of the basomedial nucleus (BMP) and parts of piriform cortex. In all four rats, the CeN was spared throughout the entire extent of the lesion. The behavioural data obtained from these four rats, and from the sham lesioned rats, was subjected to analysis. The other rats in the lesion group sustained very little, if any, damage, which was probably due to needle blockage during surgery. Their behavioural data was excluded from analysis.

Figure 11: *Schematic representations of bilateral NMDA lesions of the BLA for the four rats (98/299, 98/302, 98/303 and 98/333) included in the analysis for Experiment A. The representations are mapped onto diagrams of coronal sections of the rat brain (Paxinos and Watson (1998)), with the first coronal section at -1.40mm and the last at -4.16mm with respect to bregma. The locations of the nuclei are given in the right hand hemispheres of the coronal sections, abbreviated as follows:*

ACo	*anterior cortical amygdaloid nucleus*
AHiAL	*amygdalohippocampal area, anterolateral part*
AStr	*amygdalostriatal transition area*
BAOT	*bed nucleus of the accessory olfactory tract*
BLA	*basolateral amygdaloid nucleus, anterior part*
BLP	*basolateral amygdaloid nucleus, posterior part*
BLV	*basolateral amygdaloid nucleus, ventral part*
BMA	*basomedial amygdaloid nucleus, anterior part*
BMP	*basomedial amygdaloid nucleus, posterior part*
BSTIA	*bed nucleus of the stria terminalis, intra-amygdaloid division*
CeC	*central amygdaloid nucleus, capsular part*
CeL	*central amygdaloid nucleus, lateral division*
CeM	*central amygdaloid nucleus, medial division*
CeN	*central amygdaloid nucleus*
Cl	*claustrum*
CxA	*cortex-amygdala transition zone*
DEn	*dorsal endopiriform nucleus*
I	*intercalated nucleus of the amygdala*
IM	*intercalated amygdaloid nucleus, main part*
IMG	*intra-amygdaloid intra-medullary gray*
IPAC	*interstitial nucleus of the posterior limb of the anterior commissure*
LaDL	*lateral amygdaloid nucleus, dorsolateral part*
LaVL	*lateral amygdaloid nucleus, ventrolateral part*
LaVM	*lateral amygdaloid nucleus, ventromedial part*
LSS	*lateral stripe of the striatum*
MeAD	*medial amygdaloid nucleus, anterodorsal part*
MeAV	*medial amygdaloid nucleus, anteroventral part*
MePD	*medial amygdaloid nucleus, posterodorsal part*
MePV	*medial amygdaloid nucleus, posteroventral part*
Pir	*piriform cortex*
PLCo	*posterolateral cortical amygdaloid nucleus*
PMCo	*posteromedial cortical amygdaloid nucleus*
SI	*substantia innominata*
VEn	*ventral endopiriform nucleus*


98/299
98/302
-1.40
-2.12
-2.56
-3.14
-3.60
-4.16

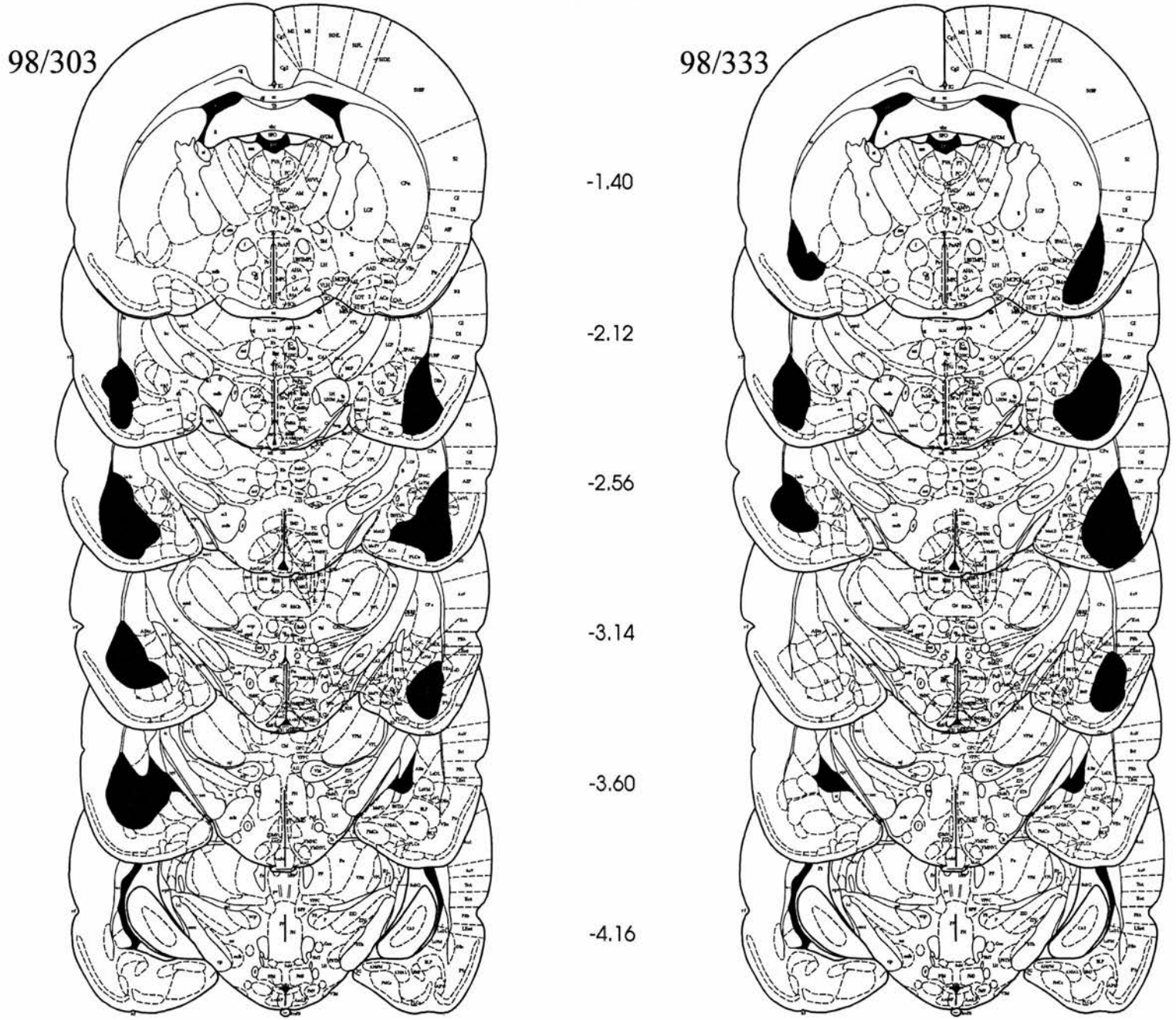

98/303
98/333
-1.40
-2.12
-2.56
-3.14
-3.60
-4.16

Figure 12: *Photomicrographs of bilateral NMDA lesions of the BLA for the four rats (98/299, 98/302, 98/303 and 98/333) included in the analysis for Experiment A. The photomicrographs are all at -2.56 with respect to bregma, and are taken with a x4 objective. Sections were stained with cresyl violet for nissl substance (see Histological procedures section for Experiment A for details). It can be seen that whilst surrounding tissue (including the CeN (central amygdaloid nucleus)) is intact, there is extensive gliosis and tissue collapse within the BLA (basolateral amygdaloid nucleus, anterior part).*

Rat 98/299	*A1 (left hemisphere)*	*A2 (right hemisphere)*
Rat 98/302	*B1 (left hemisphere)*	*B2 (right hemisphere)*
Rat 98/303	*C1 (left hemisphere)*	*C2 (right hemisphere)*
Rat 98/333	*D1 (left hemisphere)*	*D2 (right hemisphere)*

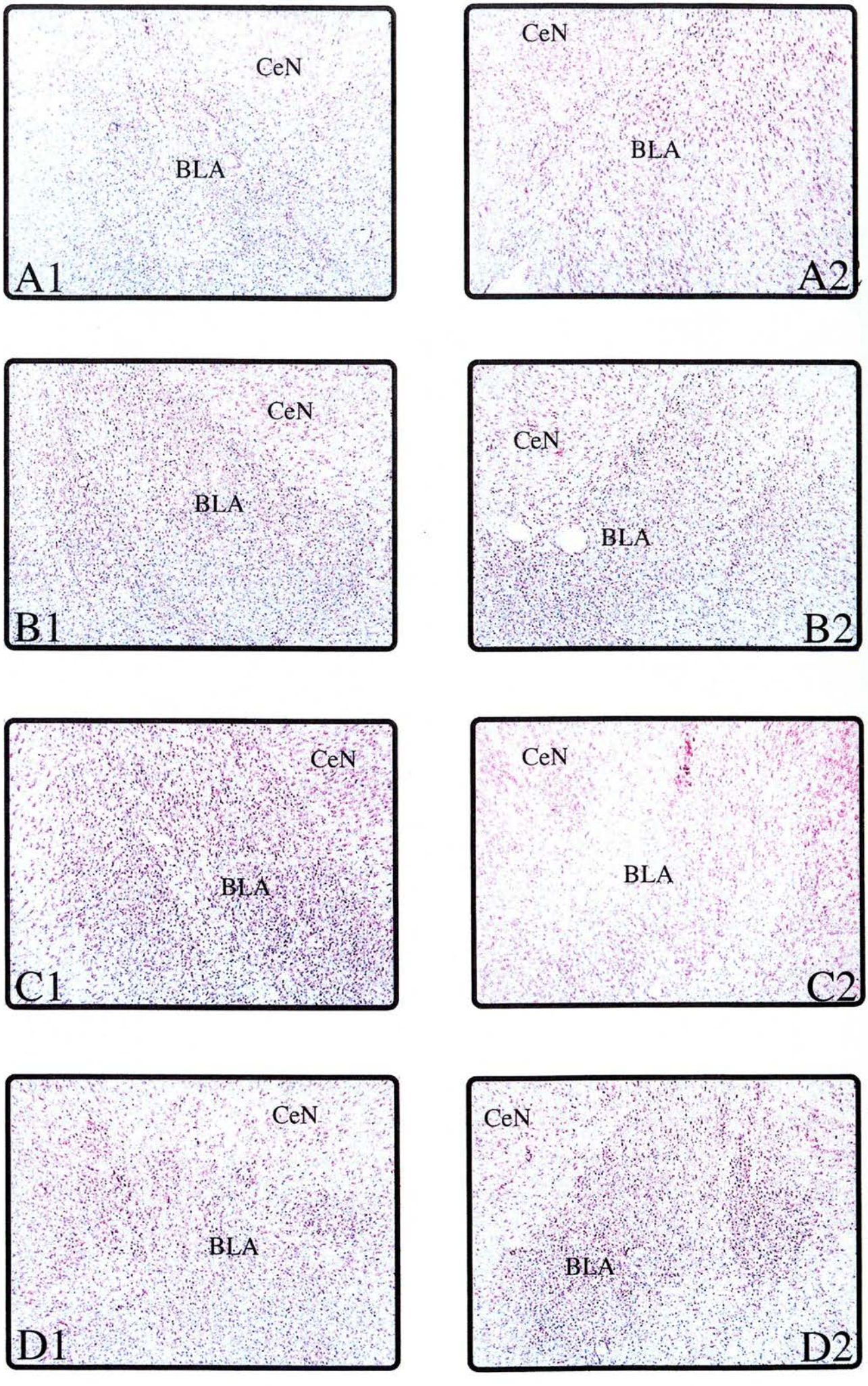
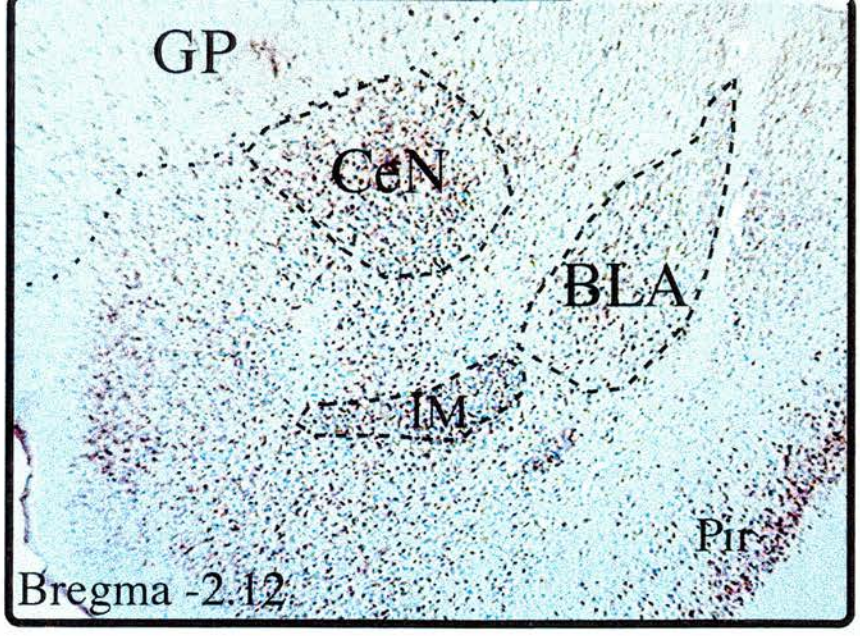

CeN
BLA
A1
CeN
BLA
A2
CeN
BLA
B1
CeN
BLA
B2
CeN
BLA
C1
CeN
BLA
C2
CeN
BLA
D1
CeN
BLA
D2

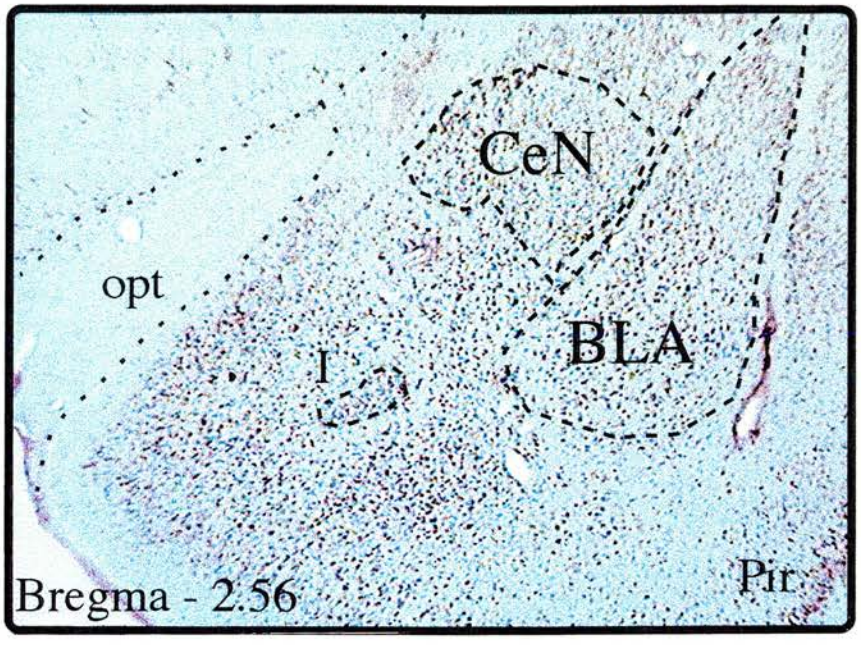
Figure 13: *The first 6 photomicrographs are taken with a x4 objective and show the BLA and CeN in the right hemisphere of an intact rat. Sections range from -2.12mm to -4.30mm with respect to bregma, and are stained with both the anti-neuronal nuclei monoclonal antibody NeuN, which reacts with most neuronal cell types but not with Purkinje, mitral and photoreceptor cells or glia, and with cresyl violet for nissl substance (see the Histological procedures section for Experiment A for details of the cresyl violet stain and the Histological procedures section for Experiment B for details of the NeuN stain). This combination of NeuN and cresyl violet gives very good structural definition and allows visualisation of gliosis. Structural locations are, abbreviated as follows:*

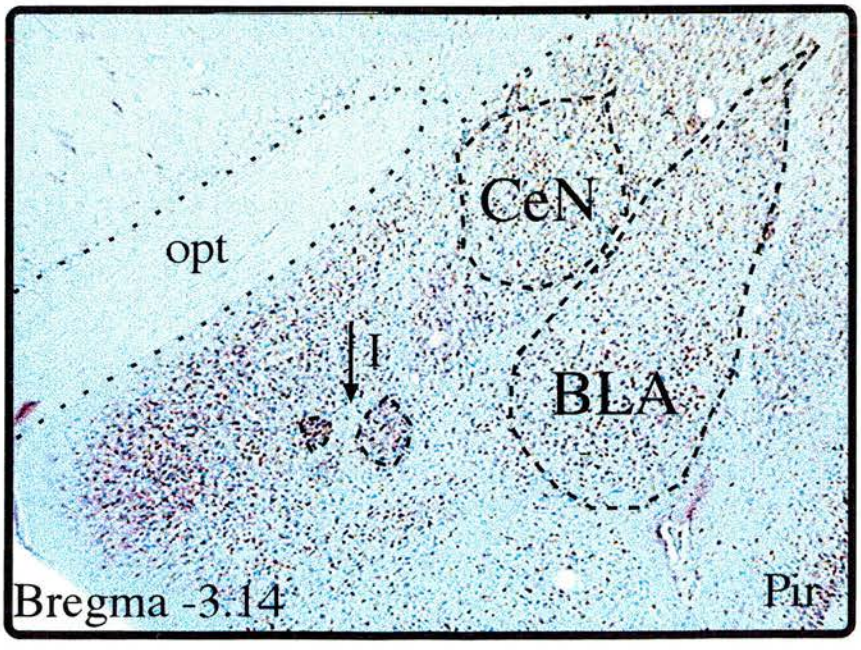
BLA	*basolateral amygdaloid nucleus, anterior part*
CeN	*central amygdaloid nucleus*
GP	*globus pallidus*
IM	*intercalated amygdaloid nucleus, main part*
Pir	*piriform cortex*

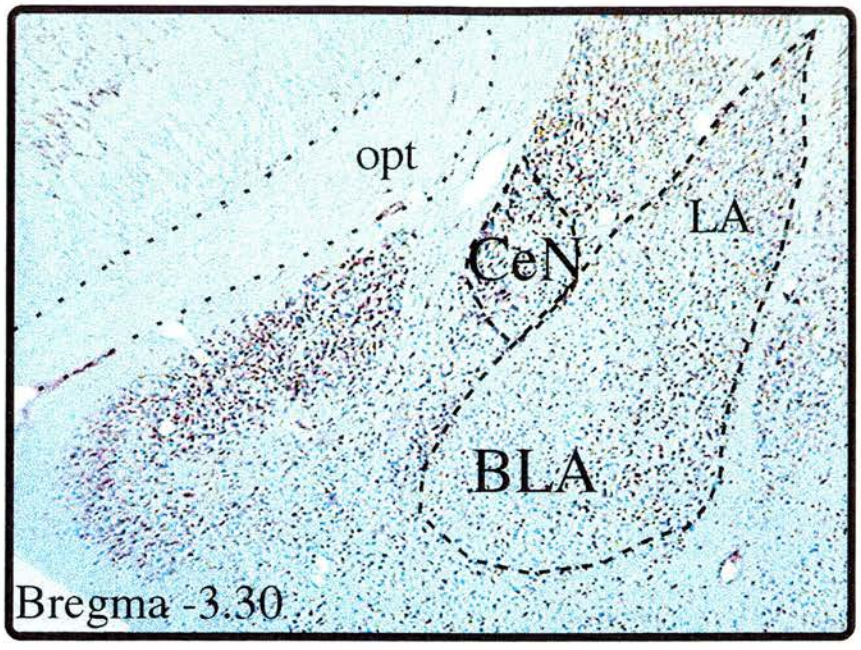
The final photomicrograph shows a section taken at -2.80mm with respect to bregma, stained with the Gallyas silver stain which shows mylelinated fibres of passage.The dense fibres surrounding the CeN are clearly visible.

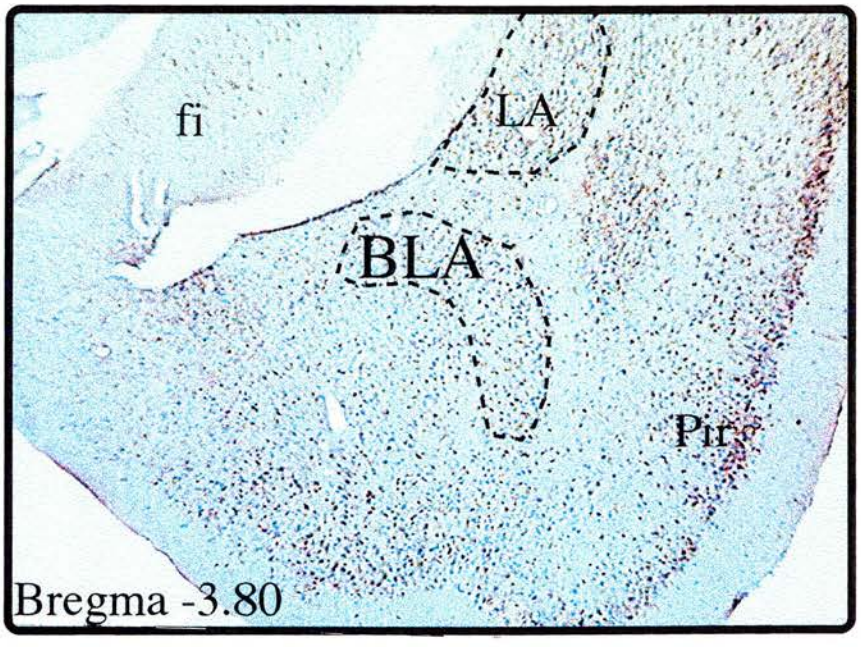
Below the photomicrographs is shown a cross-section though a diagram of the rat's brain for orientaion purposes.

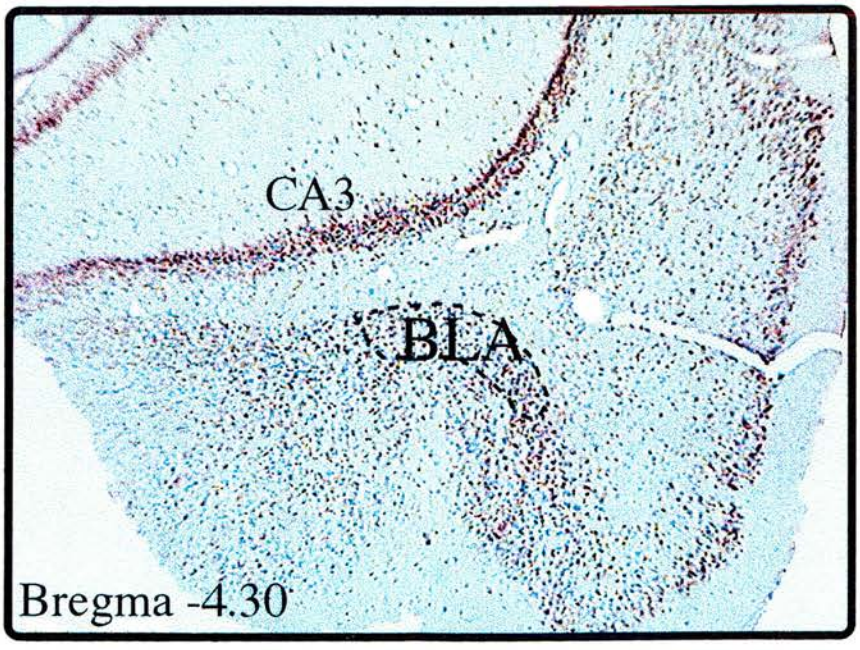

GP
CeN
BLA
IM
Pir
Bregma -2.12

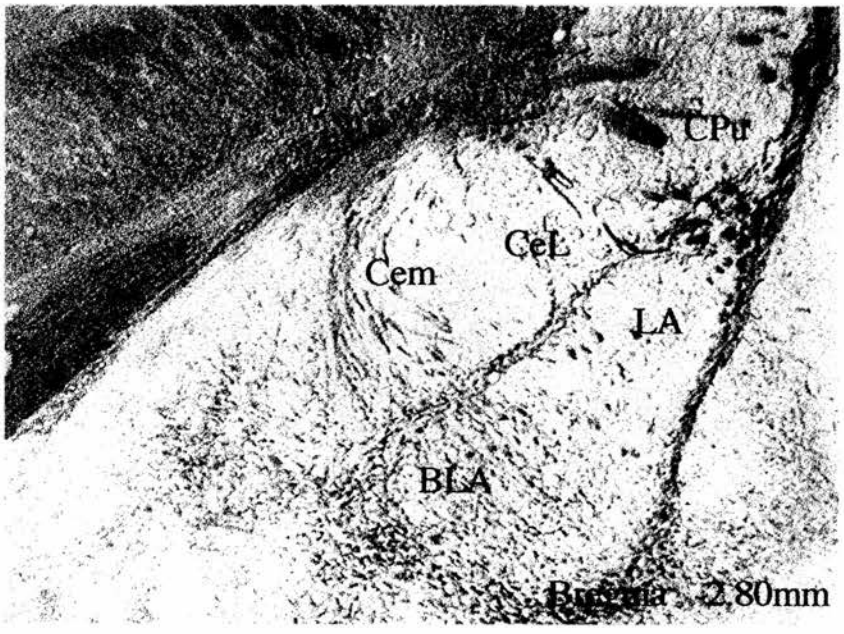

CeN
opt
BLA
I
Pir
Bregma - 2.56

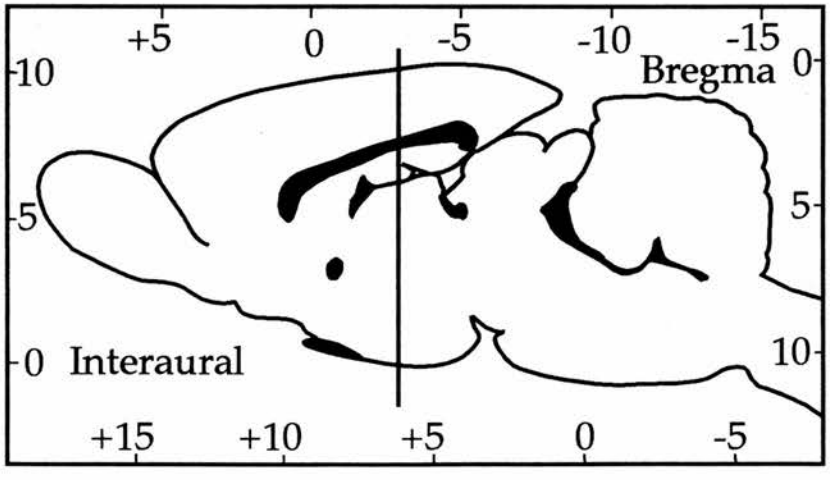

CeN
opt
I
BLA
Pir
Bregma -3.14


opt
LA
CeN
BLA
Bregma -3.30


fi
LA
BLA
Pir
Bregma -3.80


CA3
BLA
Bregma -4.30


CPu
CeL
Cem
LA
BLA
Bregma -2.80mm


+5
0
-5
-10
-15
10
Bregma
0
5
5
0 Interaural
10
+15
+10
+5
0
-5

3.3.2 Preoperative performance

3.3.2.1 Results of ANOVAs on preoperative data

3.3.2.1.1 Correct Responses

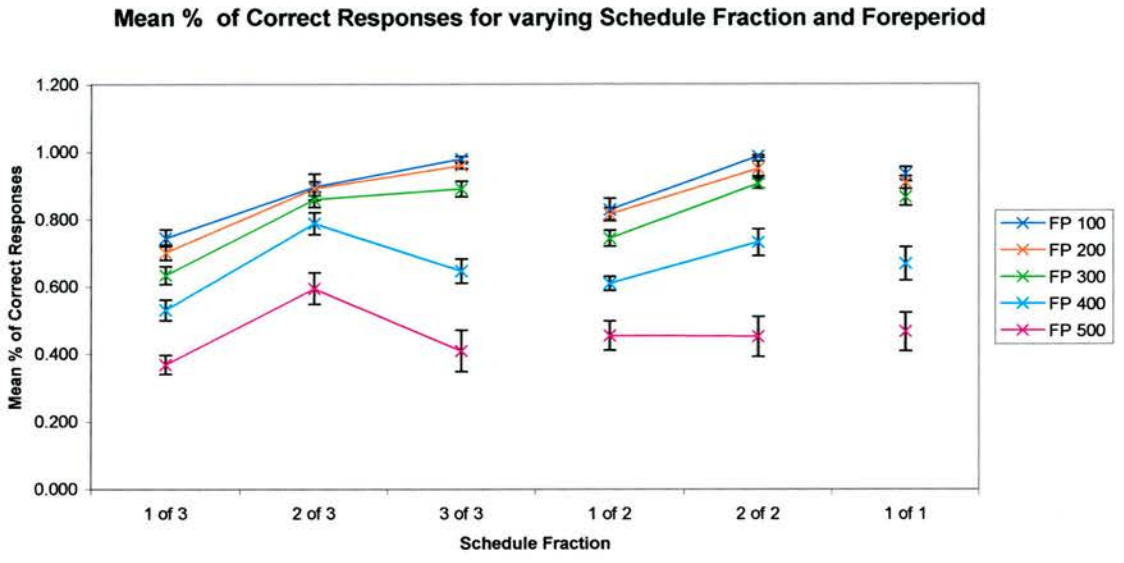


Figure 14: *Graph showing the mean (±se) percentage of correct responses made preoperatively. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Colour of line indicates length of foreperiod (100, 200, 300, 400, and 500 msecs).*

Figure 14 shows that the mean percentage of correct responses made by the rats varied across the different schedule fractions both within and between the work schedules (main effect of schedule fraction: $F(3.70, 37.02) = 27.31$, $P = 0.001$), and also according to foreperiod (main effect of foreperiod: $F(1.14, 11.46) = 61.35$, $P = 0.001$), with more correct responses made overall at shorter foreperiods. It can also be seen that two different patterns of response within the work schedules emerged according to foreperiod (interaction effect between schedule fraction and foreperiod: $(F(9.77, 97.70) = 5.31$, $P = 0.001)$. At the shorter foreperiods (100, 200 and 300) the rats made more correct responses the closer they came to achieving reward within each work schedule, but at the longer foreperiods (400 and 500) the rats made more correct responses at the second schedule fraction (2/3) of work schedule 3 and fewer at the third (rewarded) schedule fraction (3/3). Effect sizes calculated for both main effects confirmed that foreperiod (78.52%) had a more dramatic influence on behaviour than did schedule fraction

(16.73%). The effect size for the interaction is small (4.73%) but simple main effects were calculated in an attempt to pinpoint it more accurately **(Appendix C)**. However, all of the simple main effects proved significant ($p < 0.001$) making it impossible to draw any conclusions as to where exactly the interaction lies.

3.3.2.1.2 Reaction Time

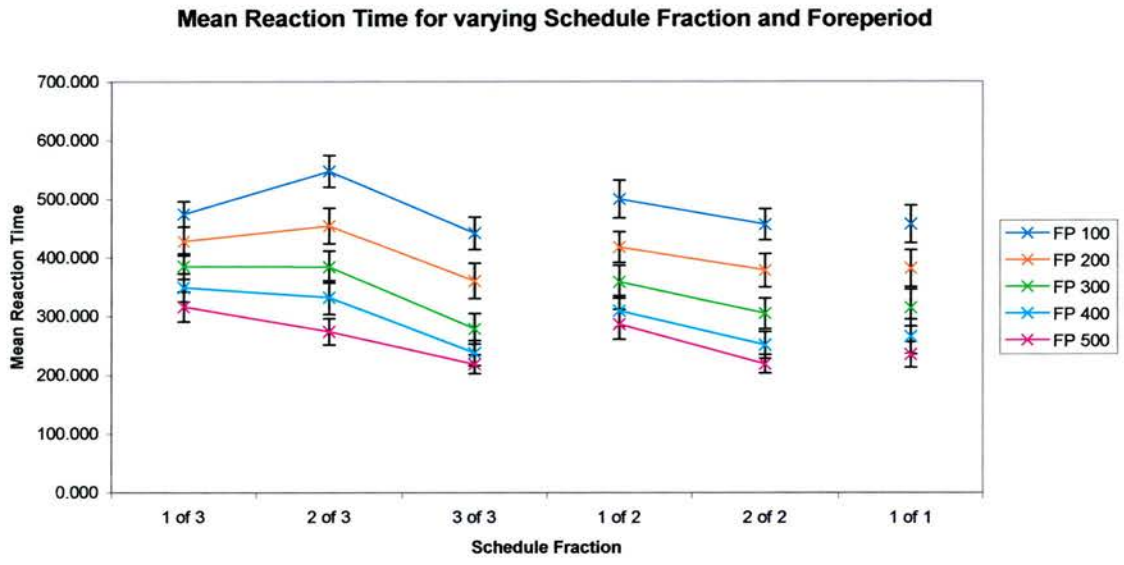


Figure 15: *Graph showing mean (±se) preoperative reaction time. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Colour of line indicates length of foreperiod (100, 200, 300, 400, and 500 msecs).*

It can be seen from ***Figure 15*** that the mean reaction time of the rats varied across the different schedule fractions both within and between the works schedules (main effect of schedule fraction: $F(2.48, 24.83) = 7.49$, $P = 0.002$), with the three rewarded schedule fractions (3/3, 2/2 and 1/1) having the fastest reaction times, all of which are very close to each other. The mean reaction time also varied according to foreperiod (main effect of foreperiod: $F(2.00, 19.98) = 383.34$, $P = 0.001$), with the rats withdrawing their snouts from the nose-poke hole more quickly at longer foreperiods. Again, it can be seen that two different patterns of response within the work schedules emerged according to foreperiod (interaction effect between schedule fraction and foreperiod: $F(10.74, 107.41) = 4.42$, $P = 0.001$). At the longer foreperiods (400 and 500) the rats showed a steady decrease in reaction time throughout each work schedule as they

came closer to achieving reward, but at the shorter foreperiods (100 and 200) the rats showed an increase in reaction time at the second schedule fraction (2/3) of work schedule 3 compared to the first and third schedule fractions (1/3 and 3/3). Effect sizes calculated for both main effects once again confirmed that foreperiod (81.78%) had a more dramatic influence on behaviour than did schedule fraction (16.04%). The effect size for the interaction is again very small (2.17%) but simple main effects were calculated for in an attempt to pinpoint it more accurately **(Appendix C)**. However, all of the simple main effects proved significant ($p < 0.01$), again making it impossible to draw any conclusions as to where exactly the interaction lies.

3.3.2.1.3 Movement Time

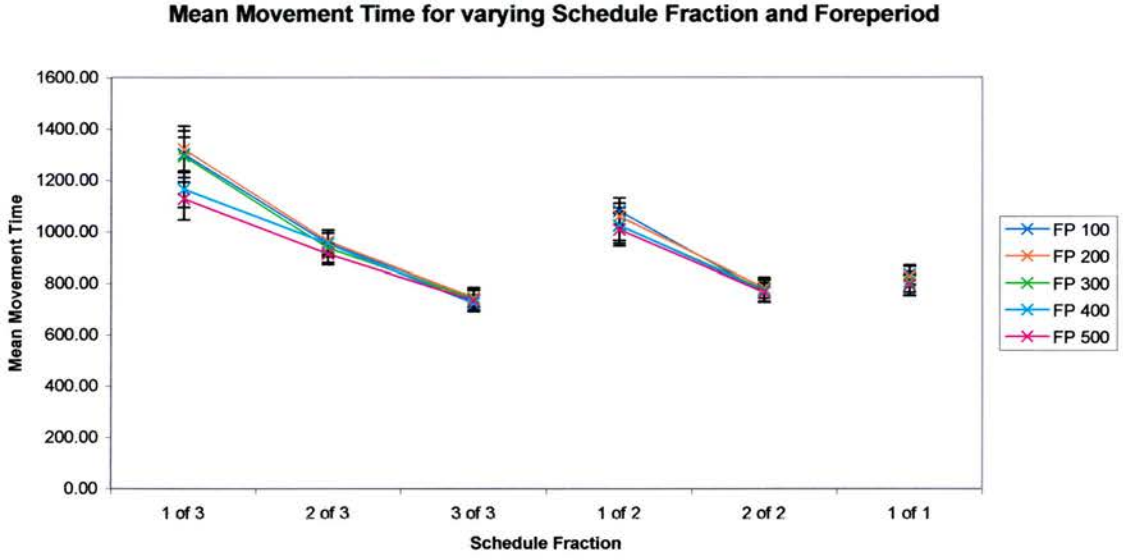


Figure 16: *Graph showing mean (±se) preoperative movement time. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Colour of line indicates length of foreperiod (100, 200, 300, 400, and 500 msecs).*

Figure 16 shows what appears to be a fairly straightforward relationship between schedule fraction and mean movement time - as the rats worked their way through a given work schedule towards reward, they took less time to move from the nose-poke hole to the hopper and open it (main effect of schedule fraction: $F(2.31, 23.09) = 39.60$, $P = 0.001$). Foreperiod, however, appears to have had very little effect on mean movement time except at schedule fraction 1/3, where mean movement time decreased as foreperiod lengthened. Nevertheless, performance of

ANOVA found a main effect for foreperiod ($F(4.00, 40.00) = 9.56$, $P = 0.001$), and also an interaction between schedule fraction and foreperiod ($F(8.52, 85.24) = 3.00$, $P = 0.004$). Calculation of effect sizes confirmed that schedule fraction (96.01%) had a much greater influence on behaviour than did foreperiod (1.67%). The effect size for the interaction is also very small (2.17%) but simple main effects were calculated in order to ascertain whether it did indeed lie within schedule fraction 1/3 **(Appendix C)**. It was found that although the simple main effects of changing levels of schedule fraction at all the foreperiods were significant ($p < 0.01$), only the simple main effect of changing levels of foreperiod at schedule fraction 1/3 was significant ($p < 0.01$), thereby supporting the theory that the interaction lies within this schedule fraction.

3.3.2.1.4 Post Response Pause

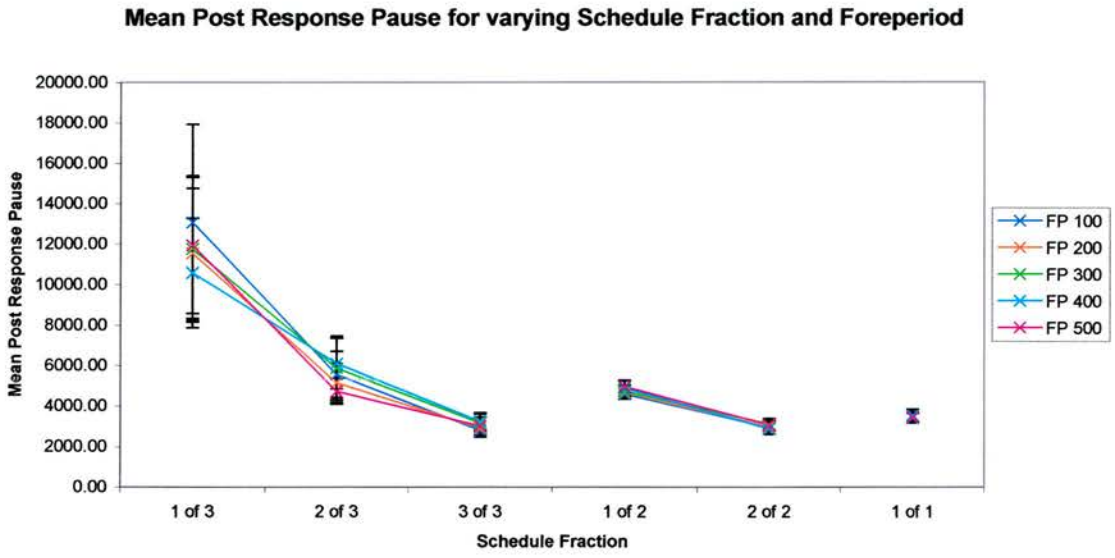


Figure 17: *Graph showing mean (±se) preoperative post response pause. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Colour of line indicates length of foreperiod (100, 200, 300, 400, and 500 msecs).*

After a complete correct work schedule, the post response pause will include the time taken to consume the reward, and it could therefore be expected that the mean post response pause would be longest for the rewarded schedule fractions (3/3, 2/2 and 1/1). However, it is clear from ***Figure 17*** that the rats were quickest to resume work at these schedule fractions, with the

post response pause for schedule fractions 2/2 and 3/3 being almost identical. Schedule fraction 1/1 has a somewhat longer mean post response pause time, perhaps because the rats were aware that they were less likely to be presented with Work Schedule 1 again rather than Work Schedules 2 or 3, which involve more work in order to obtain the reward. Schedule fraction 1/3 has by far the longest mean post response pause time, indicating that the rats were very reluctant to start the following work schedule, presumably because they were aware that they still had a lot of work to do before obtaining the next reward. Foreperiod does not appear to have any effect on mean post response pause time. Performance of ANOVA found a significant main effect for schedule fraction ($F(1.03, 10.32) = 6.26$, $P = 0.030$) but did not find a significant main effect for foreperiod or an interaction between schedule fraction and foreperiod.

3.3.2.2 Results of planned comparisons on preoperative data

3.3.2.2.1 Correct Responses

The main experimental hypothesis is that the different schedule fractions as cued by light intensity will have an effect on performance of the task. The means compared were 1/3 and 2/3, 2/3 and 3/3, and 1/2 and 2/2. Although the paired samples t-test for schedule fractions 2/3 and 3/3 is not significant, the result for 1/3 and 2/3 and for 1/2 and 2/2 is significant. Overall, these results suggest that, for this measure, the different schedule fractions do have an effect on performance of the task. In order to determine whether the rats interpret the different intensities of cue light as indicating 'progress to reward' two further sets of comparisons were made. The first set, comparisons of the means 1/3 and 3/3, 2/3 and 3/3, and 1/2 and 2/2, was to determine whether the rats perform differently according to whether it is a rewarded or an unrewarded schedule fraction. These comparisons proved to be significant, not significant and significant, suggesting that, for this measure, the rats do perform differently according to whether it is a rewarded or an unrewarded schedule fraction. The second set of comparisons, of the means 1/3 and 2/3, 1/3 and 1/2, and 2/3 and 1/2, was to determine whether the rats do use the cue lights as a means of ascertaining how close they are to reward rather than as an indication of the

availability of reward. All the comparisons proved to be significant, strongly suggesting that this is indeed the case for this measure. Together, these three sets of comparisons support the suggestion that, for the Correct Responses measure, not only is the rats' performance affected by schedule fraction, but that they do indeed interpret the different intensities of cue light as indicating 'progress to reward'. Finally, the comparison for the means 2/3 and 1/2 was significant, supporting the suggestion that differences in performance between these schedule fractions could reflect an awareness of 'cognitive distance from the goal'.

3.3.2.2.2 Reaction Time

Comparisons of the means 1/3 and 2/3, 2/3 and 3/3, and 1/2 and 2/2 proved to be not significant, significant and significant, suggesting that, overall, the different schedule fractions do have an effect on performance of the task on this measure. Comparisons of the means 1/3 and 3/3, 2/3 and 3/3, and 1/2 and 2/2 also proved to be all significant, showing that the rats perform differently according to whether it is a rewarded or an unrewarded schedule fraction. However, comparisons of the means 1/3 and 2/3, 1/3 and 1/2, and 2/3 and 1/2 all proved to be not significant, suggesting that the rats are not using the cue lights as a means of ascertaining how close they are to reward but merely as an indication of the availability of reward. The final comparison for the means 2/3 and 1/2 also was not significant, thereby indicating that the rats do not have an awareness of 'cognitive distance from the goal'.

3.3.2.2.3 Movement Time

Comparisons of the means 1/3 and 2/3, 2/3 and 3/3, and 1/2 and 2/2 all proved to be significant for this measure, suggesting that the different schedule fractions do have an effect on performance of the task. Comparisons of the means 1/3 and 3/3, 2/3 and 3/3, and 1/2 and 2/2 also all proved to be significant, showing that the rats perform differently according to whether it is a rewarded or an unrewarded schedule fraction. Likewise, comparisons of the means 1/3 and 2/3, 1/3 and 1/2, and 2/3 and 1/2 proved to be all significant, suggesting that the rats do use the cue lights as a means of ascertaining how close they are to reward rather than as an indication of the availability of reward. Together, these comparisons support the suggestion that,

for the measure of Movement Time, not only is the rats' performance affected by schedule fraction, but that they do indeed interpret the different intensities of cue light as indicating 'progress to reward'. The final comparison for the means 2/3 and 1/2 was also significant, supporting the suggestion that differences in performance between these schedule fractions could reflect an awareness of 'cognitive distance from the goal'.

3.3.2.2.4 Post Response Pause

Comparisons of the means for the main experimental hypothesis, 1/3 and 2/3, 2/3 and 3/3, and 1/2 and 2/2 proved to be all significant, suggesting that, for this measure, the different schedule fractions do have an effect on performance of the task. Comparisons of the means 1/3 and 3/3, 2/3 and 3/3, and 1/2 and 2/2 also proved to be all significant, showing that the rats perform differently according to whether it is a rewarded or an unrewarded schedule fraction. However, comparisons of the means 1/3 and 2/3, 1/3 and 1/2, and 2/3 and 1/2 proved to be significant, not significant and not significant suggesting that, overall, the rats are not using the cue lights as a means of ascertaining how close they are to reward but merely as an indication of the availability of reward. The final comparison for the means 2/3 and 1/2 also was not significant, thereby undermining the suggestion that the rats may have an awareness of 'cognitive distance from the goal'.

3.3.2.3 Discussion of preoperative performance

The results of both ANOVAs and planned comparisons suggest that the performance of the rats was influenced by the schedule fraction cues: rather than performing uniformly for all and throughout each of the different work schedules, the rats made more correct responses, and their reaction and movement times were faster for those work schedules requiring fewer correct responses, and they also performed better as they came closer to achieving reward within the work schedules. It would appear, therefore, that the cue lights signalling the onset of each work schedule and their changing intensity after every correct response had a motivational influence on the rats' performance. The results of the planned comparisons suggest that the rats also used

Means compared						Measure	*t and df*	*p*	Null hypothesis
1/3	2/3	3/3	1/2	2/2	1/1				
✓	✓					Correct Responses Reaction time Movement time Post Response Pause	t = -11.97, df = 10 t = -0.529, df = 10 t = 4.88, df = 10 t = 2.47, df = 10	0.000 not sig. 0.001 0.033	Equivalence of unrewarded SFs Equivalence of SFs within work schedule
	✓	✓				Correct Responses Reaction time Movement time Post Response Pause	t = 1.28, df = 10 t = 3.97, df = 10 t = 5.67, df = 10 t = 3.28, df = 10	not sig. 0.003 0.000 0.008	Equivalence of rewarded and unrewarded SFs
			✓	✓		Correct Responses Reaction time Movement time Post Response Pause	t = -4.62, df = 10 t = 2.38, df = 10 t = 7.64, df = 10 t = 7.99, df = 10	0.001 0.039 0.000 0.000	Equivalence of rewarded and unrewarded SFs
✓		✓				Correct Responses Reaction time Movement time Post Response Pause	t = -6.27, df = 10 t = 3.10, df = 10 t = 7.47, df = 10 t = 2.67, df = 10	0.000 0.011 0.000 0.024	Equivalence of rewarded and unrewarded SFs
✓			✓			Correct Responses Reaction time Movement time Post Response Pause	t = -4.41, df = 10 t = 0.96, df = 10 t = 3.97, df = 10 t = 2.08, df = 10	0.001 not sig. 0.003 not sig.	Equivalence of unrewarded SFs Equivalence of responses since reward
	✓		✓			Correct Responses Reaction time Movement time Post Response Pause	t = 5.56, df = 10 t = 1.46, df = 10 t = -3.15, df = 10 t = 0.79, df = 10	0.000 not sig. 0.01 not sig.	Equivalence of unrewarded SFs Equivalence of responses to reward

Table 1: *Results of planned contrasts on the main effect of schedule fraction (Preoperative data)*

the changing intensities to ascertain how close they were to achieving reward – not only did they perform differently according to whether a schedule fraction was rewarded or not rewarded, as might be expected if they used the cue lights as purely discriminative stimuli indicting the availability of reward, but they also performed differently on two of the measures (Correct Responses and Movement Time) according to which unrewarded schedule fraction they were on. It is, however, somewhat surprising that the rats did not perform differently in this respect on the Reaction Time measure: Bowman and Brown (1998) describe reaction time as "a measure of the trial by trial change in motivation induced by the cues" (p.444), but it might be that the range within which reaction times occur in this experiment (200–500 msec.) is too narrow to permit a statistical difference between the relevant schedule fractions (1/3 and 2/3, 1/3 and 1/2, and 2/3 and 1/2) to be found. It is less surprising, given the pre-emptive nature of the Post Response Pause measure, that although the rats took account of the cue lights *before* making the next response (in that they were slower to start unrewarded schedule fractions), their performance was not influenced by exactly *which* unrewarded schedule fraction they would next be on. Overall, the ANOVA and planned comparison results of this experiment would seem to be in line with the findings of Bowman and Brown (1998).

As stated previously, the use of different foreperiods in this experiment was merely intended to prevent automaticity of behavioural response and no hypothesis as to its potential effect on performance was made. However, it is now clear that foreperiod has a very dramatic effect on performance: not only were main effects of foreperiod found for the Correct Responses, Reaction Time and Movement Time measures, but the effect sizes for the first two of these were very large – much larger than the corresponding effect sizes for schedule fraction. As foreperiod is the length of time that the rats are required to sustain a nosepoke before withdrawing after the tone sounds, its effect on reaction time is perhaps the most easily explained - as foreperiod increased reaction time decreased for all the schedule fractions, presumably as a result of increased motor readiness. Such an explanation, however, also argues for the involvement of motivational factors in the influence that foreperiod has over performance. The effect of foreperiod on correct responses is rather more difficult to explain.

Correct responses decreased with lengthening foreperiod, presumably as a result of more anticipatory errors being made by the rats. This increase most likely resulted from an increase in motor readiness, with rats being more likely to 'jump the gun' and withdraw from the nosepoke hole too early at the longer foreperiods. Again, such an explanation argues for the involvement of motivational factors in the influence that foreperiod has over performance. Foreperiod appeared to have very little effect on movement time except at schedule fraction 1/3, where movement time decreased as foreperiod increased. Since the effect size is extremely small (1.67%), it would seem sensible to disregard this main effect. Likewise, foreperiod had no effect on post response pause; this can be attributed to the fact that this is measured before the rats have made a nose-poke.

Interaction effects between schedule fraction and foreperiod are also present for the Correct Responses, Reaction Time and Movement Time measures. Interaction effects normally take precedence over any main effects but there is an argument that they should not do so if the effect size for the interaction effect is very small compared to those for the main effects, as is the case here. However, given that foreperiod has had such an unexpectedly dramatic effect on performance, it would seem appropriate to discuss its interaction with schedule fraction in some detail. On the primary measure of Correct Responses the rats made more correct responses the closer they came to achieving reward within each work schedule at the shorter foreperiods (100, 200 and 300), but at the longer foreperiods (400 and 500) the rats made more correct responses at the second schedule fraction (2/3) of work schedule 3 and fewer at the initial schedule fraction (1/3) and third (rewarded) schedule fraction (3/3). It would appear, therefore, that having to both do a lot of work for the reward and hold the nosepoke for a longer period worsened performance. This pattern of response can be explained in both motor readiness and motivational terms. On the shorter foreperiods, the rats' eagerness to obtain the reward was not really affected by having to sustain the nosepoke and they made fewer anticipatory errors and therefore more correct responses. On the longer foreperiods, however, the rats made very few correct responses and therefore a very high number of anticipatory errors on the first schedule fraction of the work schedule (1/3), presumably because their impatience to obtain the reward

resulted in motor over-readiness and an inability to sustain the nosepoke. Their performance improved on the second schedule fraction of the work schedule (2/3), presumably because the 'punishment' of having to repeat the previous schedule fraction made them more careful, but by the time the rats reached the last fraction of the work schedule (3/3) they were again too impatient to wait for the statutory foreperiod and therefore made more anticipatory errors. It is more difficult to explain the pattern of results seen with the Reaction Time measure, but it might be that this too is dependent on the mean percentage of anticipatory errors made. At the longest foreperiod (500), there was a linear relationship between schedule fractions 1/3, 2/3 and 3/3, with reaction time decreasing as the rats worked through the work schedule. However, as foreperiod decreased, reaction time decreased less and less on the second schedule fraction of work schedule 3 (2/3), until, in fact, it actually increased for the shortest foreperiod (100). It might be that since the rats made a high percentage of anticipatory errors on the previous schedule fraction (1/3), they took more care on this schedule fraction (2/3) which slowed down reaction time on the shorter foreperiods but not on the longer foreperiods. Interaction effects were also found for the Movement Time measure, but it is clear that the interaction effect lies in the first schedule fraction of work schedule 3, and that the overall pattern of response is governed by the main effect of schedule fraction. No interaction effect was found for the Post Response Pause measure, which is not surprising given that no main effect of foreperiod was found.

To conclude, the results of this experiment show that not only did the rats use the different schedule fraction cues to inform them of whether a correct response would be followed by reward, but they also used them as a means of ascertaining how close they were to achieving that reward. However, it is very clear that length of foreperiod profoundly affects performance on the SFC task. For this reason, Post Response Pause might be described as the 'purest' measure of performance, since the only main effect was that of schedule fraction and there was no interaction effect between schedule fraction and foreperiod. However, the results of the planned comparisons suggest that this measure is perhaps not sensitive enough to reveal differences in performance that would support the hypothesis that the rats not only use the cue

lights as an indication of the availability of reward, but also as a means of ascertaining how close they are to achieving it. Correct Responses and Movement Time would appear to be the most effective measures in this respect, despite the influence of foreperiod on performance.

3.3.3 Postoperative performance

3.3.3.1 Results of ANOVAs on postoperative data

3.3.3.1.1 Correct Responses

Main effect / interaction	F value	P value	Effect size
Postoperative Sham – Postoperative Lesion ANOVA:			
SF	F (5, 45) = 18.25	p = 0.000	21.96 %
FP	F (1.37, 12.31) = 34.03	p = 0.000	78.03 %
Group	F (1, 9) = 0.27	p = 0.619	
SF*FP	F (14.60, 131.39) = 1.20	p = 0.283	
SF*Group	F (5, 45) = 1.22	p = 0.314	
FP*Group	F (1.37, 12.31) = 0.27	p = 0.683	
SF*FP*Group	F (14.60, 131.39) = 0.61	p = 0.858	
Preoperative Sham - Postoperative Sham ANOVA:			
SF	F (4.29, 51.46) = 31.95	p = 0.000	25.57 %
FP	F (1.26, 15.07) = 52.5	p = 0.000	74.42 %
Group	F (1. 12) = 1.49	p = 0.245	
SF*FP	F (13.69, 164.29) = 1.54	p = 0.105	
SF*Group	F (4.29, 51.46) = 0.21	p = 0.942	
FP*Group	F (1.26, 15.07) = 1.06	p = 0.338	
SF*FP*Group	F (13.69, 164.29) = 0.73	p = 0.735	
Preoperative Lesion - Postoperative Lesion ANOVA:			
SF	F (4.18, 25.09) = 18.74	p = 0.000	12.64 %
FP	F (1.41, 8.46) = 37.86	p = 0.000	80.99 %
Group	F (1, 6) = 0.05	p = 0.835	
SF*FP	F (12.51, 75.07) = 4.33	p = 0.000	06.35 %
SF*Group	F (4.18, 25.09) = 0.50	p = 0.741	
FP*Group	F (1.41, 8.46) = 0.24	p = 0.717	
SF*FP*Group	F (12.51, 75.07) = 1.35	p = 0.209	

Table 2: *Main effects and interactions obtained from the 3 sets of ANOVAs used to analyse postoperative performance on the mean percentage of correct responses measure.*

Figure 18.1.a to 18.1.e shows that not only is there very little difference in postoperative performance between the BLA-lesioned and the Sham-lesioned rats (no main effect of group was found for any of ANOVAs), but that their postoperative performance is very similar to their preoperative performance (shown in ***Figure 14***). The mean percentage of correct responses

made by both groups of rats varied across the different schedule fractions both within and between the work schedules (main effect of schedule fraction found for all three ANOVAs), and also according to foreperiod (main effect of foreperiod found for all three ANOVAs), with more correct responses made overall at shorter foreperiods. ***Figure 18.1.a to 18.1.e*** shows that both groups also continued to show two patterns of response according to length of foreperiod, with, at the shorter foreperiods, progressively more correct responses being made within work schedules 2 and 3 as the rats came closer to achieving the reward, but, at the longer foreperiods, fewer correct responses being made on the rewarded schedule fraction (3/3) of work schedule 3 compared to the preceding schedule fraction (2/3). Differences also emerge between the two groups as foreperiod increases, with the BLA-lesioned rats making fewer correct responses on schedule fraction 3/3 at foreperiod 400, and on schedule fractions 2/2, 2/3 and 3/3 at foreperiod 500. However, although the ANOVA carried out on the preoperative data for this measure resulted in a significant interaction between schedule fraction and foreperiod, this was not the case for either the main Postoperative Sham – Postoperative Lesion ANOVA or for the Preoperative Sham – Postoperative Sham ANOVA (although a significant interaction was found for the Preoperative Lesion – Postoperative Lesion ANOVA). The interaction effect for the preoperative data was very small (4.73%), and this lack can perhaps be attributed to changes in the group sizes pre- and post-operatively, to the rats having all undergone some kind of surgery and to the time lapse between when the preoperative baseline data were collected and when the postoperative data were collected.

3.3.3.1.2 Reaction Time

Figure 18.2.a to 18.2.e also shows that the postoperative performance of the BLA-lesioned and the Sham-lesioned rats is very similar (no main effect of group was found for any of ANOVAs), with the most obvious difference occurring in work schedule 2 at foreperiod 300. The postoperative performance of both groups of rats is also similar to their preoperative performance (shown in ***Figure 15***) with mean reaction time decreasing overall as foreperiod increases (main effect of foreperiod found for all three ANOVAs), and also as the rats come

Main effect / interaction	F value	P value	Effect size
Postoperative Sham – Postoperative Lesion ANOVA:			
SF	F (3.13, 28.16) = 4.74	p = 0.008	16.27 %
FP	F (3.93, 35.41) = 180.68	p = 0.000	83.72 %
Group	F (1, 9) = 0.01	p = 0.912	
SF*FP	F (18.28, 164.52) = 1.55	p = 0.078	
SF*Group	F (3.13, 28.16) = 0.12	p = 0.950	
FP*Group	F (3.93, 35.41) = 1.76	p = 0.159	
SF*FP*Group	F (18.28, 164.52) = 1.55	p = 0.229	
Preoperative Sham - Postoperative Sham ANOVA:			
SF	F (2.67, 32.05) = 4.39	p = 0.013	10.86 %
FP	F (3.32, 39.89) = 329.85	p = 0.000	86.73 %
Group	F (1, 12) = 0.37	p = 0.556	
SF*FP	F (11.73, 140.80) = 3.28	p = 0.000	02.40 %
SF*Group	F (2.67, 32.05) = 0.06	p = 0.975	
FP*Group	F (3.32, 39.89) = 0.69	p = 0.578	
SF*FP*Group	F (11.73, 140.80) = 1.17	p = 0.310	
Preoperative Lesion - Postoperative Lesion ANOVA:			
SF	F (3.20, 19.18) = 10.39	p = 0.000	23.62 %
FP	F (2.27, 13.60) = 182.66	p = 0.000	74.06 %
Group	F (1, 6) = 0.14	p = 0.725	
SF*FP	F (9.28, 55.68) = 3.03	p = 0.005	02.31 %
SF*Group	F (3.20, 19.18) = 0.34	p = 0.805	
FP*Group	F (2.27, 13.60) = 0.64	p = 0.560	
SF*FP*Group	F (9.28, 55.68) = 1.16	p = 0.337	

Table 3: *Main effects and interactions obtained from the 3 sets of ANOVAs used to analyse postoperative performance on the mean reaction time measure.*

closer to achieving reward within each work schedule (main effect of schedule fraction found for all three ANOVAs). However, in contrast to the preoperative data there is little evidence, at least in ***Figure 18.2.a to 18.2.e***, to suggest that either group responds differently according to length of foreperiod. The ANOVA carried out on the preoperative data for this measure resulted in a significant interaction between schedule fraction and foreperiod; however, whilst the two subsidiary postoperative ANOVAs also resulted in significant interactions between schedule fraction and foreperiod, the main Postoperative Sham – Postoperative Lesion ANOVA did not. But, as with the correct responses measure, the interaction effect for the preoperative data was very small (2.17%), and the lack of a significant interaction between schedule fraction and foreperiod in the Postoperative Sham – Postoperative Lesion ANOVA can be attributed to changes in the group sizes pre- and postoperatively, to the rats having all undergone some kind

of surgery and to the time lapse between when the preoperative baseline data were collected and when the postoperative data were collected.

3.3.3.1.3 Movement Time

Main effect / interaction	F value	P value	Effect size
Postoperative Sham – Postoperative Lesion ANOVA:			
SF	F (2.52, 22.72) = 25.06	p = 0.000	100. %
FP	F (4, 36) = 1.19	p = 0.330	
Group	F (1, 9) = 0.650	p = 0.441	
SF*FP	F (8.30, 74.68) = 0.72	p = 0.673	
SF*Group	F (2.52, 22.72) = 0.35	p = 0.758	
FP*Group	F (4, 36) = 0.51	p = 0.727	
SF*FP*Group	F (8.30, 74.68) = 0.73	p = 0.670	
Preoperative Sham - Postoperative Sham ANOVA:			
SF	F (2.06, 24.75) = 47.28	p = 0.013	98.98 %
FP	F (4, 48) = 4.31	p = 0.005	01.01 %
Group	F (1, 12) = 1.88	p = 0.194	
SF*FP	F (6.00, 71.98) = 1.52	p = 0.183	
SF*Group	F (2.06, 24.75) = 1.11	p = 0.346	
FP*Group	F (4, 48) = 0.56	p = 0.696	
SF*FP*Group	F (6.00, 71.98) = 1.04	p = 0.407	
Preoperative Lesion - Postoperative Lesion ANOVA:			
SF	F (1.83, 11.01) = 15.86	p = 0.001	100 %
FP	F (2.55, 15.29) = 1.48	p = 0.260	
Group	F (1, 6) = 2.44	p = 0.169	
SF*FP	F (4.24, 25.46) = 1.35	p = 0.279	
SF*Group	F (1.83, 11.02) = 0.29	p = 0.734	
FP*Group	F (2.55, 15.29) = 0.62	p = 0.590	
SF*FP*Group	F (4.24, 25.46) = 0.33	p = 0.866	

Table 4: *Main effects and interactions obtained from the 3 sets of ANOVAs used to analyse postoperative performance on the mean movement time measure.*

It can be seen from ***Figure 18.3.a to 18.3.e*** that the postoperative performance of the BLA-lesioned and Sham-lesioned rats is again fairly similar, with movement time decreasing as the rats approach reward within the work schedules. In this respect the postoperative performance of the two groups is also similar to their preoperative performance (shown in ***Figure 16***). However, the BLA-lesioned rats do appear to perform slightly better than the Sham-lesioned rats within some of the work schedules, most notably on schedule fraction 1/2 at foreperiods 300 and 400, but this observation is not supported by the results of the Postoperative Sham – Postoperative Lesion ANOVA: a significant main effect is found only for schedule fraction and not for group. The two subsidiary ANOVAs, comparing preoperative and postoperative

performance in the Sham-lesioned rats and in the BLA-lesioned rats, also resulted in main effects for schedule fraction but not for group. The ANOVA carried out on the preoperative data for this measure also resulted in a significant interaction between schedule fraction and foreperiod but this was not the case with the Postoperative Sham – Postoperative Lesion or Preoperative Lesion – Postoperative Lesion ANOVAs, though an interaction was found for the Preoperative Sham – Postoperative Sham data. However, the size of the interaction effect for the preoperative data was very small (2.30%), and the lack of interaction effects in the Postoperative Sham – Postoperative Lesion or Preoperative Lesion – Postoperative Lesion ANOVAs can again be attributed to changes in the group sizes pre- and postoperatively, to the rats having all undergone some kind of surgery and to the time lapse between when the preoperative baseline data were collected and when the postoperative data were collected.

3.3.3.1.4 Post Response Pause

Figure 18.4.a to 18.4.e shows what appear to be considerable differences in the postoperative performance of the two groups of rats, with the mean post response pause for the BLA-lesioned rats being consistently lower within work schedule 3, and more especially at schedule fraction 1/3, compared to the Sham-lesioned rats. However, the error bars are very large at schedule fraction 1/3, suggesting that it is unlikely that this difference will prove significant. This was indeed found to be the case: the main Postoperative Sham – Postoperative Lesion ANOVA gave no significant main effects of group. ***Figure 18.4.a to 18.4.e*** also shows that, postoperatively, mean post response pause decreases for both the BLA-lesioned and the Sham-lesioned rats as they come closer to achieving reward, which would seem to be in accordance with their preoperative performance (see ***Figure 17***). However, somewhat surprisingly, given the size of the effect in the preoperative ANOVA data (98.35%), and the fact that there was no effect of group, the Postoperative Sham – Postoperative Lesion ANOVA does not result in a significant main effect for schedule fraction, though the two subsidiary ANOVAs, comparing preoperative and postoperative performance in the Sham-lesioned rats and in the BLA-lesioned rats do. Once again, this difference between the results of the preoperative ANOVA and the main

Postoperative Sham – Postoperative Lesion ANOVA can be ascribed to differences in the group sizes pre- and postoperatively, the surgery itself, and the time that elapsed between collection of the preoperative data and collection of the postoperative data.

Main effect / interaction	F value	P value	Effect size
Postoperative Sham – Postoperative Lesion ANOVA:			
SF	F (1.15, 10.33) = 3.22	p = 0.099	
FP	F (2.19, 19.70) = 1.32	p = 0.292	
Group	F (1, 9) = 1.19	p = 0.303	
SF*FP	F (2.01, 18.11) = 0.55	p = 0.588	
SF*Group	F (1.15, 10.33) = 0.86	p = 0.391	
FP*Group	F (2.19, 19.70) = 0.23	p = 0.814	
SF*FP*Group	F (2.01, 18.11) = 0.37	p = 0.699	
Preoperative Sham - Postoperative Sham ANOVA:			
SF	F (1.12, 13.40) = 6.93	p = 0.018	100 %
FP	F (2.40, 28.76) = 0.42	p = 0.695	
Group	F (1, 12) = 0.57	p = 0.464	
SF*FP	F (2.04, 24.53) = 0.38	p = 0.691	
SF*Group	F (1.12, 13.40) = 0.32	p = 0.603	
FP*Group	F (2.40, 28.76) = 1.60	p = 0.216	
SF*FP*Group	F (2.04, 24.53) = 1.24	p = 0.307	
Preoperative Lesion - Postoperative Lesion ANOVA:			
SF	F (1.50, 8.98) = 15.70	p = 0.002	100 %
FP	F (4, 24) = 2.62	p = 0.100	
Group	F (1, 6) = 0.26	p = 0.629	
SF*FP	F (2.63, 15.78) = 0.48	p = 0.675	
SF*Group	F (1.50, 8.98) = 0.13	p = 0.823	
FP*Group	F (4,24) = 0.90	p = 0.447	
SF*FP*Group	F (2.63, 15.78) = 0.82	p = 0.488	

Table 5: *Main effects and interactions obtained from the 3 sets of ANOVAs used to analyse postoperative performance on the mean post response pause measure.*

3.3.3.1.5 Summary of postoperative ANOVA results

The results of the main set of ANOVAs (Postoperative sham – Postoperative Leison) showed no difference in the performance of BLA-lesioned and Sham lesioned rats on any of the dependent measures. This finding is supported by the results of the other two sets of ANOVAs there was no difference in pre- and postoperative performance for the sham-lesioined rats or for the BLA-lesioned rats. These results suggest that lesions of the BLA did not affect performance on the SFC task.

Figure 18: *Graphs comparing postoperative performance for the Sham-lesioned group and the BLA-lesioned group at each of the 5 foreperiods (100, 200, 300, 400, and 500 msecs) for all of the performance measures (Correct Responses, Reaction Time, Movement Time and Post Response Pause). Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward.*

Figure 18.1.a-e: Correct Responses

Graphs show the mean (±se) percentage of correct responses made postoperatively by the Sham-lesioned group and the BLA-lesioned group at each of the 5 foreperiods. Performance improved as the rats worked through the work schedules towards achieving reward at the shorter foreperiods, but deteriorated on the rewarded schedule fraction (3/3) of the longest work schedule compared to the previous schedule fraction (2/3) at the longer foreperiods. Differences emerged between the two groups as foreperiod increased, but these were not significant: main effects of schedule fraction and of foreperiod were found for all 3 sets of ANOVAs (Postoperative Sham – Postoperative Lesion, Preoperative Sham – Postoperative Sham and Preoperative Lesion – Postoperative Lesion), but no main effect of group. An interaction effect between schedule fraction and foreperiod was found for the Preoperative Lesion – Postoperative Lesion ANOVA. See text for details.

Figure 18.2.a-e: Reaction Time

Graphs show mean (±se) postoperative reaction time for the Sham-lesioned group and the BLA-lesioned group at each of the 5 foreperiods. Overall, reaction time decreased for both groups of rats as they worked through the work schedules towards achieving reward. Minor differences in performance between the two groups were not significant: main effects of schedule fraction and of foreperiod were found for all 3 sets of ANOVAs (Postoperative Sham – Postoperative Lesion, Preoperative Sham – Postoperative Sham and Preoperative Lesion – Postoperative Lesion), but no main effect of group. Interaction effects between schedule fraction and foreperiod were found for the Preoperative Sham – Postoperative Sham and Preoperative Lesion – Postoperative Lesion ANOVAs. See text for details.

Figure 18.3.a-e: Movement Time

Graphs show mean (±se) postoperative movement time for the Sham-lesioned group and the BLA-lesioned group at each of the 5 foreperiods. Overall, movement time decreased for both groups of rats as they worked through the work schedules towards achieving reward. Minor differences in performance between the two groups were not significant: main effects of schedule fraction were found for all 3 sets of ANOVAs (Postoperative Sham – Postoperative Lesion, Preoperative Sham – Postoperative Sham and Preoperative Lesion – Postoperative Lesion), but no main effect of group. A main effect of foreperiod was found for the Preoperative Sham – Postoperative Sham ANOVA. See text for details.

Figure 18.4.a-e: Post Response Pause

Graphs show mean (±se) postoperative post response pause for the Sham-lesioned group and the BLA-lesioned group at each of the 5 foreperiods. For both groups of rats, post response pause decreased as they worked through the work schedules towards achieving reward, but this was far less dramatic for the Sham-lesioned group. However, this difference was not significant: main effects of schedule fraction were found for the Preoperative Sham – Postoperative Sham and Preoperative Lesion – Postoperative Lesion sets of ANOVA, but no main effect of group. See text for details.

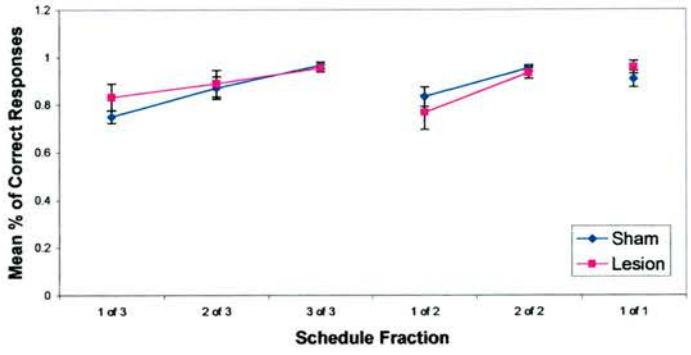


Figure 18.1.a: mean % of correct responses at foreperiod 100

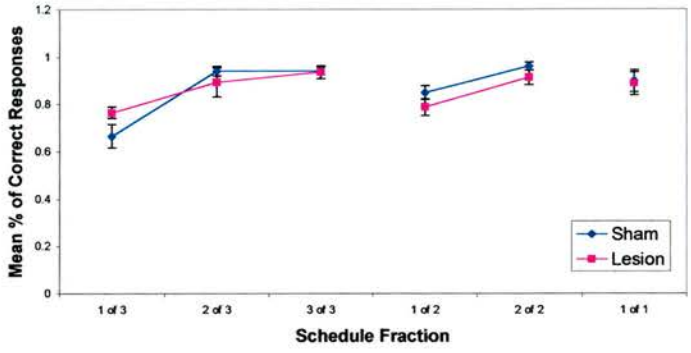


Figure 18.1.b: mean % of correct responses at foreperiod 200

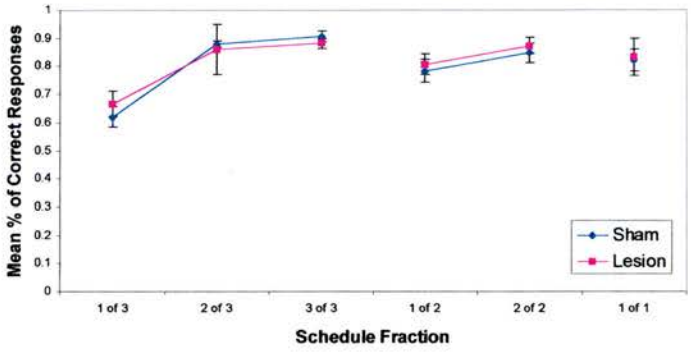


Figure 18.1.c: mean % of correct responses at foreperiod 300

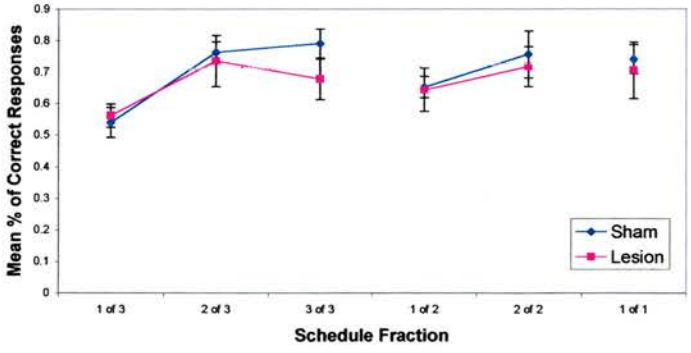


Figure 18.1.d: mean % of correct responses at foreperiod 400

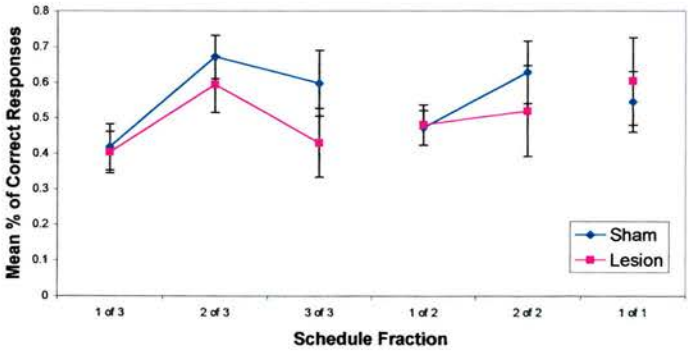


Figure 18.1.e: mean % of correct responses at foreperiod 500

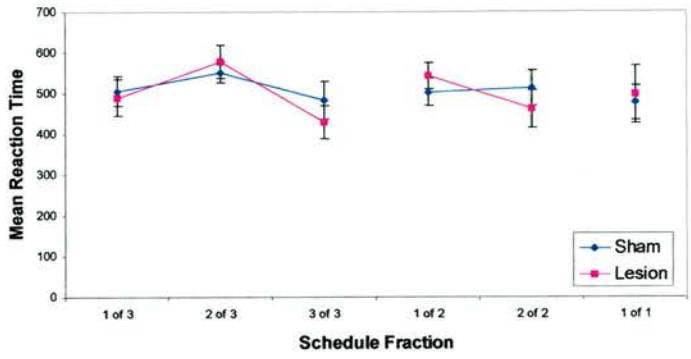


Figure 18.2.a: mean reaction time at foreperiod 100

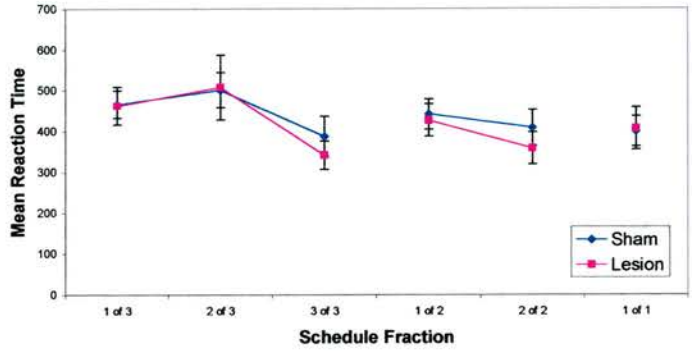


Figure 18.2.b: mean reaction time at foreperiod 200

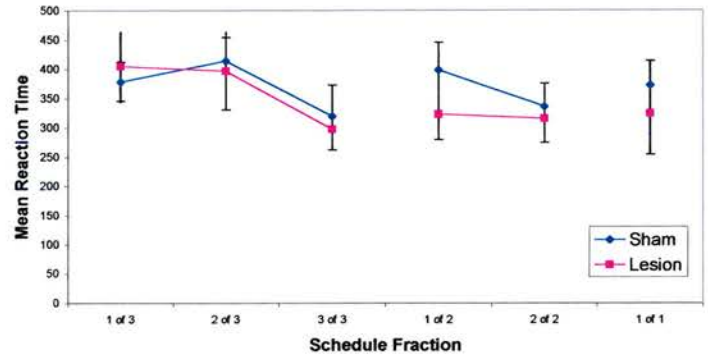


Figure 18.2.c: mean reaction time at foreperiod 300

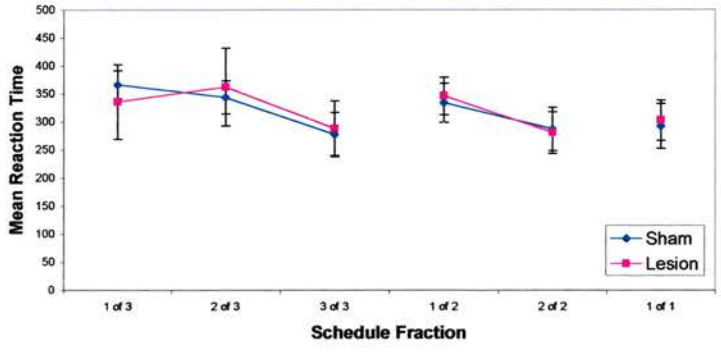


Figure 18.2.d: mean reaction time at foreperiod 400

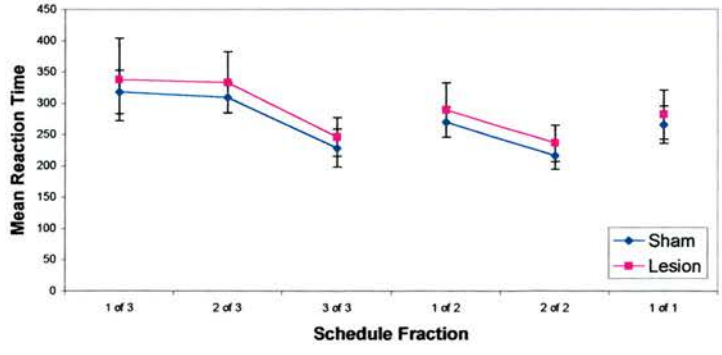


Figure 18.2.e: mean reaction time at foreperiod 500

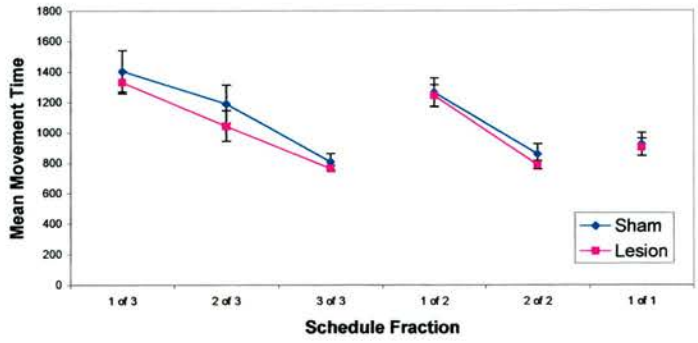


Figure 18.3.a: mean movement time at foreperiod 100

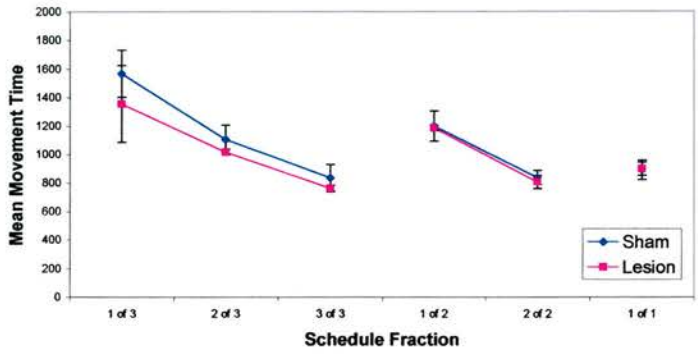


Figure 18.3.b: mean movement time at foreperiod 200

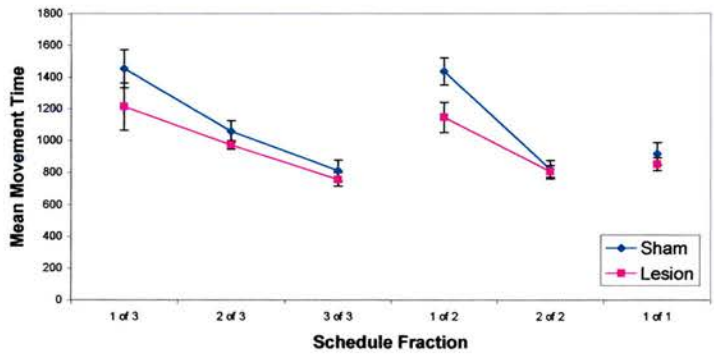


Figure 18.3.c: mean movement time at foreperiod 300

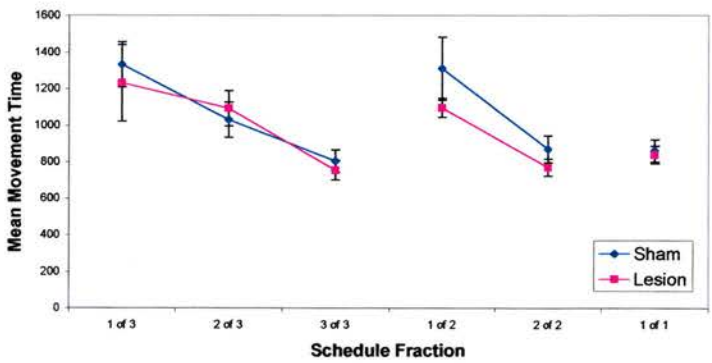


Figure 18.3.d: mean movement time at foreperiod 400

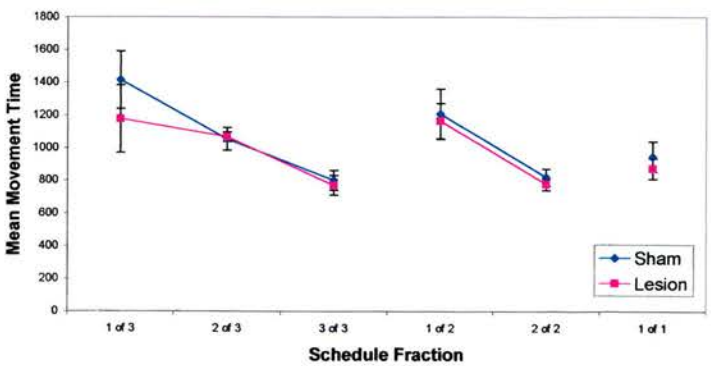


Figure 18.3.e: mean movement time at foreperiod 500

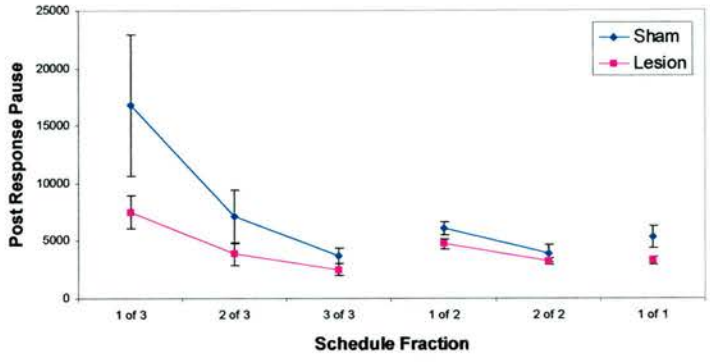


Figure 18.4.a: mean post response pause at foreperiod 100

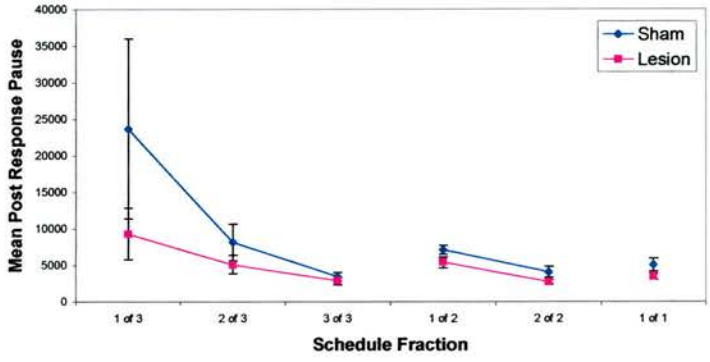


Figure 18.4.b: mean post response pause at foreperiod 200

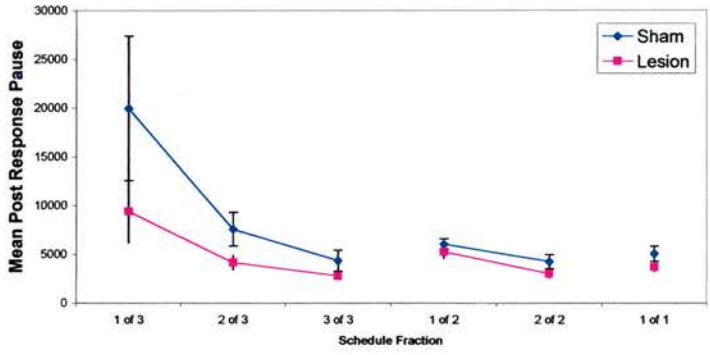


Figure 18.4.c: mean post response pause at foreperiod 300

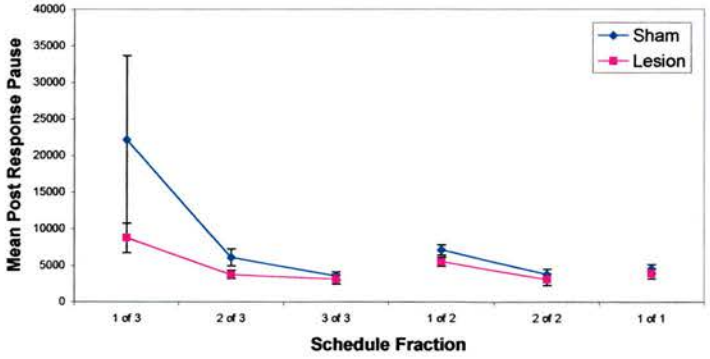


Figure 18.4.d: mean post response pause at foreperiod 400

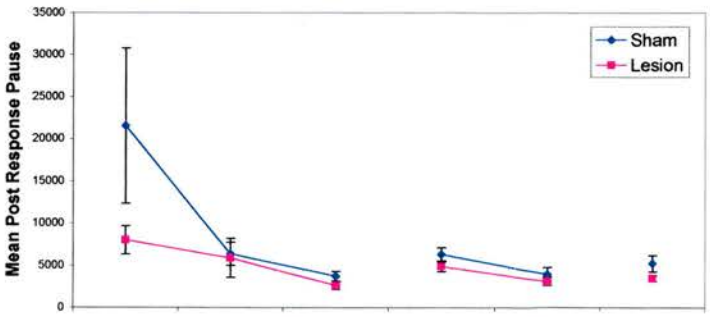


Figure 18.4.e: mean post response pause at foreperiod 500

3.3.3.2 Results of planned comparisons on postoperative data

The hypotheses are given in detail in the Analysis of preoperative performance section. It can be 135seen from ***Table 6*** (below) that for many of the planned comparisons the direction of significance is not the same postoperatively for the Sham group as for the Combined (sham and lesion) group preoperatively (results given in italics). The comparison is not a fair one in that the size of the Sham group is much smaller than that of the preoperative Combined group, but it does raise the question of how to interpret possible differences in the results of the planned comparisons for the Sham and Lesion groups postoperatively. For example, it can be seen that the planned comparison between schedule fractions 2/3 and 3/3 is significant for the Reaction Time measure for the preoperative Combined group, but that it is not significant for either the postoperative Sham or Lesion groups. It is therefore not possible to definitely attribute the change in significance of the Lesion group to the effects of the lesion. Likewise, the planned comparison between schedule fractions 1/3 and 2/3 is significant for the Post Response Pause measure for the preoperative Combined group, and for the postoperative Lesion group, but is not significant for the postoperative Sham group, making it difficult to draw any conclusions as to the effect of the lesion on performance. For this reason, it was decided to ignore the results of any planned comparisons that differ in the direction of significance between the preoperative Combined group and the postoperative Sham group in discussing whether excitotoxic lesions of the BLA as opposed to sham lesions had any effect on performance of the SFC task.

3.3.3.2.1 Correct Responses

Sham group: Two of the planned comparisons for the main hypothesis (1/3 and 2/3, and 1/2 and 2/2) were significant, but the third (2/3 and 3/3) was not. Overall, the results suggest that the rats did perform somewhat differently according to which schedule fraction they were on. Two of the planned comparisons for the first sub-hypothesis were also significant (1/3 and 3/3, and 1/2 and 2/2) but again the third (2/3 and 3/3) was not, suggesting that, overall, the rats performed differently on rewarded and unrewarded schedule fractions. All of the planned comparisons for the second sub-hypothesis were significant (1/3 and 2/3, 1/3 and 1/2, and 2/3

and 1/2), suggesting that the rats did use the different cue light intensities to ascertain how close they were to reward rather than as an indication of the availability of reward.

Lesion group: None of the planned comparisons were significant for the Lesion group except the comparison between 1/3 and 3/3. This suggests that not only were the lesioned rats not interpreting the different cue lights as indicating 'progress to reward' but that they were not regarding the schedule fractions at all.

3.3.3.2.2 Reaction Time

Sham group: The direction of significance of three of the planned comparisons (2/3 and 3/3, 1/2 and 2/2, and 1/3 and 3/3) had changed compared to the preoperative combined group and were therefore ignored. The remaining three planned comparisons were all not significant, strongly suggesting that the different schedule fractions did not have an effect on the rats' performance of the task, and that the rats did not interpret the different cue light intensities as indicating 'progress to reward'.

Lesion group: Again, the remaining three planned comparisons were all not significant, supporting the suggestion that the rats' performance was not affected by which schedule fraction they were on, and that they did not interpret the different cue light intensities as indicating 'progress to reward'.

3.3.3.2.3 Movement Time

Sham group: All of the planned comparisons were significant, strongly suggesting that the different schedule fractions did have an effect on the rats' performance of the task and that the rats did interpret the different cue light intensities as indicating 'progress to reward'.

Lesion group: The results for the Lesion group were less straightforward: two of the planned comparisons for the main hypothesis (2/3 and 3/3, and 1/2 and 2/2) were significant, whilst the third (1/3 and 2/3) was not significant. Overall, this would seem to suggest that, at least to some extent, the rats did perform differently depending on schedule fraction they were. Likewise, two of the planned comparisons for the first sub-hypothesis were significant (2/3 and 3/3, and 1/2

and 2/2) whereas the third (1/3 and 3/3) was not significant, again suggesting that, overall, the rats performed differently according to whether the schedule fraction was rewarded and unrewarded. However, none of the planned comparisons for the second sub-hypothesis (1/3 and 2/3, 1/3 and 1/2, and 2/3 and 1/2) were significant, suggesting that the rats did not use the different cue light intensities to ascertain how close they were to reward rather than as an indication of the availability of reward.

3.3.3.2.4 Post Response Pause

Sham group: The direction of significance of two of the planned comparisons (1/3 and 2/3, and 1/3 and 3/3) had changed compared to the preoperative combined group and were therefore ignored. The remaining planned comparisons for the main hypothesis (2/3 and 3/3, and 1/2 and 2/2) were significant, suggesting that, overall, the rats did perform somewhat differently according to which schedule fraction they were on. Both of the remaining planned comparisons for the first sub-hypothesis were also significant (2/3 and 3/3, and 1/2 and 2/2), confirming that the rats performed differently according to whether the schedule fraction was rewarded and unrewarded. However, none of the remaining planned comparisons for the second sub-hypothesis were significant (1/3 and 1/2, and 2/3 and 1/2), suggesting that the rats did not use the different cue light intensities to ascertain how close they were to reward rather than as an indication of the availability of reward.

Lesion group: Both of the remaining planned comparisons for the main hypothesis (2/3 and 3/3, and 1/2 and 2/2) were significant, confirming that the different schedule fractions did have an effect on performance of the task. Likewise, both of the remaining planned comparisons for the first sub-hypothesis (2/3 and 3/3, and 1/2 and 2/2) were significant, supporting the idea that the rats performed differently according to whether the schedule fraction was rewarded and unrewarded. However, neither of the remaining planned comparisons for the second sub-hypothesis (1/3 and 1/2, and 2/3 and 1/2) were significant, suggesting that the lesioned rats did not use the different cue light intensities to ascertain how close they were to reward rather than as an indication of the availability of reward.

3.3.3.2.5 Summary of results of planned comparisons on postoperative data

The results of the planned comparisons for the Correct Responses and the Movement Time measures suggest that the different schedule fractions did have an effect on the performance of the Sham-lesioned rats – not only did they perform differently according to whether a schedule fraction was rewarded or unrewarded, but they also used the different cue light intensities as a means of ascertaining how close they were to reward rather than as an indication of the availability of reward. However, the results of the planned comparisons (all of which were not significant) for the Reaction Time measure did not support this suggestion, whilst the results for the Post Response Pause measure suggested that the rats performed differently according to whether a schedule fraction was rewarded or unrewarded, but made no further use of the cue lights. These results are very much in line with those of the preoperative planned comparisons, which also showed that the rats used the cue lights as a means of ascertaining how close they were to reward on the Correct Responses and Movement Time measures, but not on the Reaction Time and Post Response Pause measures. As discussed previously (discussion of preoperative results), this is not altogether surprising with regard to the Post Response Pause measure in that its preemptive nature may make it a less subtle influence on behaviour. However, whereas preoperative performance on the Reaction Time measure showed that the rats discriminated between rewarded and unrewarded schedule fractions, postoperatively they did not discriminate at all between any of the different schedule fractions involved in the planned comparisons. It was suggested in the discussion of preoperative results that the range within which reaction times occur in this experiment (200-500msecs) is too narrow to permit a statistical difference in performance to be found between some of the schedule fractions compared, but this explanation is not sufficient to explain the complete lack of discrimination postoperatively. It might be that the rats are over-trained at this stage of the experiment, but whether this is in fact the case or not, it would appear that, in this experiment at least, Bowman and Browns's (1998) assertion that reaction time is "a measure of the trial by trial change in motivation induced by the cues" (p.444) does not hold true. With the exception of the Correct

Means compared						Correct Responses Reaction time Movement time Post Response Pause						Null hypothesis
						Preop Combined		Postop Sham		Postop Lesion		
1/3	2/3	3/3	1/2	2/2	1/1	*t* and df	*p*	*t* and df	*p*	*t* and df	*p*	
						t = -11.97, df = 10	0.000	t = -9.45, df = 6	0.000	t = -2.68, df = 3	**not sig.**	Equivalence of unrewarded SFs
						t = -5.23, df = 10	not sig.	t = -6.11, df = 6	not sig.	t = -1.12, df = 3	not sig.	
✓	✓					t = 4.88, df = 10	0.001	t = 5.78, df = 6	0.001	t = 1.34, df = 3	not sig.	Equivalence of SFs within work schedule
						t = 2.47, df = 10	0.033	t = 1.79, df = 6	*not sig.*	t = 3.26, df = 3	0.047	
						t = 1.28, df = 10	not sig.	t = -1.00, df = 6	not sig.	t = 0.47, df = 3	not sig.	Equivalence of rewarded and unrewarded SFs
						t = 3.97, df = 10	0.003	t = 2.41, df = 6	*not sig.*	t = 2.97, df = 3	not sig.	
	✓	✓				t = 5.67, df = 10	0.000	t = 3.35, df = 6	0.015	t = 8.16, df = 3	0.004	
						t = 3.28, df = 10	0.008	t = 2.85, df = 6	0.029	t = 2.71, df = 3	**not sig.**	
						t = -4.62, df = 10	0.001	t = -2.53, df = 6	0.045	t = -2.49, df = 3	**not sig.**	Equivalence of rewarded and unrewarded SFs
						t = 2.38, df = 10	0.039	t = 0.93, df = 6	*not sig.*	t = 5.25, df = 3	0.013	
			✓	✓		t = 7.64, df = 10	0.000	t = 4.27, df = 6	0.005	t = 5.79, df = 3	0.010	
						t = 7.99, df = 10	0.000	t = 5.15, df = 6	0.002	t = 6.07, df = 3	0.009	
						t = -6.27, df = 10	0.000	t = -8.19, df = 6	0.000	t = -5.09, df = 3	0.015	Equivalence of rewarded and unrewarded SFs
						t = 3.10, df = 10	0.011	t = 1.38, df = 6	*not sig.*	t = 1.97, df = 3	not sig.	
✓		✓				t = 7.47, df = 10	0.000	t = 6.87, df = 6	0.000	t = 2.73, df = 3	**not sig.**	
						t = 2.67, df = 10	0.024	t = 1.94, df = 6	*not sig.*	t = 3.16, df = 3	not sig.	
						t = -4.41, df = 10	0.001	t = -3.33, df = 6	0.016	t = -1.28, df = 3	**not sig.**	Equivalence of unrewarded SFs
						t = 0.96, df = 10	not sig.	t = 0.53, df = 6	not sig.	t = 1.03, df = 3	not sig.	
✓			✓			t = 3.97, df = 10	0.003	t = 2.99, df = 6	0.024	t = 0.56, df = 3	**not sig.**	Equivalence of responses since reward
						t = 2.08, df = 10	not sig.	t = 1.62, df = 6	not sig.	t = 1.70, df = 3	not sig.	
						t = 5.56, df = 10	0.000	t = 3.71, df = 6	0.010	t = 1.99, df = 3	**not sig.**	Equivalence of unrewarded SFs
						t = 1.46, df = 10	not sig.	t = 1.61, df = 6	not sig.	t = 2.00, df = 3	not sig.	
	✓		✓			t = -3.15, df = 10	0.01	t = -6.19, df = 6	0.001	t = -2.28, df = 3	**not sig.**	Equivalence of responses to reward
						t = 0.79, df = 10	not sig.	t = 0.41, df = 6	not sig.	t = -5.6, df = 3	not sig.	

Table 6: *Summary of planned comparison results on the main effect of schedule fraction for the preoperative Combined (Sham and Lesion) group and for the postoperative Sham-lesioned group and BLA-lesioned group. Italic font denotes that the direction of significance is not the same for the Postop Sham-lesioned group as for the Preop Combined (Sham and Lesion) group. Bold font denotes that the direction of significance is not the same for the Postop Lesion group as for the Postop Sham group.*

Responses measure, the results of the planned comparisons for the BLA-lesioned group largely resemble those of the Sham-lesioned group: on the Movement Time and Post Response Pause measures, the BLA-lesioned rats performed differently according to whether a schedule fraction was rewarded or unrewarded, but did not use the different cue light intensities as a means of ascertaining how close they were to reward rather than as an indication of the availability of reward (though the Sham-lesioned rats did do so on the Movement Time measure). Likewise, the BLA-lesioned rats also did not discriminate at all between any of the different schedule fractions involved in the planned comparisons for the Reaction Time measure. However, in contrast to the Sham-lesioned rats, the BLA-lesioned rats did not discriminate at all between any of the different schedule fractions involved in the planned comparisons for the Correct Responses measure, suggesting that the lesion did have an effect on performance of the SFC task.

3.3.3.3 Determination of lesion extent results

As can be seen from the schematic representations, the size and extent of the lesions in the four rats varied somewhat, with only two rats having substantial lesioning in the posterior part of the BLA. Since it could be argued that such variation within a group might obscure potential lesion effects, both the percentage volume of lesion within the desired structures only and the total percentage volume of lesion in each rat were estimated from the schematic representations as detailed in the Determination of lesion extent section for Experiment A. It was found that Rat 98/299 had the largest lesion both in terms of the percentage volume of lesion within the desired structures only (78.3%), and in terms of the total percentage volume of lesion (53.6%). Rat 98/302 had the third largest 'desired' lesion volume (51.3%) and second largest 'total' lesion volume (42.7%) and Rat 98/303 had the second largest 'desired' lesion volume (63.8%) and third largest 'total' lesion volume (34.1%). Rat 98/333 had both the smallest 'desired' lesion volume (42.1%) and 'total' lesion volume (31.6%) ***(Table 7)***. Separate graphs for each of the five foreperiods on the main measure of Correct Responses were drawn, showing the postoperative performance of each of the four BLA lesioned rats and the averaged postoperative

Rat	% volume of lesion within desired structures only	Total % volume of lesion (within desired structures and structures adjacent to them)
98/299	78.3	53.6
98/302	51.3	42.7
98/303	63.8	34.1
98/333	42.1	31.6

Table 7: *% volume of lesion within desired structures only and total % volume of lesion within desired structures and structures adjacent to them.*

performance of all of the Sham-lesioned rats (***Figure 19.a to 19.e***). It was then possible to compare size of lesion with performance. It was thought more likely that data points from the BLA-lesioned rats lying outwith the range of +/-2 standard deviations of the averaged Sham-lesioned rat data would belong to rats with larger rather than smaller lesions, but it can be seen from ***Figure 19.a to 19.e*** that this is not the case: Rat 98/333 has both the smallest 'desired' and the smallest 'total' lesion of the BLA-lesioned rats, but is the only rat to consistently fall outside the range of +/-2 standard deviations of the averaged Sham-lesioned rat data, and this only on one schedule fraction, SF 2/3. Otherwise, there does not appear to be any consistent relationship between extent of lesion, whether 'desired' or 'total' and performance on this measure. Given this, and the small number of rats in each group, it was decided not to carry out formal correlational analysis.

Figure 19: *Graphs showing the postoperative performance of each of the four BLA-lesioned rats (98/299, 98/302, 98/303 and 98/333) and the averaged (+/-2 standard deviations) postoperative performance of all of the Sham-lesioned rats at each of the five foreperiods (100, 200, 300, 400, and 500 msecs) on the main measure of mean percentage of Correct Responses. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. See text for details.*

Figure 19.a: *foreperiod 100*
Figure 19.b: *foreperiod 200*
Figure 19c: *foreperiod 300*
Figure 19.d: *foreperiod 400*
Figure 19.e: *foreperiod 500*

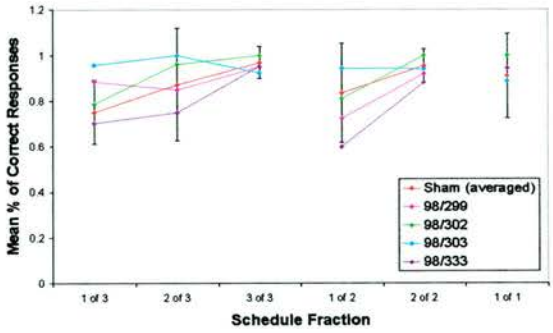


Figure 19.a: mean % of correct responses at foreperiod 100

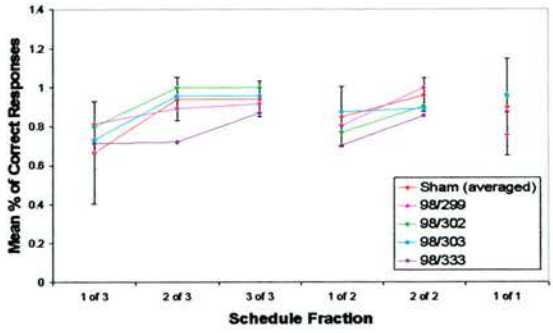


Figure 19.b: mean % of correct responses at foreperiod 200

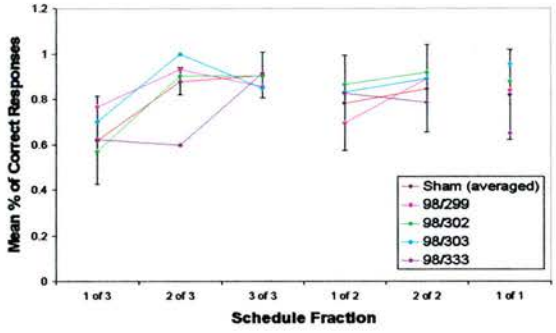


Figure 19.c: mean % of correct responses at foreperiod 300

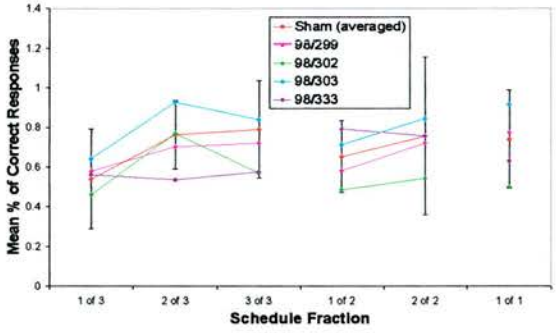


Figure 19.d: mean % of correct responses at foreperiod 400

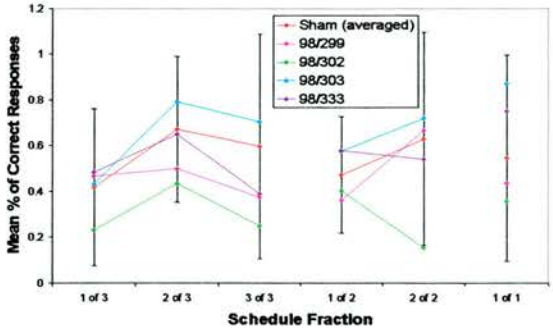


Figure 19.e: mean % of correct responses at foreperiod 500

3.4 Discussion

In the introduction to this experiment it was suggested that the BLA is critical for the acquisition of positive incentive value by formerly neutral stimuli. It was also suggested that Pavlovian conditioned stimuli could exert a motivational influence on instrumental behaviour. The premise to this experiment, therefore, was that since the SFC task appears to involve Pavlovian cues having a motivational impact on instrumental performance, BLA-lesioned rats would be impaired in their performance of the task, or, rather, would perform the task differently compared to normal rats. However, this does not appear to be wholly the case. The results of the first set of ANOVAs, between postoperative Sham-lesioned and postoperative BLA-lesioned rats, showed no difference in performance of the SFC task on any of the dependent measures. This finding was supported by the results of the other two sets of ANOVAs in that there was no difference in pre- and postoperative performance for either the Sham-lesioned rats or for the BLA-lesioned rats. These results suggest that BLA lesions did not affect performance on the SFC task. On the other hand, although it is difficult to draw any overall conclusions from the results of the planned comparisons, it would certainly appear that, at least on some of the measures, BLA lesions did affect performance on the SFC task. This is most apparent for the Correct Responses measure, for which all but one of the planned comparisons were significant for the Sham-lesioned group, but only one (1/3 and 3/3) was significant for the BLA-lesioned group. Since the direction of significance for all of the planned comparisons was the same for the Sham-lesioned group as for the preoperative combined (sham and lesion) group, it would appear that the different schedule fractions were continuing to have an effect on the Sham-lesioned rats' performance of the task, and that they were still able to interpret the different cue light intensities as indicating 'progress to reward', but that this was not the case for the lesioned rats.

It could perhaps be argued that this discrepancy between the results of the ANOVAs and of the planned comparisons is due to the small number of rats in the BLA lesion group. This is certainly a possibility, but the fact that the lesion extent graphs do not appear to show any

consistent relationship between extent of lesion, whether 'desired' or 'total' and performance on the Correct Responses measure argues against this. Also, other studies, which have been published recently, provide some support for these results. As mentioned in the introduction, other procedures in which Pavlovian cues are thought to influence instrumental responding are acquisition of a new response with conditioned reinforcement, second-order instrumental associative learning and Pavlovian-to-instrumental transfer. Recent studies involving heroin self-administration under a second-order schedule of reinforcement (Alderson, Robbins et al. 2000) and Pavlovian-to-instrumental transfer (Hall, Parkinson et al. 2001) have found that BLA lesions have no effect on performance. However, Alderson et al (2000) suggest that their results, which contrast with those of a previous study showing that the acquisition of cocaine self-administration under a second-order schedule of reinforcement is severely impaired by lesions of the BLA (Whitelaw, Markou et al. 1996), might be due to heroin-seeking behaviour being maintained by the experimental context rather than by the light CS in their experiment. Other studies have found that heroin-seeking behaviour is dependent on contextual rather than discrete drug-associated cues (Selden, Everitt et al. 1991), and it is known that spatial and contextual conditioning are dependent on the hippocampal formation (Phillips and LeDoux 1992) rather than the amygdala. Hall et al (2001) do not discuss their findings in relation to the BLA, but concentrate on the fact that lesioning the central nucleus of the amygdala does appear to abolish the Pavlovian-to-instrumental transfer effect.

In the introduction to this experiment it was also suggested that the schedule fraction cues could be regarded as discriminative stimuli that indicate the availability of reward, i.e. that a bright cue light signals no reward, a dim cue light signals no reward and no cue light signals reward. Previously, studies using discriminative stimuli have found that amygdala lesions impair performance, but these studies have largely involved aspirative or electrolytic lesions - Gaffan and Harrison (1987), for instance, showed that bilateral aspiration lesions of the amygdala severely impaired visual discrimination learning for auditory secondary reinforcement in rhesus monkeys. However, aspiration lesions of the amygdala in monkeys typically take out not only the amygdaloid complex, but also entorhinal, piriform and periamygdaloid cortex and

fibres of passage, and the impairment could be due to the removal of these rather than to the removal of the amygdala itself. More recently, Malkova et al (1997) used the same task as Gaffan and Harrison (1987) to investigate whether bilateral excitotoxic lesions of the amygdala would result in similar deficits and found that there was no effect on the rate at which monkeys solved a series of visual discrimination problems on the basis of auditory secondary reinforcement. On the other hand, Burns et al (1999) examined the effects of excitotoxic BLA lesions on the acquisition, performance and extinction of an instrumental conditional visuospatial discrimination task in rats and found differences, albeit small and transitory, between control and BLA-lesioned rats during performance and extinction.

Although the tasks of Malkova et al (1997) and Burns et al (1999) and the SFC task all involve visual discrimination learning, the form that this learning takes is somewhat different for each procedure. In Malkova et al's procedure, the visual discrimination came only in the test phase, when the monkeys had to choose which of two previously rewarded stimuli to touch based only on feedback of an auditory stimulus that had also previously been associated with reward. The monkeys had to choose the correct stimulus four times in a row, receiving feedback of the auditory stimulus each time, in order to obtain a food reward. The test phase was therefore very similar to a second-order schedule of reinforcement, with the auditory stimulus acting as both discriminative stimulus and conditioned reinforcer. In Burns et al's procedure the visual discrimination formed part of the training phase, in that rats were presented with a visual stimulus that informed them on which of two levers pressing would be rewarded by the presentation of another visual stimulus and delivery of a sucrose reward. It was this second visual stimulus that became the conditioned reinforcer for the second-order schedule which was then imposed, with rats having to make five, then ten, and then twenty correct responses in order to obtain a sucrose reward which also increased commensurately. In the SFC task the schedule fraction cue lights serve as both discriminative stimuli, in that they inform the rat how many nose-pokes are necessary in order to achieve reward within that particular work schedule, and as conditioned reinforcers within work schedules three and two, in that they are presented after every correct response which is not followed by reward (though, of course, at a different

intensity). All three procedures are therefore similar in their use of a conditioned reinforcer to maintain instrumental responding, but, unlike previous studies which have shown that lesioning the (basolateral) amygdala impairs performance (Cador, Robbins et al. 1989; Everitt, Cador et al. 1989; Burns, Robbins et al. 1993), BLA lesions have had, at most, a very transitory effect on performance.

Different explanations have been proposed by Malkova et al 1997) and by Burns et al (1999) as to why their results differ from those of previous experiments. Malkova et al explain the discrepancy by suggesting that the amygdala is not necessary for maintaining the value of secondary reinforcers once they have been learnt, but that damage to the BLA disrupts learning when a new secondary reinforcement (Malkova, Gaffan et al. 1997; Burns, Everitt et al. 1999) or a new second-order conditioning schedule (Hatfield, Han et al. 1996) is introduced. They further suggest that these findings are consistent with previous studies showing that the amygdala is important during the learning of emotionally charged events but not after the memory for those events has been consolidated (McGaugh, Introini-Collison et al. 1993; Salinas, Packard et al. 1993).

Burns et al (1999) explain their results with reference to Mishkin's proposal that memories and habits are subserved by different neural systems (Mishkin, Malamut et al. 1984). They suggest that their conditional discrimination task exemplifies visual habit learning as described by Mishkin et al (1984) in that it includes a strong element of stimulus–response learning of the type 'if stimulus A respond left: if stimulus B, respond right' rather than stimulus-reward associations, and in that acquisition is gradual and incremental, typically taking many sessions. Burns et al (1999) also note that, in accordance with Mishkin's theory of habit formation, the kind of learning underlying their conditional discrimination task would appear not to be dependent on 'limbic' structures since performance of the task is unimpaired by excitotoxic hippocampal lesions (Marston, Everitt et al. 1993), but to be dependent on the cortico-striatal system since performance of the task is impaired by lesions of the NAcc (Reading, Dunnett et al. 1991). However, despite their suggestion that their conditional discrimination task exemplifies visual habit learning, Burns et al (1999) propose that the control

rats were not simply relying on stimulus-response associations in order to maintain the accuracy of their performance, but were in fact utilising the conditioned reinforcer since its omission during the first of the five extinction sessions (during which the sucrose reward continued to be provided) resulted in an increased number of errors of commission (i.e. wrong lever presses). They go on to suggest that this was not the case with the BLA-lesioned rats: these were as well able to acquire the conditional discrimination as the control rats, and showed only minor transient impairments in performance after the second-order schedule was imposed, being initially slower both to respond correctly and to collect reward. During the first extinction session the BLA-lesioned rats also achieved a higher percentage of correct responses and made fewer errors of commission compared to the control rats, and showed an enhanced resistance to extinction during subsequent extinction sessions. Moreover, although the control rats responded increasingly slowly throughout the four final sessions (removal of the conditioned reinforcer, reward reduction and removal of reward altogether), the BLA-lesioned rats responded equally throughout them. Burns et al (1999) explain these results by arguing that in their conditional discrimination task the reward-related stimuli are acting not only as informative feedback stimuli for discriminative performance but also in a motivational capacity to energise and maintain performance. Lesioning the BLA attenuated the rats' ability to utilise the conditioned stimulus as a conditioned reinforcer, as has been shown to be the case in previous experiments (Cador, Robbins et al. 1989; Everitt, Cador et al. 1989; Burns, Robbins et al. 1993), but they were able to compensate for this by an greater reliance on stimulus-response associations maintained by primary reinforcement. Given the similarities previously noted between Burns et al's (1999) conditional discrimination task and the SFC task, it might be that a similar argument could be made for the lack of impairment seen in the BLA-lesioned rats' performance of the SFC task – they remain able to utilise the schedule fraction cues as feedback stimuli that inform them of how much work they have to do, but are no longer motivated or energised by the schedule fraction cues in their other capacity of conditioned reinforcers. It could be that AVOVA is not sensitive enough to differentiate between these two capacities, but that the

planned comparisons are, at least to some extent, able to do so, hence the discrepancy in the results obtained by the two different analyses.

There is some evidence to support this hypothesis. It has been suggested that it is the central nucleus of the amygdala (CeN) rather than the BLA that is involved in stimulus-response association (Everitt, Cardinal et al. 2000). Burns et al (1999) explain their findings with reference to Mishkin's theory of habit formation (Mishkin, Malamut et al. 1984), but Mishkin and his colleagues removed the entire amygdala (and hippocampus) from their monkeys, and their findings may therefore have resulted from destruction of the CeN rather than the BLA. (Given the volume of damage typically caused by aspirative lesions of the amygdala and hippocampus, it might even be that Mishkin et al's findings result from damage to another structure altogether – there is recent evidence, for instance, which implicates perirhinal cortex in appetitive associative learning (Liu, Murray et al. 2000; Liu and Richmond 2000).) It is of interest that lesions of the CeN but not of the BLA have been shown to abolish the Pavlovian-to-instrumental transfer effect (Hall, Parkinson et al. 2001), and that stimulus-response learning is also thought to underlie this task (Everitt, Cardinal et al. 2000) - perhaps lesions of the CeN would likewise lead to impaired performance on Burns et al's conditional discrimination task and on the SFC task. However, it has also been shown that performance on both the conditional discrimination task (Reading, Dunnett et al. 1991) and the Pavlovian-to-instrumental transfer task (Hall, Parkinson et al. 2001) is impaired by lesioning the NAcc, whereas performance of the SFC task is unimpaired (Bowman and Brown 1998). It might reasonably be expected that if similar processes in all three tasks are underlying the retention of performance after the BLA has been lesioned, then similar effects should result from lesioning other connected structures. Burns et al (1999) argue that these impairments in performance of the conditional discrimination task are unrelated to processing in the amygdala, since amygdalo-striatal interactions appear to underlie other forms of appetitive learning such as conditioned place preference (Everitt, Morris et al. 1991) and acquisition of a new response with conditioned reinforcement (Cador, Robbins et al. 1989; Burns, Robbins et al. 1993), but Hall et al (2001) suggest that the central nucleus of the amygdala and the core region of the NAcc may interact

functionally with regard to the Pavlovian-to-instrumental transfer effect. There are no direct connections between the central nucleus of the amygdala and the core region of the NAcc (Price and Amaral 1981), but it has been demonstrated that the CeN projects substantially to the dopaminergic neurons of the SNc and the VTA (Price and Amaral 1981; Haber, Fudge et al. 2000). Hall et al (2001) suggest, therefore, that the CeN might influence the Pavlovian-to-instrumental transfer effect by regulating dopaminergic innervation of the NAcc.

Given the contradictions present in the literature regarding the effect of BLA lesions on tasks employing conditioned reinforcers, and given that the results of the analysis of the data in the present experiment do not lead to any clear conclusions, it was thought best to repeat the experiment with the intention of obtaining a more definitive outcome. Since lesions of the CeN have been shown to abolish the Pavlovian-to-instrumental transfer effect (Hall, Parkinson et al. 2001), and since this procedure, which bears similarities to the SFC task, is thought to rely on stimulus-response learning (Everitt, Cardinal et al. 2000), it was also decided to run a group of CeN-lesioned rats alongside the BLA-lesioned rats in the repeated experiment. If conditioned discrimination tasks such as those used by Burns et al (1999) and by Malkova et al (1997) and the SFC task do, in fact, depend on stimulus-response learning, then it might be that a dissociation with regard to the effects of BLA and CeN lesions on performance of the SFC task will be obtained.

Chapter 4 - The Schedule Fraction Cue Task

Experiment B

4.1 General methods

4.1.1 Materials and methods

4.1.1.1 Animals

36 naïve adult male Lister hooded rats were used in this experiment. They were maintained, trained, and tested as described in the previous experiment. During the course of the experiment 2 rats were used to make practice lesions, 1 rat dies under anaesthesia, and 4 rats were killed due to poor recovery from surgery.

4.1.1.2 Apparatus

The apparatus was as described for the previous experiment.

4.1.1.3 Experimental procedure

All the rats underwent the same training procedures as described for the previous experiment. However, a programming error resulted in half of the rats being run on a version of the SFC task with only 3 foreperiods of 100, 200 and 300 msec rather than the 5 foreperiods that were used in the first experiment. The remaining rats were run on the usual version with 5 foreperiods of 100, 200, 300, 400 and 500 msec. Once performance had stabilised, preoperative data were collected over 7 daily sessions. The rats then underwent surgery, and, after a week's recovery period, postoperative data were collected over a further 7 daily sessions. They then underwent a reversal stage, with the schedule fraction cues being reversed for each rat. In other words, those rats for which no cue lights indicated that there were 3 trials before reward, dim cue lights indicated that there were 2 trials before reward, and bright cue lights indicated that the current trial would be rewarded found that bright cue lights indicated 3 trials before reward, dim cue

lights indicated that there were 2 trials before reward, and no cue lights indicated that the current trial would be rewarded. Reversal data were collected over 10 days, with each rat undergoing one daily session lasting up to 30 min.

4.1.1.4 Surgery

Anaesthetic and surgical procedures were as described for the previous experiment. After extensive piloting (detailed in **Appendix D)** the co-ordinates used for the BLA lesions were AP -2.3 and -3.1mm with respect to bregma and ML +/-5mm from the midline and DV -8.6mm (lower) and -7.7mm (upper) from the skull surface, with the skull level, giving 4 sites per hemisphere and 8 sites in all. The BLA-lesioned group received injections of 0.07µl quinolinate (0.06 M) into the lower sites and 0.03 µl quinolinate (0.06 M) into the upper sites. The injections were made over 2 min for the lower sites, with the needle then left for 3 min in situ, and over 1 min for the upper sites, with the needle again left for 3 min in situ. The control (BLA Sham-lesioned) group underwent the same procedure, but was injected with phosphate buffer vehicle instead of the neurotoxin. The co-ordinates used for the CeN lesions were AP -2.4 and -3.2mm with respect to bregma and ML +/-3.9 and +/-4.3mm from the midline, and DV -8.2mm (lower) and -7.9mm (upper) from dura, with the skull level, giving 2 sites per hemisphere and 4 sites in all. The lesion group received injections of 0.1µl ibotenate (0.03 M) per site with infusion taking place over 2 min and the needle then being left in situ for a further 3 minutes. The control (CeN Sham-lesioned) group underwent the same procedure but with phosphate buffer vehicle being used in place of the neurotoxin.

4.1.1.5 Histological procedures

The rats were killed and perfused and their brains removed and post-fixed in 4% para-formaldehyde as described for the first experiment. After a few hours, the brains were transferred into 20% phosphate-buffered sucrose solution to await further processing. The brains were then mounted in egg yolk as follows in order to stabilise the brains as much as possible before cutting, and thus make it easier to mount the sections on slides. First, the front

third and the cerebellum of each brain were removed, leaving the region of interest. These remaining portions were very thoroughly washed in distilled water in order to remove excess sucrose before being carefully dried with absorbent tissue. Plastic trays containing small wells of about 1.5cm diameter and 1.5cm depth were lightly greased with WD40 and a brain portion placed in each well, front – uppermost. Egg yolk was then pipetted around each brain until it was completely covered, with care being taken to prevent the formation of bubbles. The trays were then placed in a formalin bath for a minimum of 24 h, until the egg yolk had set. The brains, encased in the rubbery egg yolk, were then removed from the wells and cut using a freezing microtome as described for the previous experiment, and the first section in every series mounted on gelatine-coated slides and stained with cresyl violet, again as described for the previous experiment. The second section in every series was immunohistochemically stained as described below using the mouse anti-neuronal nuclei monoclonal antibody, NeuN (Chemicon International, Inc. CAT No. MAB377); this immunoglobulin reacts with most neuronal cell types throughout the nervous system of vertebrates, including those in cerebellum, cerebral cortex, hippocampus and thalamus, but it does not react with Purkinje, mitral and photoreceptor cells, or glia.

First, the sections were sorted into a net basket and washed in 0.1M phosphate-buffered saline (PBS) for 5 min on a flatbed shaker; this was repeated a further 3 times using fresh 0.1M PBS. The sections were then placed in 1% sodium borohydride in distilled water for 15 min in order to remove unreacted formaldehyde from the tissue. They were then washed in 0.1M PBS for 8 x 5 min on a shaker, using fresh 0.1M PBS at each interval, until all the bubbles had disappeared. The sections were then transferred from the net basket into wells in a tissue culture plate containing NeuN primary antibody (1:1000 in Antibody diluting Solution (ADS), 0.4ml per well) and left on a flatbed shaker in the fridge (4°C) overnight. The following day the sections were transferred back into the net basket and washed in 0.1M PBS for 30 min on a flatbed shaker before being transferred into wells in a tissue culture plate containing the first NeuN secondary antibody (anti-mouse IgG, 1:200 in ADS, 0.4ml per well) and incubated at room temperature on a shaker for 60 min. They were then transferred back into the net basket

and washed for a further 4 x 5 min in 0.1M PBS before being transferred into wells in a tissue culture plate containing the second NeuN secondary antibody (mouse PAP, 1:200, 0.4ml per well) and incubated at room temperature on a flatbed shaker for 60 min. Following this, the sections were transferred back into the net basket and washed for 4 x 5 min in 0.1M PBS before being transferred into wells in a tissue culture plate containing diaminobenzidine (DAB) solution (2 tablets in 30ml of distilled water) and allowed to incubate at room temperature on a flatbed shaker until an acceptable depth of stain was achieved - staining occurs primarily in the nucleus of neurons, with lighter staining in the cytoplasm. They were then given a final 4 x 5 min wash in 0.1M PBS before being mounted onto gelatine-coated slides, and allowed to dry thoroughly. The mounted sections were then stained, lightly, with cresyl violet for nissl substance; this combination of NeuN and cresyl violet gives very good structural definition and allows visualisation of gliosis.

The sections were examined using a Leitz "Diaplan" light microscope fitted with a Sony DXC-3000P video camera and connected to a high-resolution monitor, and the extent of excitotoxic damage determined according to the level of neuronal loss and associated gliosis throughout the region of interest. For each rat, sections were selected and schematic representations of the lesions at each of the six given co-ordinates (-1.40, -2.12, -2.56, -3.14, -3.60 and -4.16 mm relative to bregma) were made according to the criteria given in the previous experiment. Photographic representations of some of the lesions were made using a Pixera camera (PVC 100C) connected to a Power Macintosh 7300/200 using the Pixera VCS 1.2 program.

4.1.2 Analysis of data

In this experiment there were 3 stages: the preoperative stage and the postoperative stage, each consisting of 7 daily sessions, and the reversal stage consisting of 10 daily sessions. As described in more detail in the previous experiment, four different measures of performance were used (mean percentage of correct responses, mean reaction time (csec), mean movement

time (csec), and mean post response pause (csec). Performance at these stages was analysed as follows:

4.1.2.1 Analysis of preoperative performance

Given the unexpected differences between pre- and postoperative performance for the Sham-lesioned rats in the previous experiment, and given that this task is fairly novel, it was thought sensible to look at the preoperative performance of the rats in this experiment and compare it to that of the rats in the previous experiment. This is given in **Appendix E**.

4.1.2.2 Analysis of preoperative/postoperative performance

The results of the previous experiment showed that normal rats use the different intensities of cue light in the SFC task to measure how close they are to receiving reward, or, in other words, as an indication of "progress to reward'. The main question was whether or not the BLA- and CeN-lesioned rats would use the changing cue light intensities in the same way. The preoperative and postoperative data for each measure were subjected to 4-way mixed-subject ANOVAs, with group (BLA-lesioned, CeN-lesioned or Sham-lesioned rats) as the between-subjects factor and pre/post (preoperative performance / postoperative performance), schedule fraction (6 levels), and foreperiod (3 levels) as the within-subjects factors. Homogeneity of covariance was tested using the Mauchly sphericity test **(Appendix B)** and the Huynh-Feldt approximation applied to correct for any violations. Effect sizes were calculated for every significant main effect and interaction in order to assess the impact of each factor on performance. Simple main effects **(Appendix C)** were also calculated since all the ANOVAs were within-subjects, thus making the use of post-hoc tests inadvisable. The ANOVA results and effect sizes are given in tabulated form for ease of reference. Some of the more interesting significant main effects and interactions arising out of the ANOVAs were depicted using either graphs or bar charts in order to aid understanding of performance.

As in the previous experiment, planned comparisons were also performed on pairs of conditions from the main effect of schedule fraction for the preoperative Combined group and

for the postoperative Sham-, BLA- and CeN-lesioned groups in order to test various hypotheses as to how the rats interpret the schedule fraction cue lights. These hypotheses are given in more detail in the Analysis of performance section for Experiment A and the results are tabulated for ease of reference.

4.1.2.3 Analysis of reversal performance

The performance of the Sham-, BLA- and CeN-lesioned rats on the mean percentage of Correct Responses measure after the meaning of schedule fraction cues had been reversed for each rat was also analysed. During the reversal stage, those rats for which no cue lights indicated that there were three trials before reward, dim cue lights indicated that there were two trials, and bright cue lights indicated that the current trial would be rewarded found that bright cue lights now indicated that there were three trials before reward, dim cue lights indicated that there were two trials, and no cue lights indicated that the current trial would be rewarded, and vice versa. The reversal stage was intended to ascertain whether lesions of the BLA or CeN impaired re-learning the meaning of the cues and was analysed by subjecting the data for each measure to four sets of 4-way mixed ANOVAs, with Group (BLA-lesioned, CeN-lesioned and Sham-lesioned rats) as the between-subjects factor, and schedule fraction (6 levels), foreperiod (3 levels) and either post/reverse (postoperative performance and reversal performance) or last/first (last day of postoperative performance and first day of reversal performance) or day1/day10 (first day of reversal performance and last day of reversal performance) or last/last (last day of postoperative performance and last day of reversal performance) as the within-subjects factors. The first, and main, set of ANOVAs (Postop – Reversal) was intended to compare performance during the reversal stage with pre-reversal performance, and was carried out on data that had been collected over the ten consecutive days of the reversal stage and then averaged. Since very little difference had been found in the rats' postoperative and preoperative performance, it was decided to use the data from the postoperative stage as the indicator of pre-reversal performance, rather than the data from the preoperative stage, as the former was closer in time to the reversal stage. However, since the reversal stage data consists of data that has been

averaged over the 10 days immediately following the reversal of the cues, it was extremely likely that the rats' performance would not remain stable during this period. For example, it was very possible that the rats' performance would be aversely affected by the reversal of the cues initially, and would subsequently improve over time as they learnt the new meaning of the cues, but it would be impossible to determine whether this was indeed the case from this set of ANOVAs alone. Further sets of ANOVAs were therefore performed, which utilised data from the last postoperative session, and the first and last reversal sessions, and were intended to give a 'snapshot' of what was happening at different times during the reversal process. However, it must be borne in mind that since these sets of ANOVAs were carried out using data from single sessions, the results are necessarily somewhat more 'noisy' and less reliable than the other ANOVAs reported in this experiment, which have used data that has been averaged over several sessions. The second set of ANOVAs (Last day Postop – First day Reversal) therefore compared the data from the last postoperative session with the data from the first reversal session. This set of ANOVAs was intended to provide an insight into the immediate effects on performance of reversing the cues. The third set of ANOVAs (First day Reversal – Last day Reversal) compared the data from the first reversal session with that from the last in order to ascertain whether there was any change in performance over the ten days of the reversal stage. The final set of ANOVAs (Last day Postop – Last day Reversal) compared the data from the last postoperative session with that from the last reversal session. The potential usefulness of this set of ANOVAs is largely dependent on the results of the previous two sets of ANOVAs; for instance, it would be interesting to see if any significant differences in performance found between the last day of the psostoperative stage and the first day of the reversal stage had disappeared by the last day of the reversal stage.

The results of all the sets of ANOVAs are given in tabulated form for ease of reference. The Mauchly sphericity test **(Appendix B)** was used to test for homogeneity of covariance, and effect sizes (given in brackets when referred to in text) were calculated for every significant main effect and interaction in order to assess the impact of each on performance. Only the

results for the main measure of mean percentage of correct responses are fully dealt with in the thesis.

4.1.3 Determination of lesion extent

Finally, since it could be argued that variations in the size and extent of the lesions of the rats included in the both the BLA- and CeN-lesioned groups might obscure potential lesion effects, both the percentage volume of lesion within the desired structures only and the total percentage volume of lesion in each rat were estimated from the schematic representations (see Determination of lesion extent section in previous chapter). Graphs were drawn showing the postoperative performance of the rats with the largest and the smallest BLA lesions (both desired and total), and also of the rats with the largest and the smallest CeN lesions (again, both desired and total), alongside the averaged postoperative performance of all of the Sham-lesioned rats for each of the 5 foreperiods on the main measure of Correct Responses. It was assumed that any data point from the BLA- or CeN-lesioned rats lying outwith the range of +/-2 standard deviations of the averaged sham-lesioned rat data indicated a possible lesion effect.

4.2 Results

4.2.1 Histological analysis

4.2.1.1 BLA lesions

11 animals in all were assigned to the BLA lesion group, and are given below. However, only 7 of the 11 rats were deemed to have reasonable bilateral lesions of the BLA; these are in bold:

01/001	01/010	**01/031**	**01/035**
01/003	01/012	**01/032**	**01/036**
01/005	01/014	**01/033**	

4 animals in all were assigned to the Sham BLA lesion group. These were rats:

01/002	01/009	01/020	01/025

The brains from the BLA lesion group and the Sham BLA lesion group were subjected to histological procedures as described in the Histological procedures section for Experiment A. Schematic representations of the lesions sustained by each rat included in the lesion group were drawn and are given in ***Figure 20***, and also photomicrographs of each lesion at -2.56 with respect to bregma ***(Figure 21)***. Overall, in all the rats, the lateral part of the BLA (LA) was lesioned completely, but there was some sparing of neurons in the BLA itself, which varied in extent between individual rats. These lesions of the BLA can only, therefore, be described as partial. In the anterior part of the BLA there is some damage to dorsal endopiriform nucleus (DEn) in some of the rats, and to overlying parts of globus pallidus in others, but, importantly, the CeN is spared in all cases. Towards the posterior part of the BLA (from -3.14mm from bregma onwards) the lateral part of the BLA (LA) continues to be lesioned, but in several of the rats the other structures are largely intact, i.e. the ventromedial part of the lateral amygdala (LaVM), ventrolateral part of the lateral amygdala (LaVL), the posterior part of the basolateral nucleus (BLP), the posterior part of the basomedial nucleus (BMP) and the ventral part of the basolateral nucleus (BLV).

Discussion of BLA lesion results

As noted above, the BLA lesions were all 'partial' in that there was sparing of neurons in the BLA although the LA was completely lesioned in all cases. This is most likely due to the concentration of the neurotoxin, quinolinate, being too low. It may also be that the large magnocellular neurons found in the BLA, as opposed to the LA, are more resistant to quinolinate. However, it should be noted that this low concentration (0.06 M) gave complete lesions in the practice rats. The partiality of the lesions may also have been due to the co-ordinates being rather too high, so that the main body of the toxin was infused into LA rather than BLA. However, in several rats, the needle track can be seen going into the BLA ***(Figure 21: I1, J2, K1)***. There is some evidence to suggest that the problem may be due to the structure of the brain itself rather than the co-ordinates as such – in several cases the needle tracks appear to curve around the external capsule, and so come into the LA rather than the BLA because of the extra distance covered. It is, of course, unlikely that the needle itself is bending around the

curve of the external capsule; instead the needle is failing to penetrate the external capsule and the brain is shifting laterally in order to allow it to descend. Although an obvious solution to this problem would be to make the co-ordinates slightly deeper and more medial, this would not necessarily prevent the needle from sliding around the external capsule rather than penetrating it, and might perhaps cause CeN to be lesioned as well. However, as discussed below, the CeN appears to be remarkably resilient to neurotoxins. Another possibility would be to enter the brain far more medially and go down through the brain diagonally; this would have the advantage of allowing the needle to meet the external capsule 'straight on', thus making it more likely to penetrate, but would be technically more difficult in that the stereotaxic arms would have to be positioned on the diagonal as well.

4.2.1.2 CeN lesions

11 animals in all were assigned to the CeN lesion group, and are given below. However, for reasons that are given below, only 6 rats were included in the lesion group; these are in bold:

01/006	**01/013**	01/022	**01/027**
01/008	**01/018**	**01/023**	**01/029**
01/011	**01/019**	01/026	

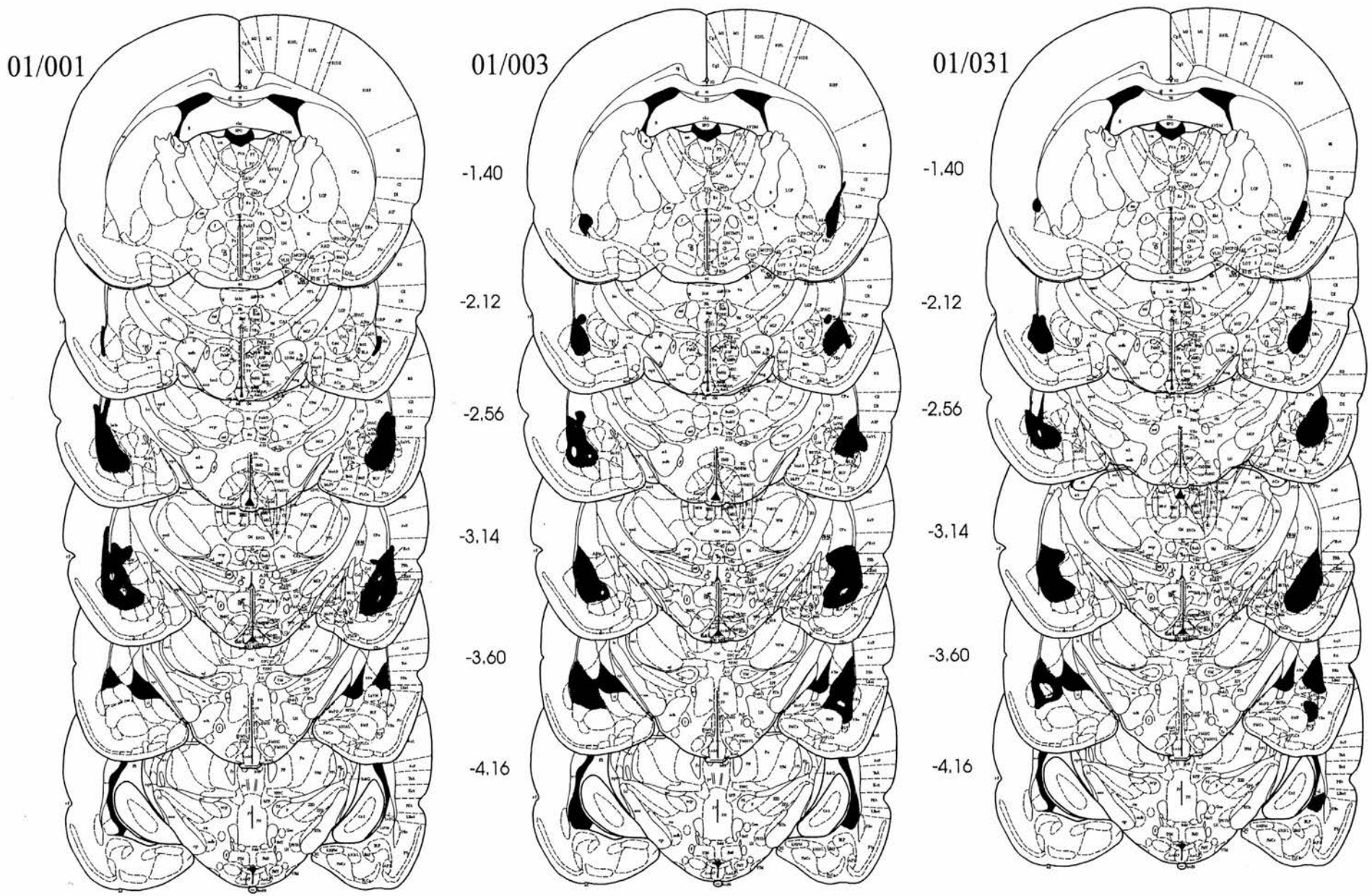
3 animals in all were assigned to the Sham CeN lesion group. These were rats:

01/007	01/017	01/028

Schematic representations of the lesions sustained by each rat included in the lesion group were drawn and are given in ***Figure 22***. However, it should be borne in mind that these schematics are even less representative of the lesions than is usually the case because of the degree of enlargement of the ventricles seen in these rats - it was not possible to incorporate any enlargement into the schematics, which therefore show the size and approximate location of damaged tissue as if there were no enlargement. Enlargement of the ventricles also made it difficult to assess the extent of lesioning in some of the rats. For example, rat 01/027 section 20 left hemisphere (approx. –3.60) and rat 01/029 section 21 (approx. –3.14) have huge ventricles. Photomicrographs of each lesion at -2.56 with respect to bregma are given in ***Figure 23***.

Figure 20: *Schematic representations of bilateral quinolinate lesions of the BLA for the seven rats (01/001, 01/003, 01/031, 01/032, 01/033, 01/035 and 01/036) included in the analysis for Experiment B. The representations are mapped onto diagrams of coronal sections of the rat brain (Paxinos and Watson 1998), with the first coronal section at -1.40mm and the last at -4.16mm with respect to bregma. The locations of the nuclei are given in the right hand hemispheres of the coronal sections, abbreviated as follows:*

ACo anterior cortical amygdaloid nucleus
AHiAL amygdalohippocampal area, anterolateral part
AStr amygdalostriatal transition area
BAOT bed nucleus of the accessory olfactory tract
BLA basolateral amygdaloid nucleus, anterior part
BLP basolateral amygdaloid nucleus, posterior part
BLV basolateral amygdaloid nucleus, ventral part
BMA basomedial amygdaloid nucleus, anterior part
BMP basomedial amygdaloid nucleus, posterior part
BSTIA bed nucleus of the stria terminalis, intra-amygdaloid division
CeC central amygdaloid nucleus, capsular part
CeL central amygdaloid nucleus, lateral division
CeM central amygdaloid nucleus, medial division
CeN central amygdaloid nucleus
Cl claustrum
CxA cortex-amygdala transition zone
DEn dorsal endopiriform nucleus
I intercalated nucleus of the amygdala
IM intercalated amygdaloid nucleus, main part
IMG intra-amygdaloid intra-medullary gray
IPAC interstitial nucleus of the posterior limb of the anterior commissure
LaDL lateral amygdaloid nucleus, dorsolateral part
LaVL lateral amygdaloid nucleus, ventrolateral part
LaVM lateral amygdaloid nucleus, ventromedial part
LSS lateral stripe of the striatum
MeAD medial amygdaloid nucleus, anterodorsal part
MeAV medial amygdaloid nucleus, anteroventral part
MePD medial amygdaloid nucleus, posterodorsal part
MePV medial amygdaloid nucleus, posteroventral part
Pir piriform cortex
PLCo posterolateral cortical amygdaloid nucleusPMCo posteromedial cortical amygdaloid nucleus
SI substantia innominata
VEn ventral endopiriform nucleus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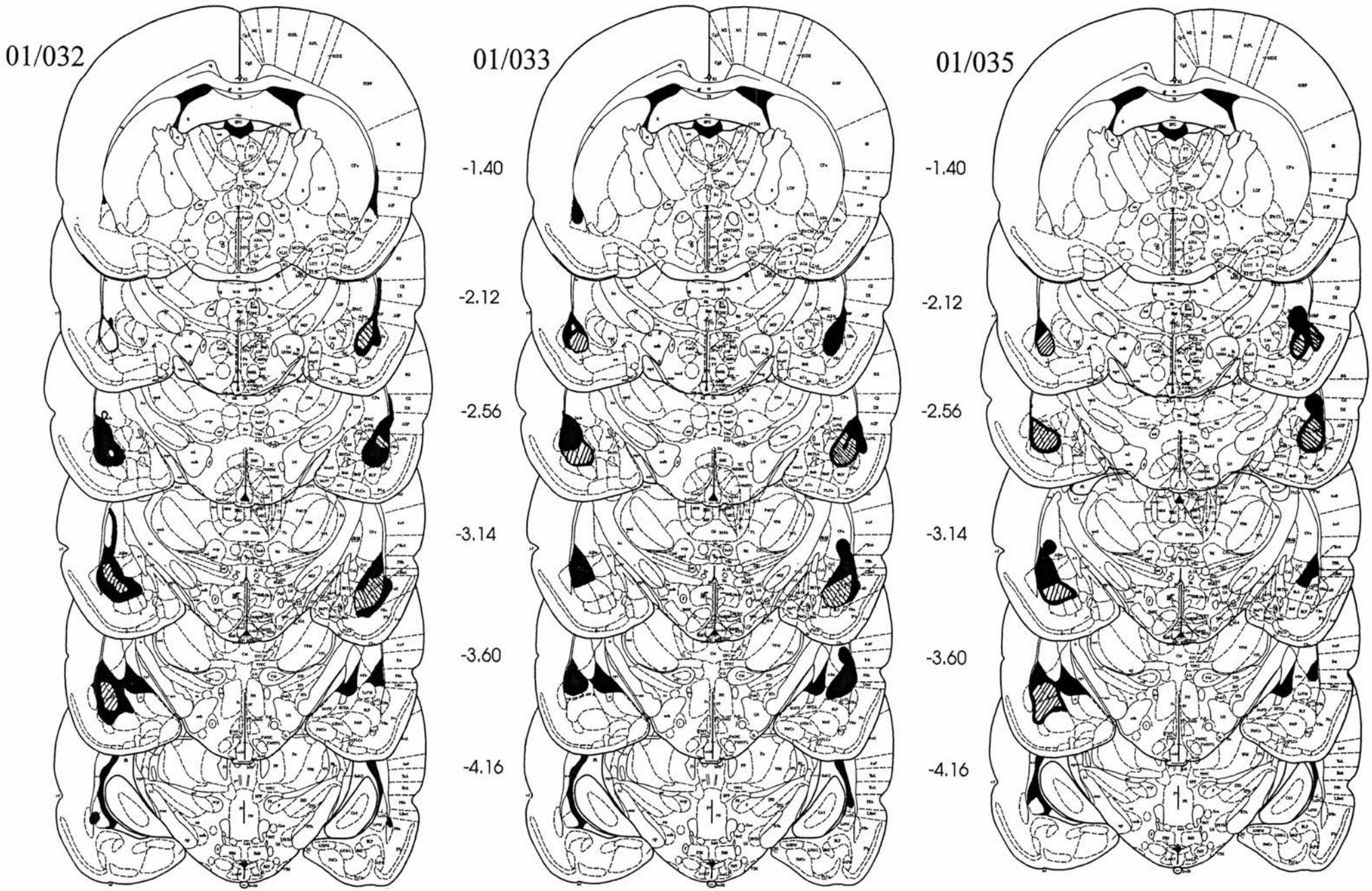
-4.16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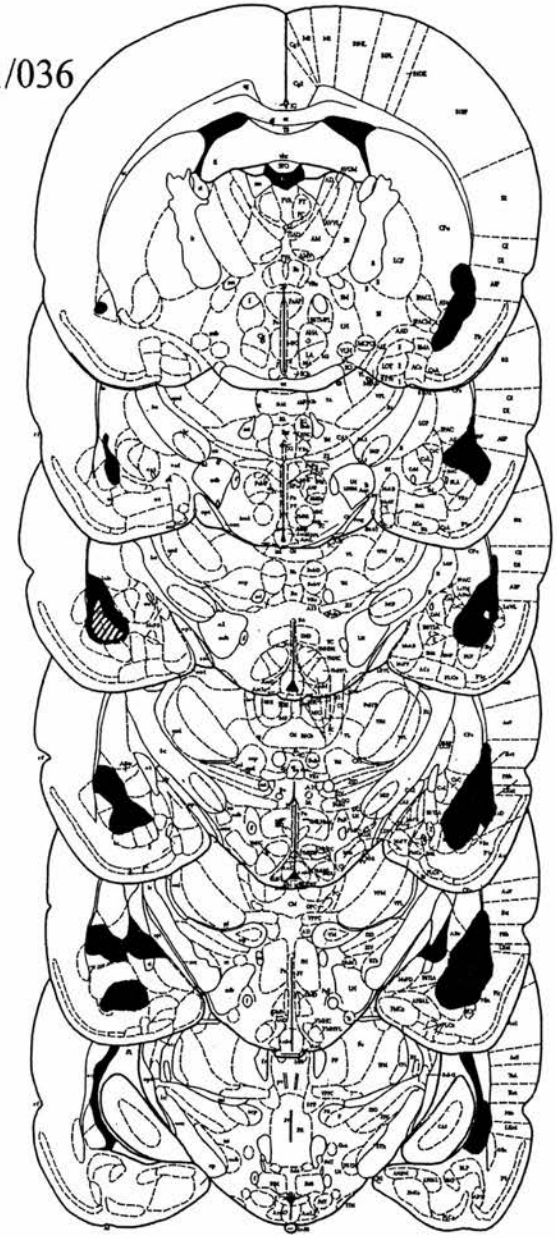
01/036



-1.40

-2.12

-2.56

-3.14

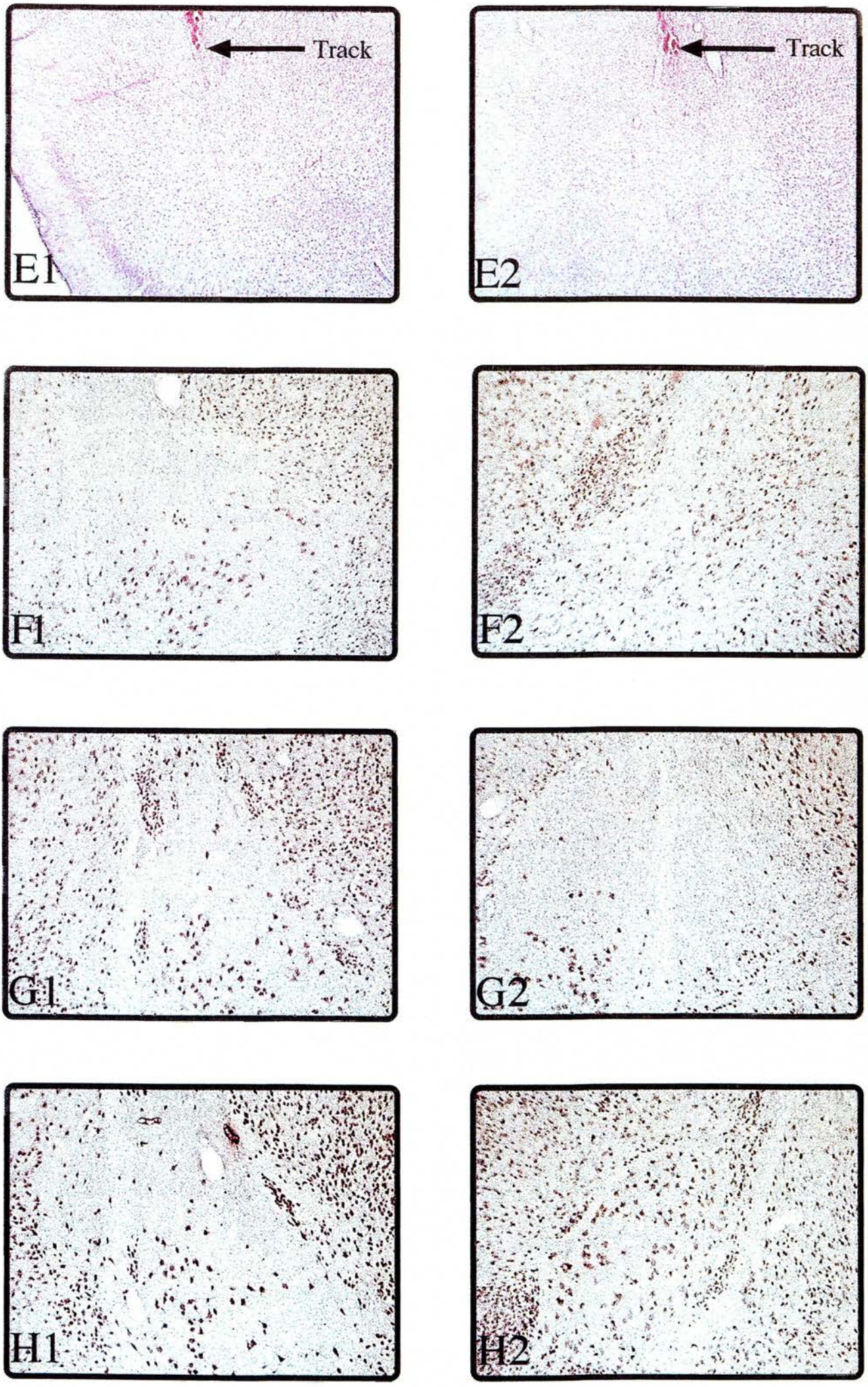
-3.60

-4.16

Figure 21: *Photomicrographs of bilateral quinolinate lesions of the BLA for two practice rats (01/024 and 01/028) and for the seven rats (01/001, 01/003, 01/031, 01/032, 01/033, 01/035 and 01/036) included in the analysis for Experiment B. The photomicrographs are all at -2.56 with respect to bregma, and are taken with a x4 objective. Sections were stained with cresyl violet for nissl substance and with NeuN, which reacts with most neuronal cell types but not with glia (see the Histological procedures section for Experiment A for details of the cresy violet stain and the Histological procedures chapter for Experiment B for details of the NeuN stain).*

It can be seen that whilst surrounding tissue (including the CeN (central amygdaloid nucleus)) is intact, there is extensive gliosis and tissue collapse within the BLA (basolateral amygdaloid nucleus, anterior part).

Rat 01/024	*F1 (left hemisphere)*	
Rat 01/028	*F2 (right hemisphere)*	
Rat 01/001	*F1 (left hemisphere)*	*F2 (right hemisphere)*
Rat 01/003	*G1 (left hemisphere)*	*G2 (right hemisphere)*
Rat 01/031	*H1 (left hemisphere)*	*H2 (right hemisphere)*
Rat 01/032	*I1 (left hemisphere)*	*I2 (right hemisphere)*
Rat 01/033	*J1 (left hemisphere)*	*J2 (right hemisphere)*
Rat 01/035	*K1 (left hemisphere)*	*K2 (right hemisphere)*
Rat 01/0336	*L1 (left hemisphere)*	*L2 (right hemisphere)*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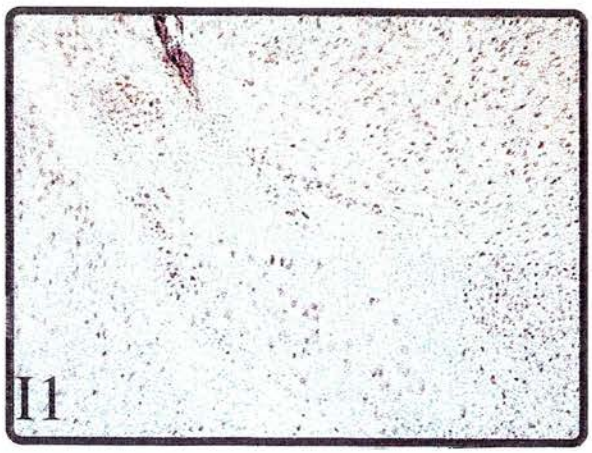
E1
Track
E2
F1
F2
G1
G2
H1
H2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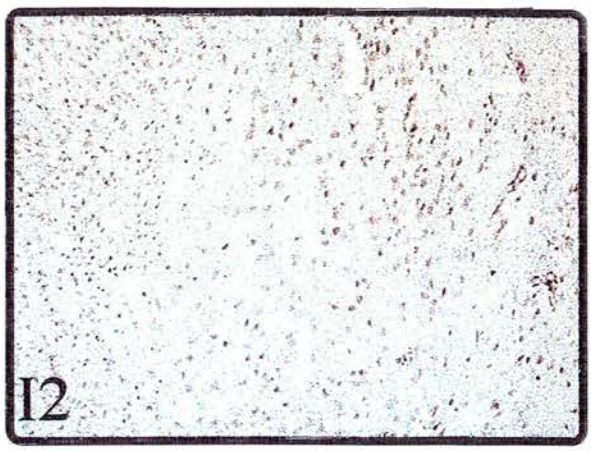
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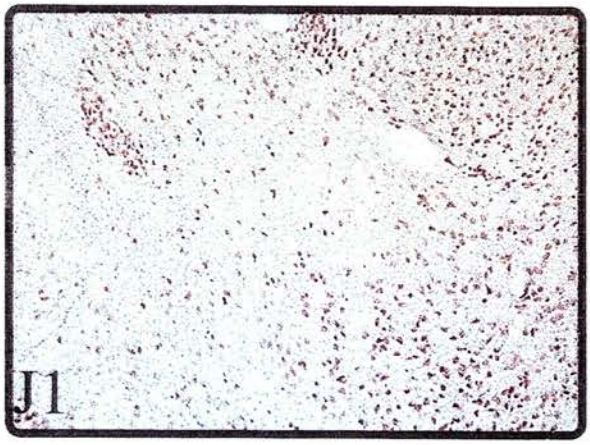
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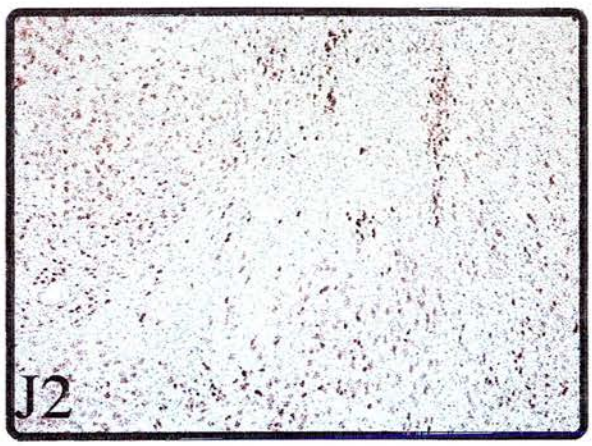
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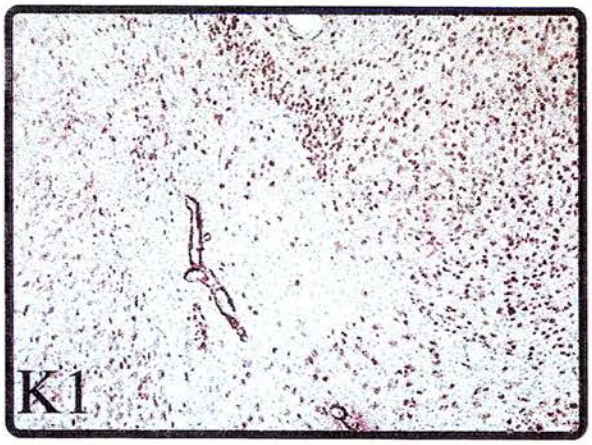
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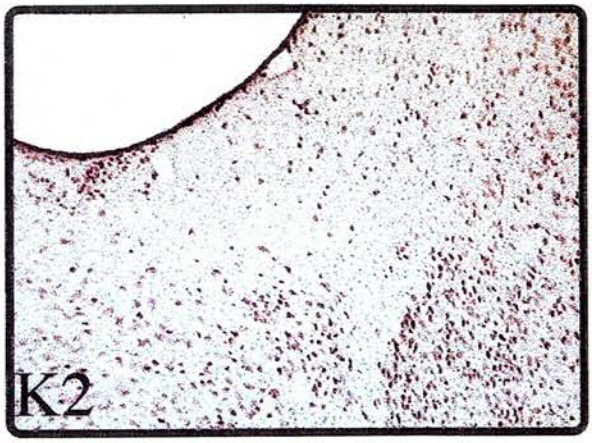
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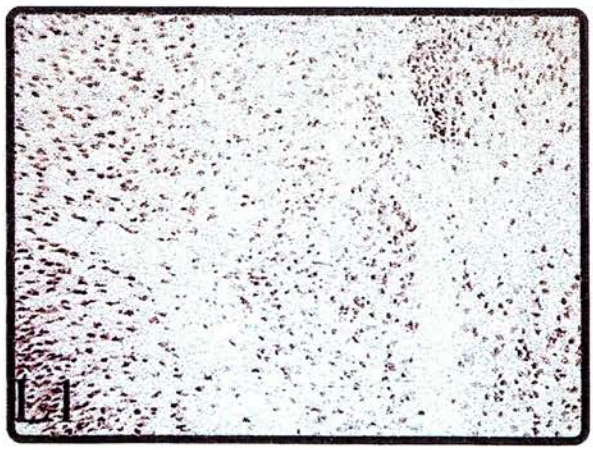
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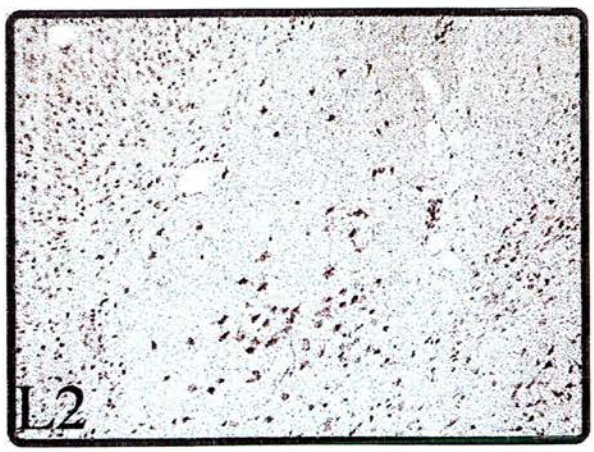

L2

Unfortunately, no bilateral discrete lesions of the CeN were obtained. All of the rats sustained bilateral lesions, but these varied considerably in location, with some being too lateral or medial and others too low relative to the CeN. One rat (01/023, ***Figure 23: B1 and B2***) sustained large bilateral lesions of the CeN that encompassed much of the BLA as well, and five other rats (01/013, 01/018, 01/019, 01/027 and 01/029, ***Figure 23: C1 – H2***) sustained large unilateral lesions of the CeN that also encompassed much of the BLA. The remaining rats (01/006, 01/008, 01/011, 01/022 and 01/026) sustained very little or no damage at all to the CeN, whether bilateral or unilateral, although they all sustained damage to the BLA.

Bilateral lesions of the CeN:

01/023 The CeN is almost completely lesioned in the left hemisphere and completely lesioned in the right hemisphere. The lesioned areas also encompass large parts of the basolateral complex (but not LA).

Partial unilateral lesions of the CeN:

01/013 The CeN is not lesioned in the more anterior part of the right hemisphere, but is lesioned from approximately –2.56mm from bregma back. The lesioned area also encompasses large parts of the basolateral complex (but not LA).

01/018 The CeN is not lesioned in the more anterior part of the left hemisphere, but is lesioned from approximately –2.56mm back. The lesioned area also encompasses large parts of the basolateral complex (but not LA).

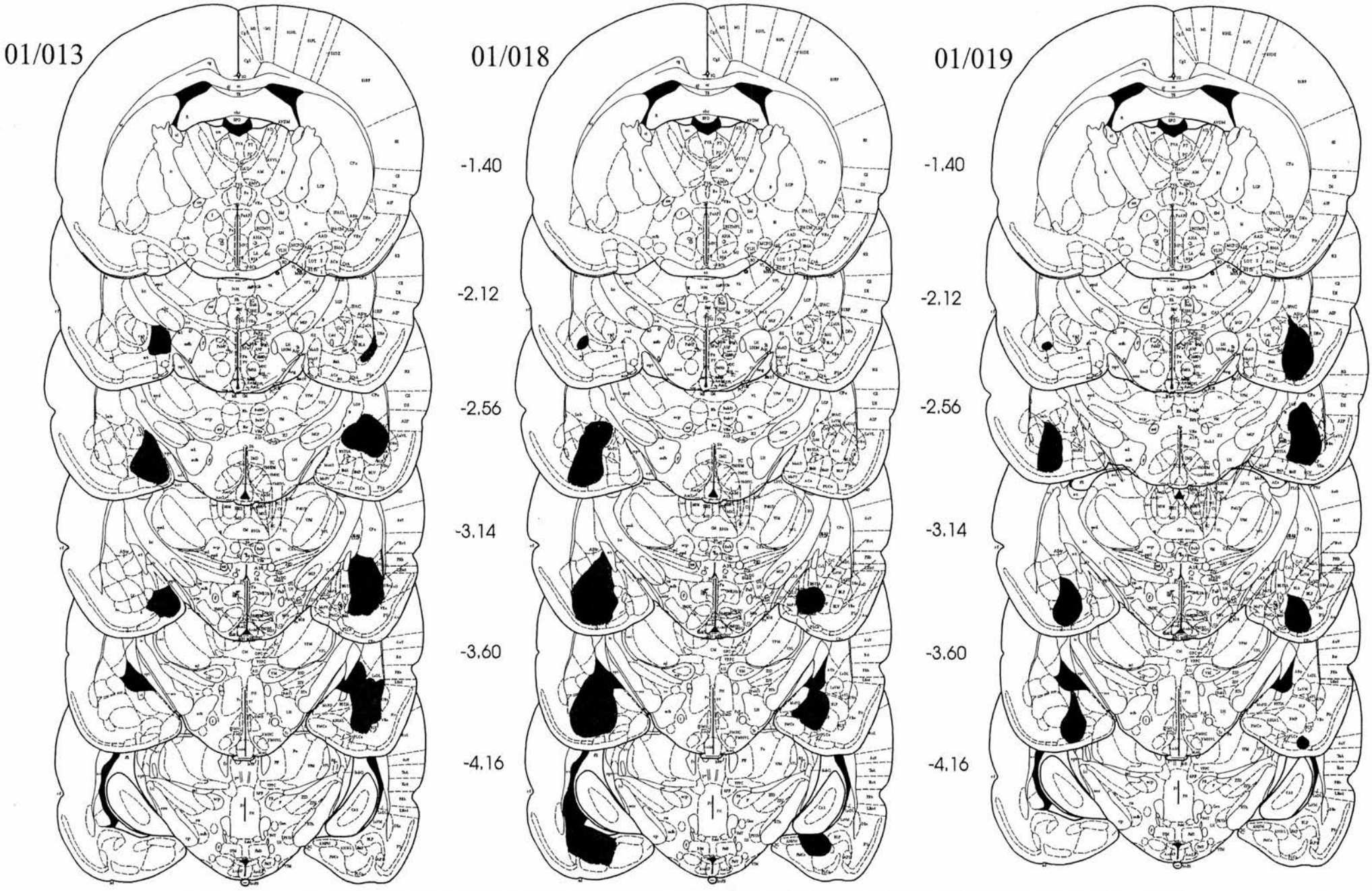
01/019 The CeN is not lesioned in the more anterior part of the right hemisphere, but the CeC and CeL subdivisions of the CeN are lesioned from approximately –2.56mm to –3.14mm. The lesioned area also encompasses large parts of the basolateral complex (but not LA). The lesioned area is therefore not quite as extensive as in 01/013 and 01/018.

01/027 The CeN is lesioned throughout its entire extent in the left hemisphere, from approximately –1.40mm to –3.60mm. The lesioned area also encompasses large parts of the basolateral complex (but not LA).

01/029 The CeN is not lesioned in the anterior part of the left hemisphere, but is lesioned from approximately –3.14mm back. In the right hemisphere, only the CeM division of CeN is lesioned in the anterior part, and further back, the CeL and the CeM divisions. The lesioned areas encompass most of the basolateral complex.

Figure 22: *Schematic representations of bilateral ibotenate lesions of the CeN for the six rats (01/013, 01/018, 01/019, 01/023, 01/027 and 01/029) included in the analysis for Experiment B. The representations are mapped onto diagrams of coronal sections of the rat brain (Paxinos and Watson 1998), with the first coronal section at -1.40mm and the last at -4.16mm with respect to bregma. The locations of the nuclei are given in the right hand hemispheres of the coronal sections, abbreviated as follows:*

ACo	*anterior cortical amygdaloid nucleus*
AHiAL	*amygdalohippocampal area, anterolateral part*
AStr	*amygdalostriatal transition area*
BAOT	*bed nucleus of the accessory olfactory tract*
BLA	*basolateral amygdaloid nucleus, anterior part*
BLP	*basolateral amygdaloid nucleus, posterior part*
BLV	*basolateral amygdaloid nucleus, ventral part*
BMA	*basomedial amygdaloid nucleus, anterior part*
BMP	*basomedial amygdaloid nucleus, posterior part*
BSTIA	*bed nucleus of the stria terminalis, intra-amygdaloid division*
CeC	*central amygdaloid nucleus, capsular part*
CeL	*central amygdaloid nucleus, lateral division*
CeM	*central amygdaloid nucleus, medial division*
CeN	*central amygdaloid nucleus*
Cl	*claustrum*
CxA	*cortex-amygdala transition zone*
DEn	*dorsal endopiriform nucleus*
I	*intercalated nucleus of the amygdala*
IM	*intercalated amygdaloid nucleus, main part*
IMG	*intra-amygdaloid intra-medullary gray*
IPAC	*interstitial nucleus of the posterior limb of the anterior commissure*
LaDL	*lateral amygdaloid nucleus, dorsolateral part*
LaVL	*lateral amygdaloid nucleus, ventrolateral part*
LaVM	*lateral amygdaloid nucleus, ventromedial part*
LSS	*lateral stripe of the striatum*
MeAD	*medial amygdaloid nucleus, anterodorsal part*
MeAV	*medial amygdaloid nucleus, anteroventral part*
MePD	*medial amygdaloid nucleus, posterodorsal part*
MePV	*medial amygdaloid nucleus, posteroventral part*
Pir	*piriform cortex*
PLCo	*posterolateral cortical amygdaloid nucleus*
PMCo	*posteromedial cortical amygdaloid nucleus*
SI	*substantia innominata*
VEn	*ventral endopiriform nucleus*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-2.56
-3.14
-3.60
-4.16

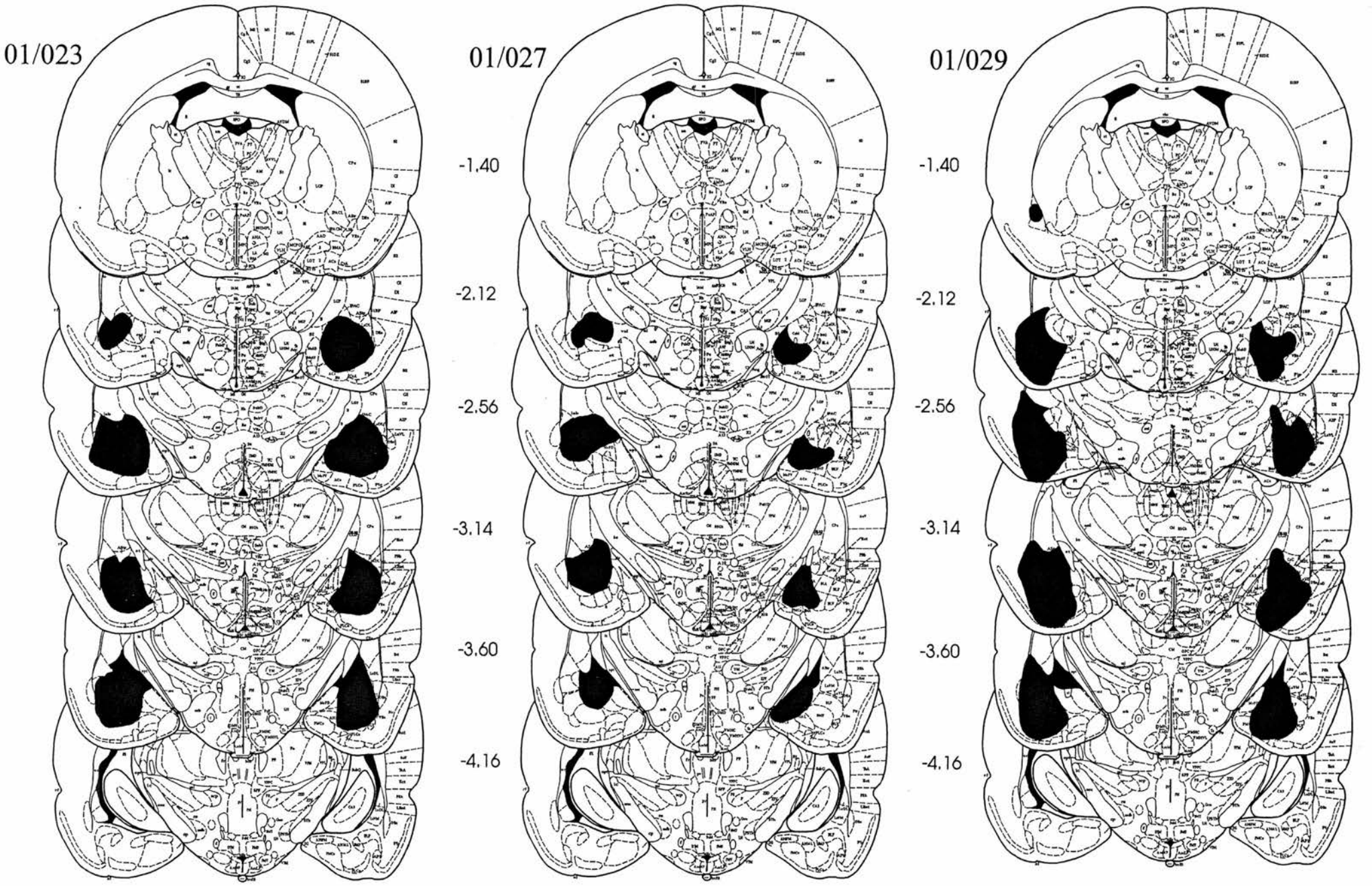
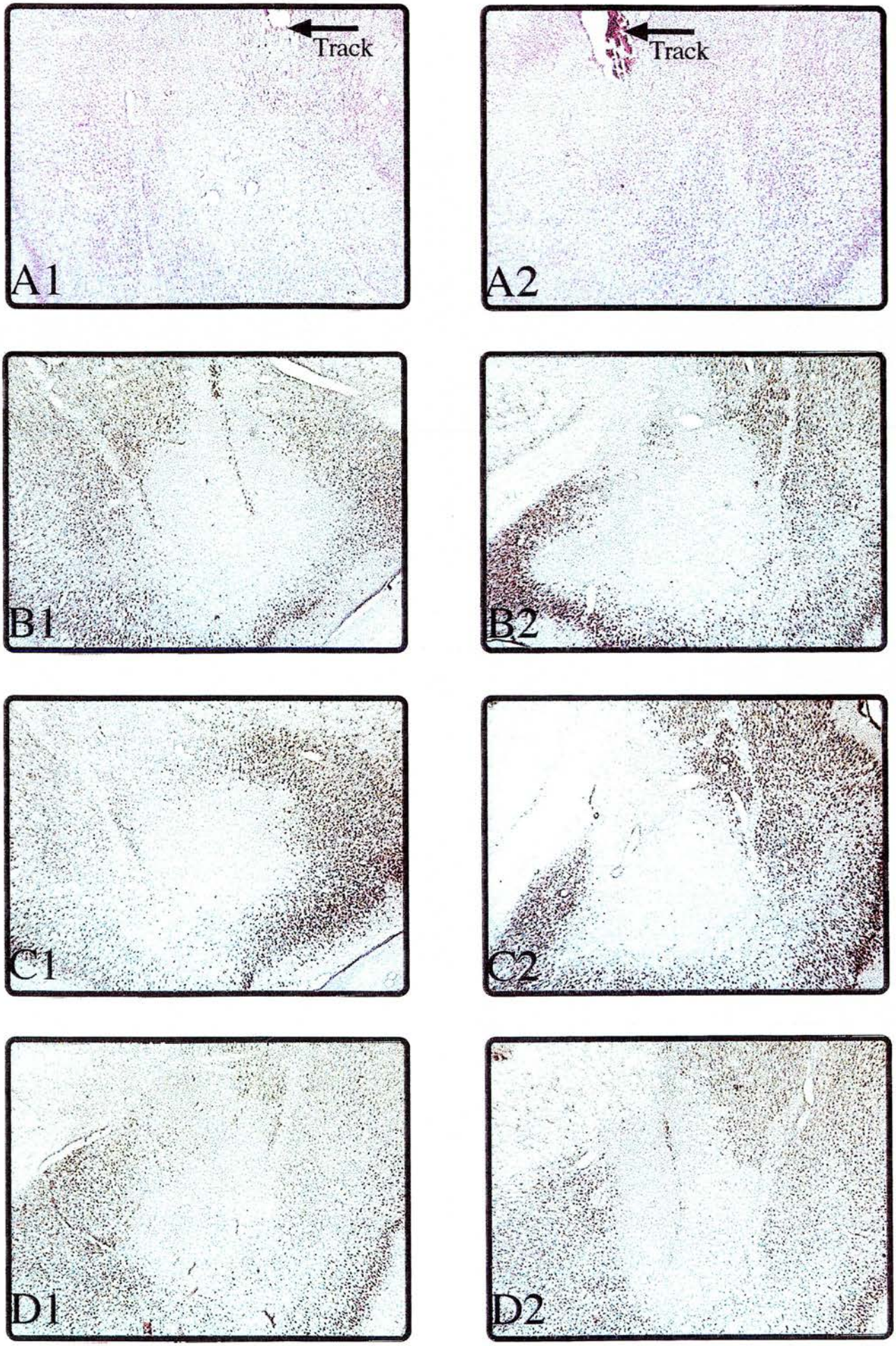
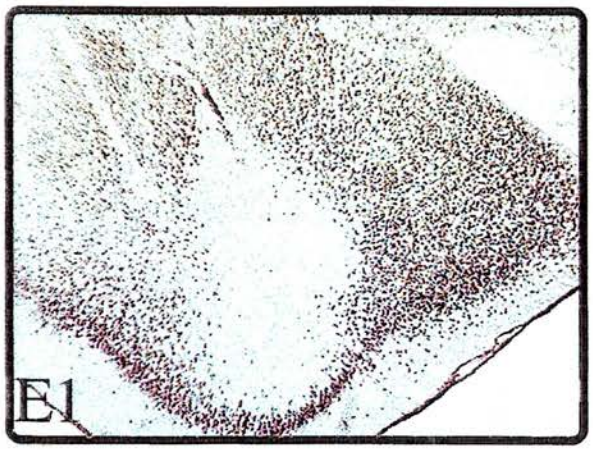

01/023
01/027
01/029
-1.40
-2.12
-2.56
-3.14
-3.60
-4.16

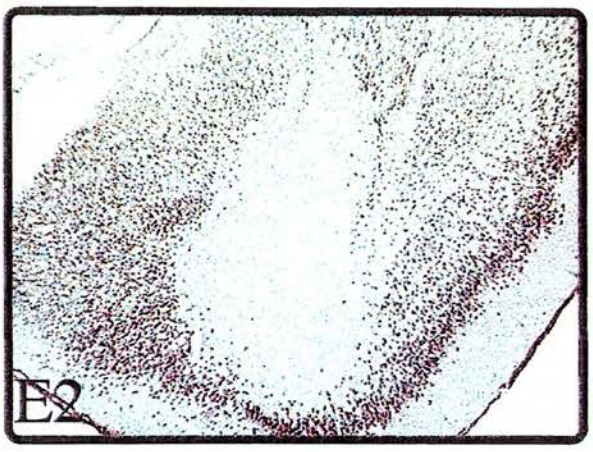
Figure 23: *Photomicrographs of bilateral ibotenate lesions of the CeN for two practice rats (00/831 and 00/836) and for the six rats (01/013, 01/018, 01/019, 01/023, 01/027 and 01/029) included in the analysis for the second SFC task experiment. The photomicrographs are all at -2.56 with respect to bregma, and are taken with a x4 objective. Sections were stained with cresyl violet for nissl substance and with NeuN, which reacts with most neuronal cell types but not with glia (see the Histological procedures section for Experiment A for details of the cresy violet stain and the Histological procedures chapter for Experiment B for details of the NeuN stain).*

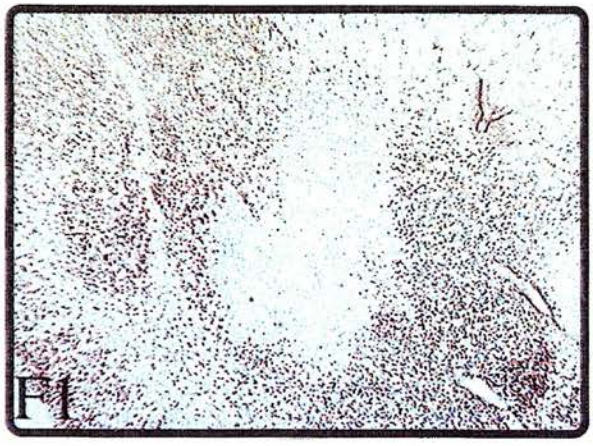
It can be seen that whilst surrounding tissue (including the CeN (central amygdaloid nucleus)) is intact, there is extensive gliosis and tissue collapse within the BLA (basolateral amygdaloid nucleus, anterior part).

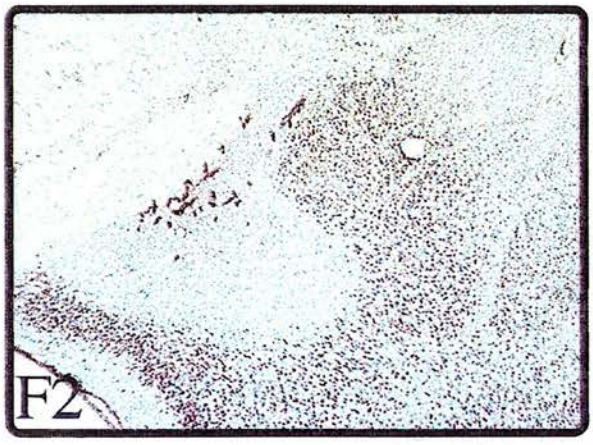
Rat 00/831	*A1 (left hemisphere)*	
Rat 00/836	*A2 (right hemisphere)*	
Rat 01/023	*B1 (left hemisphere)*	*B2 (right hemisphere)*
Rat 01/013	*C1 (left hemisphere)*	*C2 (right hemisphere)*
Rat 01/018	*D1 (left hemisphere)*	*D2 (right hemisphere)*
Rat 01/019	*E1 (left hemisphere)*	*E2 (right hemisphere)*
Rat 01/027	*F1 (left hemisphere)*	*F2 (right hemisphere)*
Rat 01/029	*H1 (left hemisphere)*	*H2 (right hemisphere)*


Track
A1
Track
A2
B1
B2
C1
C2
D1
D2


E1


E2


F1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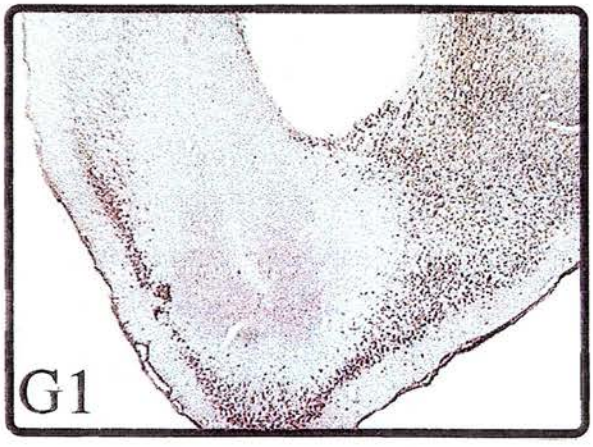
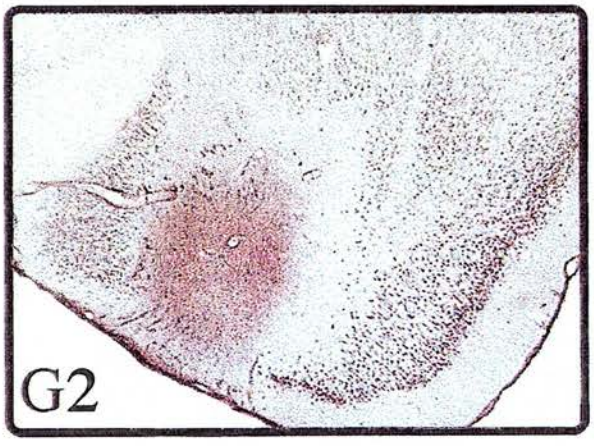
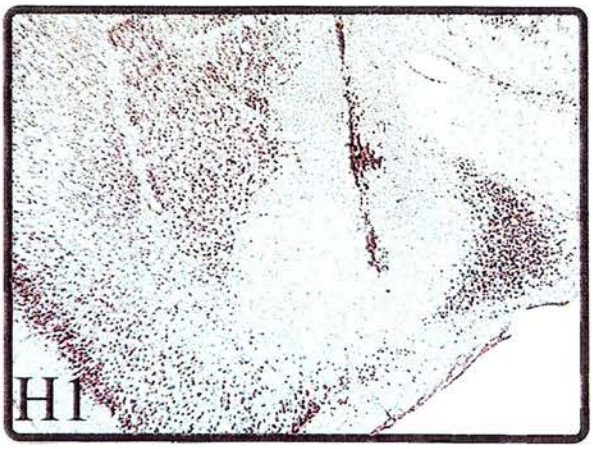
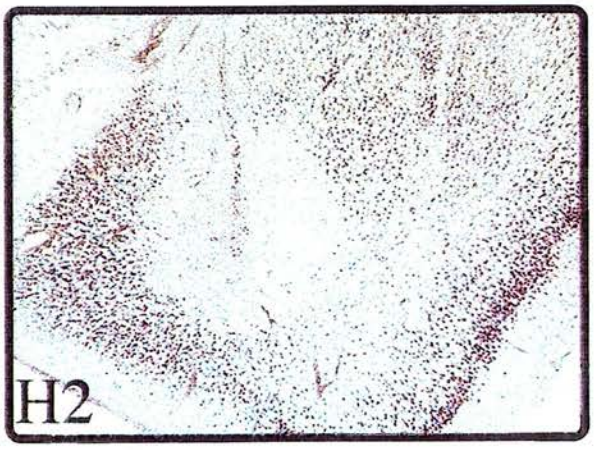

G1


G2


H1


H2

Discussion of CeN lesion results

Failure to achieve reasonable bilateral lesions of the CeN in this group of rats can be attributed to three main reasons: difficulty in locating the lesions to the CeN, difficulty in actually penetrating the CeN, and the size of lesions:

Location: Despite extensive piloting, the lesions varied considerably in placement, with some being too lateral / medial (i.e. the lesion in one hemisphere being too lateral whilst that in the other hemisphere is too medial relative to CeN) and others too low relative to CeN. In some of the rats the area of lesion also seems to start rather too far back, missing the onset of CeN. Laterality / mediality of the lesions in some of the rats (e.g. 01/013 and 01/026) can be ascribed to difficulty in locating bregma - many rats in this cohort had very 'messy' intersections between the coronal and sagittal sutures. It would perhaps have been better to have taken the co-ordinates from the mid-line sinus, but this would have meant drilling away another large, central, area of skull in addition to the injection holes on each side of the skull. One possibility, however, would be to use the hand-held drill to open up a large hole over the midline sinus, and to use a stereotaxic drill, which would make much smaller holes, for the injection holes. In other cases (e.g. 01/008, 01/011 and 01/022) the lesioned area is too low. This is most probably due to the co-ordinates themselves being too low, but in some cases (Practice rat 00/836 section , 01/009 section 16 left hemisphere, and section 16 right hemisphere) needle track marks can be seen coming into CeN or just deflected by it, which suggests that, at least in these cases, the co-ordinates are reasonable. In some of the rats (e.g. 01/008 and 01/018) the area of lesion also seems to start rather too far back, missing the onset of CeN. This may be due to experimental error in setting the co-ordinates, but may also reflect variability between rats as to where BLA and CeN are located. A possibility would be to take the co-ordinates from the inter-aural line rather than bregma, which would perhaps afford more consistency between rats. Likewise, positioning the incisor bar at –3.3mm (approximately level skull) rather than attempting to achieve level skull with each rat by adjusting bregma and lambda until equal would perhaps also afford more consistency between rats. However, on the whole, it would seem wise to re-define the co-ordinates.

Difficulty in actually penetrating the CeN: It was described above how, in some of the BLA lesions, the needle came down the outside of the external capsule rather than penetrating through it. A similar problem was encountered with these lesions: the CeN is surrounded by what appears to be a very resilient fibre bundle (see *Figure 13*), which not only protects the nucleus from physical penetration by the needle, but also from infiltration by the neurotoxin. In several rats, it is evident that the needle has failed to penetrate this fibre bundle but has instead skirted around it and entered the BLA instead (see 01/006 section 20 right hemisphere but flipped, 01/011 section 18 right hemisphere, and 01/019 section 14 right hemisphere), whilst in other rats there is evidence of toxin damage to the areas surrounding the CeN, which is spared (see 01/019 section 17 right hemisphere, and 01/026 section 17 left hemisphere).

Size of the lesions: The lesions were all very large and showed very little sparing of neurons, suggesting that the concentration of the neurotoxin used, ibotenic acid, was too high. However, both the concentration and the quantity of ibotenic acid used in this study is far lower than that reported by others such as Parkinson et al (2000). One possibility is that the greater extent of the lesions in this experiment could be due to a longer time elapsing between surgery and sacrifice - the practice rats, which had smaller lesions, received the same quantity and concentration of toxin as the experimental rats but were killed after 3 days rather than after a month. This possibility could be investigated by infusing a given amount of ibotenic acid into the brains of a group of rats and then sacrificing individual rats every few days over the course of a month. Whatever the outcome of such a study, further reduction in the concentration / quantity of ibotenic acid is necessary. Another possibility is that the size of lesion co-varies with the depth of anaesthesia and type of anaesthetic – many of the rats were resistant to barbiturate, and had to be 'topped up' with halothane during surgery. It is possible that those rats which were more lightly anaesthetised, or which recovered more quickly may have sustained larger lesions. Likewise, larger lesions may be sustained when halothane is used as the anaesthetic, since animals usually recover from it more quickly than they do from barbiturate.

Overall, it is most likely that most of the problems are due to the lesion co-ordinates being slightly too low and the propensity of the needle to skirt around CeN, thus bringing it into

or close to BLA. This, combined with too great a concentration of toxin, has lead to these disappointing results.

4.2.2 Results of ANOVAs on preoperative/postoperative performance

4.2.2.1 Correct Responses

Main effect / interaction	F value	P value	Effect size
SF	F (4.95, 84.18) = 55.26	p = 0.000	16.36 %
FP	F (1.35, 22.93) = 152.5	p = 0.000	75.50 %
Pre/Post	F (1.00, 17.00) = 4.64	p = 0.046	00.43 %
Group	F (2, 17) = 1.46	p = 0.259	
SF*FP	F (6.10, 103.79) = 2.79	p = 0.014	00.96 %
SF*Pre/Post	F (4.04, 68.65) = 1.95	p = 0.112	
SF*Group	F (9.90, 84.18) = 2.16	p = 0.028	01.28 %
FP*Pre/Post	F (1.70, 28.90) = 0.01	p = 0.985	
FP*Group	F (2.70, 22.93) = 1.04	p = 0.388	
Pre/Post*Group	F (2.00, 17.00) = 4.16	p = 0.034	00.78 %
SF*FP*Pre/Post	F (10.00, 170.00) = 2.33	p = 0.013	00.54 %
SF*FP*Group	F (12.21, 103.79) = 1.00	p = 0.452	
SF*Pre/Post*Group	F (8.08, 68.65) = 1.12	p = 0.363	
FP*Pre/Post*Group	F (3.40, 28.90) = 1.81	p = 0.162	
SF*FP*Pre/Post*Group	F (20.00, 170.00) = 0.69	p = 0.831	

Table 8: *Main effects, interactions and effect sizes resulting from the 4-way ANOVA on the mean percentage of correct responses measure which compares the different lesion groups' (Group) preoperative and postoperative performance (Pre/Post) according to schedule fraction (SF) and foreperiod (FP)*

This ANOVA produced no significant 4-way interactions, one significant 3-way interaction (SF*FP*pre/post), three significant 2-way interactions, of which only two (SF*group and pre/post*group) need, theoretically, be considered since the other (SF*FP) is superseded by the 3-way interaction, and three significant main effects (SF, FP and pre/post). Importantly, there is no significant main effect of Group. The three significant main effects, again theoretically, need not be considered since they are superseded by the interactions; however, the effect sizes of the latter are very small compared to those for the main effects. It can be seen from ***Table 8*** that foreperiod, with an effect size of 75.50%, most dramatically influences performance, whilst schedule fraction has a much smaller influence (16.36%). These results are very similar to the preoperative findings. The interactions and the pre/post main effect have very little influence on performance since their effect sizes are all extremely small; however the pre/post*group

interaction (0.78%) is of obvious interest even though performance is collapsed across schedule fraction and foreperiod, and is therefore graphed out and discussed below. (The 3-way interaction is also graphed out and discussed in **Appendix F** in order to get some kind of overall picture of what is happening.)

Pre/Post * Group interaction

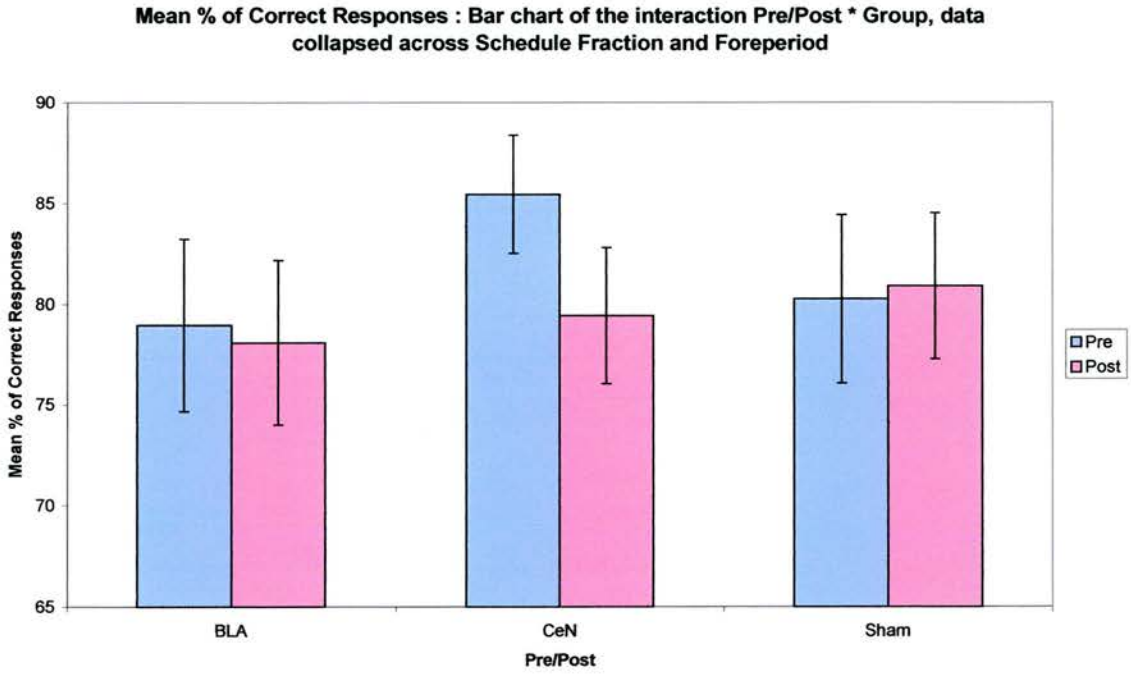


Figure 24: *Bar chart of the interaction pre/post*group, data collapsed across schedule fraction and foreperiod, showing the mean (±se) percentage of correct responses made preoperatively and postoperatively by the BLA-lesioned, CeN-lesioned and Sham-lesioned rats. Blue bars indicate preoperative performance and pink bars indicate postoperative performance.*

Figure 24 shows that the mean percentage of correct responses made by the BLA-lesioned and Sham-lesioned rats is very similar preoperatively, and remained much the same postoperatively. The CeN-lesioned rats, however, made more correct responses preoperatively than did the other two groups, though this decreased to about the same level postoperatively. The interaction would therefore appear to be due to differences between the groups in the preoperative stage. Since all the rats were assumed to be homogeneous preoperatively, boxplots were constructed to see if there were any outliers or extreme values in the preoperative data for the CeN-lesion group. This proved to be the case: 2 extreme values were found at SF1/2 FP300, and 1 extreme value was found at SF2/3 FP100, whilst single outlier values were found SF1/1 FP500, SF2/3

FP500 and 2 outlier values at SF3/3 FP500. This compares with four outliers and no extreme values in the BLA-lesion group and four outliers and two extreme values in the Sham-lesion group. The incident of extreme values is therefore slightly higher in the CeN-lesion group, possibly because there are only 6 rats in this group, compared to 7 rats in the BLA-lesion and Sham-lesion groups.

4.2.2.2 Reaction Time

Main effect / interaction	F value	P value	Effect size
SF	F (3.42, 58.15) = 10.07	p = 0.000	40.58 %
FP	F (1.63, 27.69) = 13.57	p = 0.000	22.82 %
Pre/Post	F (1.00, 17.00) = 2.88	p = 0.108	
Group	F (2, 17) = 0.24	p = 0.793	
SF*FP	F (7.09, 120.58) = 2.99	p = 0.006	06.84 %
SF*Pre/Post	F (3.74, 63.55) = 2.01	p = 0.108	
SF*Group	F (6.84, 58.15) = 1.39	p = 0.228	
FP*Pre/Post	F (2.00, 34.00) = 1.18	p = 0.320	
FP*Group	F (3.26, 27.69) = 0.23	p = 0.887	
Pre/Post*Group	F (2.00, 17.00) = 1.44	p = 0.264	
SF*FP*Pre/Post	F (6.87, 116.88) = 0.96	p = 0.461	
SF*FP*Group	F (14.19, 120.58) = 0.69	p = 0.781	
SF*Pre/Post*Group	F (7.48, 63.55) = 1.09	p = 0.380	
FP*Pre/Post*Group	F (4.00, 34.00) = 0.14	p = 0.964	
SF*FP*Pre/Post*Group	F (13.75, 116.88) = 0.36	p = 0.982	

***Table 9**: Main effects, interactions and effect sizes resulting from the 4-way ANOVA on the mean reaction time measure which compares the different lesion group's (Group) preoperative and postoperative performance (Pre/Post) according to schedule fraction (SF) and foreperiod (FP)*

This ANOVA produced no significant 4-way interactions, no significant 3-way interactions, one significant 2-way interaction (SF*FP) and two significant main effects (SF and FP). There is no significant main effect of Group. Given the anomalous preoperative data (see ***Figure 34 in* Appendix E**), the 2-way interaction was graphed in order to see what was happening postoperatively; this is given in **Appendix F**.

4.2.2.3 Movement Time

This ANOVA produced no significant 4-way interactions, no significant 3-way interactions, two significant 2-way interactions (SF*FP and SF*pre/post) and three significant main effects SF, FP and pre/post) which are superseded by the interactions. The main effect of Group was not

significant. It can be seen from ***Table 10*** that schedule fraction, with an effect size of 82.87%, most strongly influences performance, whilst foreperiod (02.06 %) has a very small influence. These results are very similar to the preoperative findings. The pre/post main effect and the interactions have a very little influence on performance since their effect sizes are all small; however the SF*pre/post interaction is of some interest, and is therefore given in **Appendix F**.

Main effect / interaction	F value	P value	Effect size
SF	F (2.35, 40.00) = 41.82	p = 0.000	82.87 %
FP	F (2.00, 34.00) = 35.80	p = 0.000	02.06 %
Pre/Post	F (1.00, 17.00) = 41.69	p = 0.000	05.02 %
Group	F (2, 17) = 0.22	p = 0.808	
SF*FP	F (4.00, 68.08) = 7.94	p = 0.000	01.75 %
SF*Pre/Post	F (2.84, 48.24) = 14.24	p = 0.000	02.84 %
SF*Group	F (4.71, 40.00) = 0.43	p = 0.816	
FP*Pre/Post	F (1.62, 27.53) = 1.71	p = 0.203	
FP*Group	F (4.00, 34.00) = 1.38	p = 0.261	
Pre/Post*Group	F (2.00, 17.00) = 2.22	p = 0.140	
SF*FP*Pre/Post	F (5.03, 85.57) = 1.15	p = 0.339	
SF*FP*Group	F (8.01, 68.08) = 1.98	p = 0.062	
SF*Pre/Post*Group	F (5.67, 48.24) = 1.81	p = 0.121	
FP*Pre/Post*Group	F (3.24, 27.53) = 0.66	p = 0.591	
SF*FP*Pre/Post*Group	F (10.07, 85.57) = 1.13	p = 0.179	

Table10: *Main effects, interactions and effect sizes resulting from the 4-way ANOVA on the mean movement time measure which compares the different lesion groups' (Group) preoperative and postoperative performance (pre/post) according to schedule fraction (SF) and foreperiod (FP)*

4.2.2.4 Post Response Pause

Main effect / interaction	F value	P value	Effect size
SF	F (2.10, 35.62) = 39.65	p = 0.000	87.99 %
FP	F (1.95, 33.16) = 2.06	p = 0.144	
Pre/Post	F (1.00, 17.00) = 24.98	p = 0.000	01.81 %
Group	F (2, 17) = 0.80	p = 0.464	
SF*FP	F (3.44, 58.55) = 0.25	p = 0.884	
SF*Pre/Post	F (1.55, 26.36) = 2.20	p = 0.140	
SF*Group	F (4.19, 35.62) = 0.73	p = 0.580	
FP*Pre/Post	F (2.00, 34.00) = 0.75	p = 0.482	
FP*Group	F (3.90, 33.16) = 2.35	p = 0.076	
Pre/Post*Group	F (2.00, 17.00) = 7.08	p = 0.006	01.02 %
SF*FP*Pre/Post	F (3.33, 56.69) = 1.84	p = 0.144	
SF*FP*Group	F (6.89, 58.55) =1.39	p = 0.227	
SF*Pre/Post*Group	F (3.10,26.36) =3.89	p = 0.019	01.91 %
FP*Pre/Post*Group	F (4.00, 34.00) = 1.83	p = 0.146	
SF*FP*Pre/Post*Group	F (6.67, 56.69) = 0.52	p = 0.809	

Table 11: *Main effects, interactions and effect sizes resulting from the 4-way ANOVA on the mean post response pause measure which compares the different lesion groups' (Group) preoperative and postoperative performance (pre/post) according to schedule fraction (SF) and foreperiod (FP)*

This ANOVA produced no significant 4-way interactions, one significant 3-way interaction (SF*pre/post*group), one significant 2-way interaction (pre/post*group) and two significant main effects (SF and pre/post). There is no significant main effect of Group. It can be seen from ***Table 11*** that schedule fraction, with an effect size of 87.99%, is much the strongest influence on performance, which is in accordance with the preoperative data. The pre/post main effect and the interactions have very little influence on performance since their effect sizes are very small, but the SF*pre/post*group interaction is of obvious interest and is therefore graphed out and discussed below:

SF * Pre/Post * Group interaction

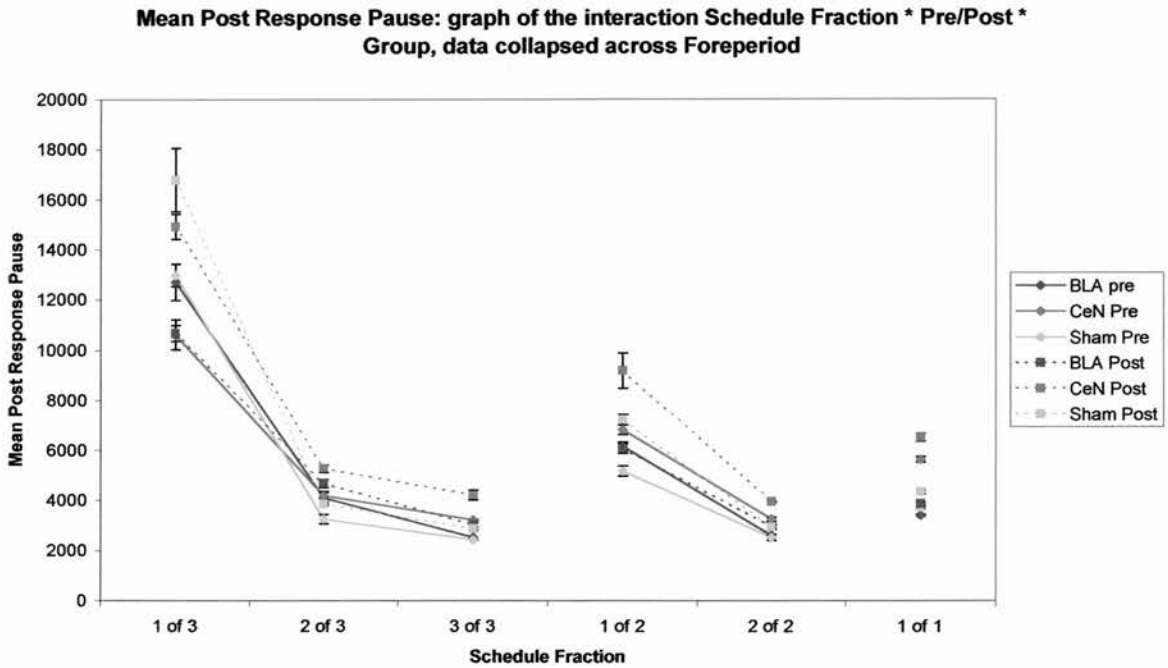


Figure 25: *Graph of the interaction SF*pre/post*group, data collapsed across foreperiod, showing mean (±se) preoperative and postoperative post response pause for the BLA-lesioned, CeN-lesioned and Sham-lesioned rats. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Colour of line indicates group; solid lines indicate preoperative performance and dotted lines indicate postoperative performance*

This interaction effect, though small (1.91 %), is interesting because it involves both pre/post and Group, indicating that there are differences in performance between the different groups after surgery. It is, however, very difficult to ascertain where the interaction lies from the interaction graph. It is clear that, for all the groups, mean post response pause decreases as the

rats work their way through the work schedules towards reward. For both the Sham-lesioned and the CeN-lesioned groups, the mean post response pause is longer postoperatively than it is preoperatively, and this difference performance is most evident at schedule fractions 1/3 and 1/2. Overall, the difference between pre- and postoperative performance is greatest for the CeN-lesioned group, and may result from the lesion, but the difference in performance by the Sham-lesioned rats is harder to explain. For the BLA-lesioned group, pre- and postoperative performance seems to vary according to schedule fraction: at SF1/3 the mean post response pause is shorter postoperatively than it is preoperatively, at SF2/3 and again at SF3/3 it is longer postoperatively. At schedule fractions 1/2, 2/2 and 1/1, postoperative performance is the same or slightly slower than it is preoperatively. The interaction would therefore appear to lie largely in schedule fractions 1/3 and 1/2, where differences between pre- and postoperative performance are largest, and where the performance of the BLA-lesioned group differs from that of the CeN-lesioned and Sham-lesioned groups.

4.2.3 Results of planned comparisons on preoperative and postoperative performance

Planned comparisons were run on the preoperative stage data (all the rats, discussed in **Appendix E)** and on the postoperative stage data (Sham-lesioned, BLA-lesioned and CeN-lesioned rats separately). The hypotheses are given in detail in the Analysis of data: preoperative data section for Experiment A. It can be seen from ***Table 12*** that, as with many of the postoperative planned comparisons in the previous experiment, the direction of significance is not always the same for the postoperative Sham-lesioned group as for the preoperative Combined (Sham and Lesion) group. This again raises the question of how to interpret possible differences in the results of the planned comparisons for the Sham-lesioned, BLA-lesioned and CeN-lesioned groups. For instance, it is not possible to attribute the change in direction of significance from significant in the preoperative Combined group to not significant in the two lesion groups for the Reaction Time measure planned comparison between 1/3 and 2/3 to the effect of the lesions since the result for the postoperative Sham-lesioned group is also not

significant. Likewise, for the Correct Responses measure, it is difficult to interpret the change in direction of significance from significant in the preoperative Combined group to not significant in the postoperative Sham-lesioned group, given that the results for both the lesion groups are also significant. It was therefore decided to ignore the results of any of the planned comparisons that differ in the direction of significance between the preoperative Combined groups and the postoperative Sham-lesioned group in discussing whether lesioning the BLA or the CeN had any effect on performance of the SFC task.

4.2.3.1 Correct Responses (see *Table 12*)

Postoperative data (Sham-, BLA-, and CeN-lesioned groups): The significance of the result of one of the planned comparisons (1/2 and 2/2) had changed direction for the Sham-lesioned group compared to the preoperative Combined group, and this result was therefore ignored. For all three groups, the result of one of the two remaining planned comparisons for the main hypothesis (1/3 and 2/3) was significant but the result of the other (2/3 and 3/3) was not, making it rather difficult to know whether the different schedule fractions did have an effect on performance of the task. Likewise, for all groups, the result of one of the two remaining planned comparisons for the first sub-hypothesis (1/3 and 3/3) was significant whereas the result of the other (2/3 and 3/3) was not, again making it difficult to know whether the rats performed differently for rewarded and unrewarded schedule fractions. However, the results of all three of the planned comparisons for the second sub-hypothesis (1/3 and 2/3, 1/3 and 1/2, and 2/3 and 1/2) were significant for the Sham- and BLA-lesioned groups, suggesting that the rats did use the different cue light intensities to ascertain how close they were to achieving reward rather than as an indication of the availability of reward. The results of only two of these planned comparisons (1/3 and 2/3, and 2/3 and 1/2) were significant for the CeN-lesioned group, whereas the result of the third (1/3 and 1/2) was not, but overall it would appear that these rats were also able to use the different cue light intensities to ascertain how close they were to achieving reward. The results of the postoperative planned comparisons for the three groups are

Means compared						Correct Responses Reaction Time Movement Time Post Response Pause								Null hypothesis
1/3	2/3	3/3	1/2	2/2	1/1	Preop Combined (S + L)		Postop Sham		Postop BLA		Postop CeN		
						t and df	*p*	*t* and df	*p*	*t* and df	*p*	*t* and df	*p*	
✓	✓					t = -10.14, df = 19 t = 2.18, df = 19 t = 3.46, df = 19 t = 5.74, df = 19	0.000 *0.042* 0.003 0.000	t = -4.53, df = 6 t = 2.25, df = 6 t = 4.21, df = 6 t = 8.14, df = 6	0.004 *not sig* 0.006 0.000	t = -9.77, df = 7 t = 1.66, df = 7 t = 2.73, df = 7 t = 3.68, df = 7	0.000 not sig 0.034 0.010	t = -4.67, df = 6 t = 1.01, df = 6 t = 2.31, df = 6 t = 3.13, df = 6	0.005 not sig **not sig** 0.026	Equivalence of unrewarded SFs Equivalence of SFs within work schedule
	✓	✓				t = 1.82, df = 19 t = 3.54, df = 19 t = 6.04, df = 19 t = 6.31, df = 19	not sig *0.002* 0.000 0.000	t = 0.48, df = 6 t = 0.92, df = 6 t = 2.82, df = 6 t = 4.20, df = 6	not sig *not sig* 0.030 0.006	t = -1.38, df = 7 t = 5.22, df = 7 t = 5.15, df = 7 t = 3.52, df = 7	not sig **0.002** 0.002 0.013	t = 0.00, df = 6 t = 0.43, df = 6 t = 2.68, df = 6 t = 2.26, df = 6	not sig not sig 0.043 **not sig**	Equivalence of rewarded and unrewarded SFs
			✓	✓		t = -3.33, df = 19 t = 0.65, df = 19 t = 7.27, df = 19 t = 4.58, df = 19	*0.004* not sig 0.000 0.000	t = -1.56, df = 6 t = -0.41, df = 6 t = 3.43, df = 6 t = 3.90, df = 6	*not sig* not sig 0.014 0.008	t = -5.79, df = 7 t = 2.77, df = 7 t = 4.67, df = 7 t = 4.07, df = 7	**0.001** **0.032** 0.003 0.007	t = -3.23, df = 6 t = 2.27, df = 6 t = 3.13, df = 6 t = 2.67, df = 6	**0.023** not sig 0.026 0.044	Equivalence of rewarded and unrewarded SFs
✓		✓				t = -9.35, df = 19 t = 3.96, df = 19 t = 7.71, df = 19 t = 6.16, df = 19	0.000 0.001 0.000 0.000	t = -3.74, df = 6 t = 2.62, df = 6 t = 7.75, df = 6 t = 8.83, df = 6	0.010 0.039 0.000 0.000	t = -8.14, df = 7 t = 3.76, df = 7 t = 4.14, df = 7 t = 3.82, df = 7	0.000 0.010 0.006 0.009	t = -4.52, df = 6 t = 1.06, df = 6 t = 4.14, df = 6 t = 3.45, df = 6	0.006 **not sig** 0.009 0.018	Equivalence of rewarded and unrewarded SFs
✓			✓			t = 7.51, df = 19 t = -2.70, df = 19 t = -3.03, df = 19 t = -4.39, df = 19	0.000 0.014 0.007 0.000	t = 2.47, df = 6 t = -3.19, df = 6 t = -4.06, df = 6 t = -6.04, df = 6	0.048 0.019 0.007 0.001	t = 3.69, df = 7 t = -0.65, df = 7 t = -1.88, df = 7 t = -2.36, df = 7	0.010 **not sig** **not sig** **not sig**	t = 2.46, df = 6 t = 0.32, df = 6 t = -2.16, df = 6 t = -2.33, df = 6	**not sig** **not sig** **not sig** **not sig**	Equivalence of unrewarded SFs Equivalence of responses since reward
	✓		✓			t = -4.92, df = 19 t = -0.46, df = 19 t = 2.62, df = 19 t = 3.26, df = 19	0.000 not sig *0.017* 0.004	t = -2.71, df = 6 t = -0.88, df = 6 t = 2.30, df = 6 t = 3.13, df = 6	0.035 not sig *not sig* 0.020	t = -3.74, df = 7 t = 1.17, df = 7 t = 4.41, df = 7 t = 1.76, df = 7	0.010 not sig **0.004** **not sig**	t = -2.63, df = 6 t = 0.85, df = 6 t = 1.12, df = 6 t = 2.08, df = 6	0.046 not sig not sig **not sig**	Equivalence of unrewarded SFs Equivalence of responses to reward

Table 12: *Summary of planned comparison results on the main effect of schedule fraction for the preoperative Combined (Sham and Lesion) group and for the postoperative Sham-lesioned group, BLA-lesioned group and CeN-lesioned group. Italic font denotes that the direction of significance is not the same for the Postop Sham-lesioned group as for the Preop Combined (sham and lesion groups). Bold font denotes that the direction of significance is not the same for the Postop BLA-lesioned or CeN-lesioned group as for the Postop Sham-lesioned group.*

very similar in terms of significance / non-significance to each other and to the preoperative Combined group results. However, this pattern of results is of concern since the second sub-hypothesis is premised on the main and first sub-hypothesis being true.

4.2.3.2 Reaction Time (see Table 12)

Postoperative data (Sham-, BLA-, and CeN-lesioned groups): The significance of the results of two of the planned comparisons (1/3 and 2/3, and 2/3 and 3/3) for the Sham-lesioned group had changed direction compared to the preoperative Combined group, and these results were therefore ignored. For the Sham-lesioned group, the result of the only remaining planned comparison for the main hypothesis (1/2 and 2/2) was not significant, suggesting that the different schedule fractions did not have an effect on performance of the task. The result of one of the two remaining planned comparisons for the first sub-hypothesis (1/3 and 3/3) was significant whereas the result of the other (1/2 and 2/2) was not, making it difficult to know whether these rats performed differently for rewarded and unrewarded schedule fractions. Likewise, the result of one of the two remaining planned comparisons for the second sub-hypothesis (1/3 and 1/2) was significant whereas the result of the other (2/3 and 1/2) was not, again making it difficult to know whether these rats did use the different cue light intensities to ascertain how close they were to achieving reward rather than as an indication of the availability of reward.

In contrast to the Sham-lesioned group, the result of the only remaining planned comparison for the main hypothesis (1/2 and 2/2) was significant for BLA-lesioned group, suggesting that the different schedule fractions might have had an effect on performance of the task. However, such factors do not sufficiently explain the difference in preoperative performance on the Reaction Time measure. The results of both of the two remaining planned comparisons for the first sub-hypothesis (1/2 and 2/2 and 1/3 and 3/3) were also significant, suggesting that the rats performed differently for rewarded and unrewarded schedule fractions. However, neither of the results of the two remaining planned comparisons for the second sub-hypothesis (1/3 and 1/2 and 2/3 and 1/2) were significant, suggesting that the rats did not use the

different cue light intensities to ascertain how close they were to achieving reward rather than as an indication of the availability of reward.

For the CeN-lesioned group, none of the results of the planned comparisons were significant, suggesting that not only were these rats not interpreting the different cue lights as indicating 'progress to reward', but that they were not regarding the schedule fractions at all.

Performance would therefore appear to differ between the groups on this measure. However, the pattern of results for the Sham-lesioned group is again of concern since the second sub-hypothesis is premised on the main and first sub-hypothesis being true. Also, the preoperative results obtained from ANOVA for this measure are very different in this experiment from those obtained in the previous experiment.

4.2.3.3 Movement Time (see Table 12)

Postoperative data (Sham-, BLA-, and CeN-lesioned groups): The significance of the result of the planned comparison between 2/3 and 1/2 had changed direction for the Sham-lesioned group compared to the preoperative Combined group, and this result was therefore ignored. With one exception (the planned comparison between 1/3 and 2/3 is not significant for the CeN-lesioned group) the results of the postoperative planned comparisons for the main and first sub-hypothesis are all significant for all of the groups, and are therefore identical to the preoperative Combined group results. This suggests that, overall, the lesions had no effect on performance of the task in that the rats from all three groups were able to differentiate between the different schedule fractions and to distinguish between rewarded and unrewarded schedule fractions. However, although the remaining two planned comparisons (1/3 and 2/3, and 1/3 and 1/2) for the second sub-hypothesis are also significant for the Sham-lesioned rats, suggesting that they were able to use the different cue light intensities to ascertain how close they were to achieving reward rather than as an indication of the availability of reward, this was not the case for the BLA-lesioned and the CeN-lesioned rats. For the BLA-lesioned rats, only one of the remaining two planned comparisons for the second sub-hypothesis is significant (1/3 and 2/3), thus making it difficult to know whether they were using the different cue light intensities as an indication of

the availability of reward rather than merely to ascertain how close they were to achieving it. For the CeN-lesioned rats, neither of the remaining two planned comparisons for the second sub-hypothesis are significant, suggesting that they did not use the different cue light intensities to ascertain how close they were to achieving reward rather than as an indication of the availability of reward.

4.2.3.4 Post Response Pause (see Table 12)

Postoperative data (Sham-, BLA-, and CeN-lesioned groups): All of the results of the planned comparisons were significant for the Combined group, suggesting that the different schedule fractions did have an effect on the performance of the task, and that these rats did interpret the different cue light intensities as indicting 'progress to reward'. The results of the planned comparisons for the main hypothesis (1/3 and 2/3, 2/3 and 3/3, and 1/2 and 2/2) and for the first sub-hypothesis (2/3 and 3/3, 1/2 and 2/2, and 1/3 and 3/3) were likewise significant for the BLA- and CeN-lesioned groups (with the exception, for the latter, of the result of the planned comparison between 2/3 and 3/3, which was not significant), suggesting that, on the whole, the different schedule fractions did have an effect on the lesioned rats' performance of the task and that they were able to discriminate between rewarded and unrewarded schedule fractions. However, for both lesion groups, the result for only one of the planned comparisons for the second sub-hypothesis (1/3 and 2/3) was significant, whereas the results of the other two planned comparisons (1/3 and 1/2, and 2/3 and 1/2) were not significant, suggesting that, overall, the rats did not use the different cue light intensities to ascertain how close they were to achieving reward rather than as an indication of the availability of reward.

4.2.4 Results of ANOVAs on reversal performance (Correct responses measure only)

4.2.4.1 Postoperative – Reversal ANOVA

This ANOVA produced no significant 4-way interactions, one significant 3-way interaction (FP*post/reverse*group), three significant 2-way interactions, of which only two

Main effect / interaction	F value	P value	Effect size
SF	F (5.00, 85.00) = 53.48	p = 0.000	08.30 %
FP	F (1.28, 21.87) = 171.51	p = 0.000	80.68 %
Post/Reverse	F (1, 17) = 2.86	p = 0.109	
Group	F (2, 17) = 1.24	p = 0.314	
SF*FP	F (9.76, 165.89) = 3.53	p = 0.000	00.66 %
SF*Post/Reverse	F (3.72, 63.19) = 13.73	p = 0.000	02.45 %
SF*Group	F (10, 85) = 1.63	p = 0.111	
FP*Post/Reverse	F (1.95, 33.18) = 7.73	p = 0.002	00.59 %
FP*Group	F (2.57, 21.87) = 1.75	p = 0.192	
Post/Reverse*Group	F (2.00, 17.00) = 5.61	p = 0.013	01.35 %
SF*FP*Post/Reverse	F (8.71, 148.12) = 1.90	p = 0.058	
SF*FP*Group	F (19.52, 165.89) = 1.01	p = 0.455	
SF*Post/Reverse*Group	F (7.43, 63.19) = 0.95	p = 0.476	
FP*Post/Reverse*Group	F (3.90, 33.18) = 5.61	p = 0.002	00.86 %
SF*FP*Post/Reverse*Group	F (17.43, 148.12) = 0.50	p = 0.952	

Table 13: *Main effects, interactions and effect sizes resulting from the 4-way ANOVA on the mean percentage of correct responses measure which compares the different lesion groups' (Group) postoperative and reversal performance (Post/Reverse) according to schedule fraction (SF) and foreperiod (FP).*

(SF*post/reverse and FP*post/reverse) need, theoretically, be considered since the third (post/reverseerse*group) is superseded by the 3-way interaction, and two significant main effects (SF and FP) which are also superseded by the 2- and 3-way interactions. There was no significant main effect of Group. It can be seen from ***Table 13*** that foreperiod, with an effect size of 80.68%, has the most dramatic influence on performance, whilst schedule fraction has a much smaller influence (8.30%). The effect sizes for the interactions are all extremely small showing that they have very little influence on performance, but the FP*post/reverse*group interaction is of obvious interest and is therefore graphed out and discussed below. The SF*post/reverse interaction is also of some interest, even though it is collapsed across group as well as foreperiod, since it does confirm that there are differences between postoperative and reversal performance. For this reason it is given in **Appendix G**.

FP * Post/Reverse * Group interaction

Although the effect size is very small (0.86%), this interaction is interesting because it compares the performance of the different groups between themselves and between the postoperative and reversal stages. The bar chart of the interaction (***Figure 26***) shows that, overall, as foreperiod increased the mean percentage of correct responses decreased for all three groups in both the

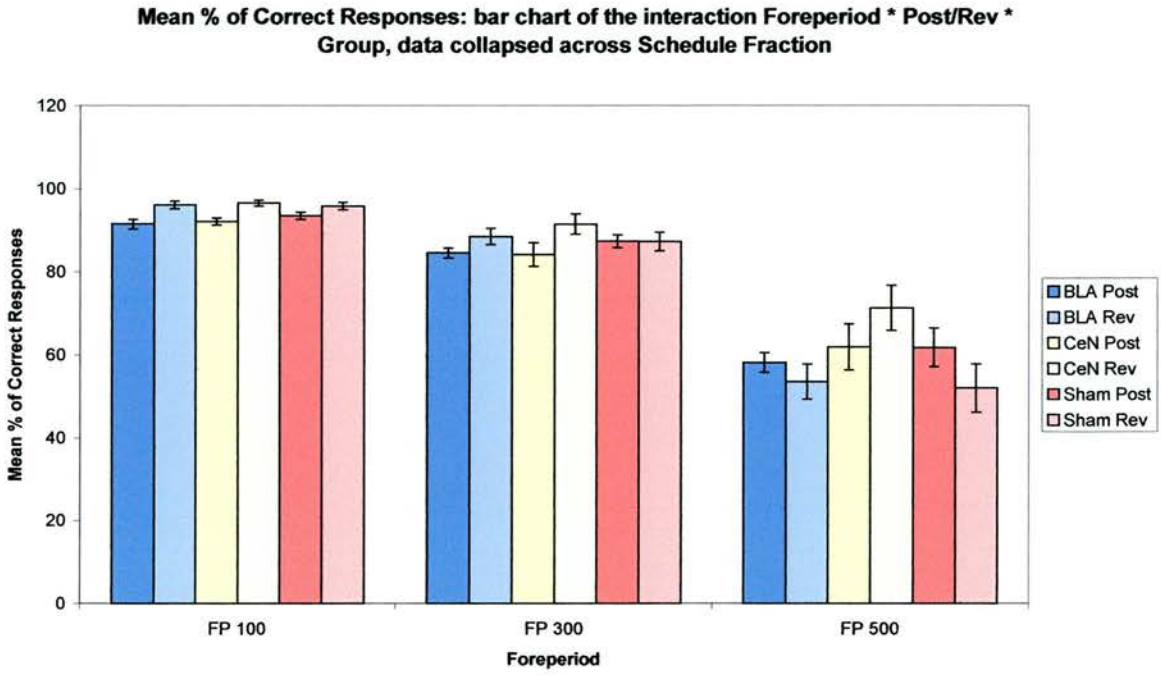


Figure 26: *Bar chart of the interaction FP*post/reverse*group, data collapsed across schedule fraction, showing the mean (±se) percentage of correct responses made postoperatively and during reversal at foreperiods 100, 300, and 500. Colour of bar indicates group, solid colouring indicates postoperative performance and hatching indicates reversal performance*

postoperative stage and the reversal stage. It would also appear from the bar chart that most of the interaction effect lies within foreperiod 500: at foreperiods 100 and 300 the rats in all three groups achieved a slightly higher mean percentage of correct responses in the reversal stage, but at foreperiod 500 the BLA- and Sham-lesioned rats showed a decrease in the mean percentage of correct responses in the reversal stage compared to the postoperative stage, whereas the CeN-lesioned rats continued to show an increase. Boxplots were constructed in order to see whether the decrease in the mean percentage of correct responses shown by the BLA- and Sham-lesioned groups or the increase shown by the CeN-lesioned group during the reversal stage could be explained by outlying or extreme values in the data set. The boxplots showed that this could be the case – no group had any outlying or extreme values in the postoperative data sets, but the CeN-lesioned group had two outlying values in the reversal data set, and the Sham-lesioned group had one outlying value.

4.2.4.2 Last day Postoperative - First day Reversal

This ANOVA (Last day Postop – First day Reversal) produced no 4-way interactions, one 3-way interaction (SF*FP*last/first), two 2-way interactions, only one of which need be considered (last/first*group) since the other (SF*last/first) is superseded by the 3-way

Main effect / interaction	F value	P value	Effect size
SF	F (5.00, 85.00) = 11.44	p = 0.000	10.26 %
FP	F (1.59, 27.11) = 63.11	p = 0.000	49.58 %
Last/First	F (1, 17) = 0.01	p = 0.925	
Group	F (2, 17) = 1.15	p = 0.341	
SF*FP	F (10, 170) = 1.39	p = 0.187	
SF*Last/First	F (5.00, 85.00) = 8.15	p = 0.000	07.45 %
SF*Group	F (10, 85) = 0.68	p = 0.736	
FP*Last/First	F (2, 34) = 1.78	p = 0.184	
FP*Group	F (3.19, 27.11) = 2.87	p = 0.052	
Last/First*Group	F (2.00, 17.00) = 12.66	p = 0.000	05.29 %
SF*FP*Last/First	F (8.70, 147.98) = 2.44	p = 0.014	04.81 %
SF*FP*Group	F (20, 170) = 1.49	p = 0.092	
SF*Last/First*Group	F (10, 85) = 0.68	p = 0.740	
FP*Last/First*Group	F (4, 34) = 1.91	p = 0.131	
SF*FP*Last/First*Group	F (17.41, 147.98) = 0.99	p = 0.478	

Table 14: *Main effects, interactions and effect sizes resulting from the 4-way ANOVA on the mean percentage of correct responses measure which compares the different lesion groups' (Group) last day of postoperative performance and first day of reversal performance (Last/First) according to schedule fraction (SF) and foreperiod (FP).*

interaction, and two main effects (SF and FP) which are also superseded by the 3-way interaction. There was no signifiant main effect of Group. It can be seen from ***Table 14*** that foreperiod, with an effect size of 49.58%, most strongly influences performance, whilst schedule fraction has a much smaller influence (10.26%). The interactions have very little influence on performance since their effect sizes are all small; however the last/first*group interaction is of some interest, and is therefore graphed out and discussed below. The 3-way interaction (SF*FP*last/first) provides a more informative picture of what is happening overall, and is therefore given in **Appendix G.**

Last/First * Group interaction

In this interaction the data are collapsed across both schedule fraction and foreperiod. Although the effect size is again quite small (5.29%), this interaction is interesting because it compares the performance of the different groups during the postoperative and reversal stages. It can be

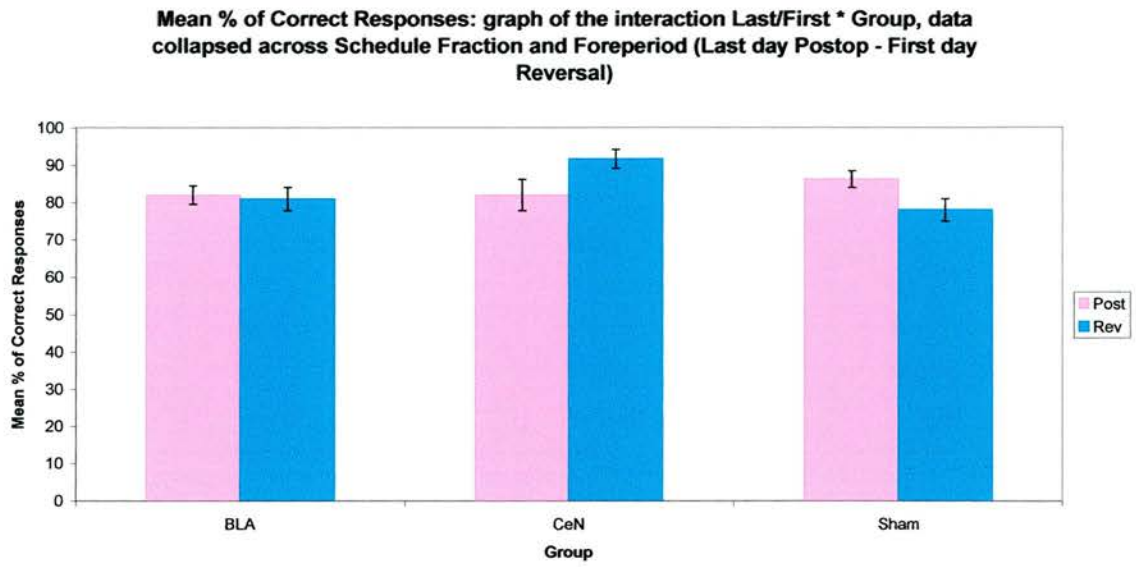


Figure 27: *Graph of the interaction last/first*group, data collapsed across schedule fraction and foreperiod, showing the mean (±se) percentage of correct responses made on the last day of postoperative performance and the first day of reversal performance. Pink bars indicate postoperative performance and green bars indicate reversal performance.*

seen that the mean percentage of correct responses is about the same for the BLA- and CeN-lesioned groups during the postoperative stage, but slightly higher for the Sham-lesioned group ***(Figure 27)***. In the reversal stage, the mean percentage of correct responses is about the same for the BLA- and Sham-lesioned groups, and is comparable to the mean percentage of correct responses for the postoperative stage for the BLA- and CeN-lesioned group, but is higher for the CeN-lesioned group. Boxplots were therefore constructed in order to see whether the increase in the mean percentage of correct responses for the Sham-lesioned group in the postoperative stage and the CeN-lesioned group in the reversal stage could be explained by outlying or extreme values in the data sets. This proved to be the case - the boxplots revealed that the Sham-lesioned group did indeed have one outlying value in the postoperative stage, and that the CeN-lesioned group had one outlying value in the reversal stage.

4.2.4.3 First day Reversal – Last day Reversal ANOVA

This ANOVA for the mean percentage of correct responses made on the first and the last day of reversal produced no significant 4-way interactions, one significant 3-way interaction (FP*day1/day10*group), two significant 2-way interactions (FP*day1/day10 and

Main effect / interaction	F value	P value	Effect size
SF	F (5.00, 90.00) = 3.27	p = 0.009	09.93 %
FP	F (1.35, 24.33) = 11.80	p = 0.001	33.95 %
Day1/Day10	F (1.00, 18.00) = 5.17	p = 0.035	04.66 %
Group	F (1, 18) = 0.17	p = 0.679	
SF*FP	F (10, 180) = 1.51	p = 0.138	
SF*Day1/Day10	F (5, 90) = 0.48	p = 0.789	
SF*Group	F (5, 90) = 0.96	p = 0.442	
FP*Day1/Day10	F (2.00, 36.00) = 5.48	p = 0.008	08.90 %
FP*Group	F (1.35, 24.33) = 0.08	p = 0.848	
Day1/Day10*Group	F (1.00, 18.00) = 4.75	p = 0.043	04.28 %
SF*FP*Day1/Day10	F (9.66, 173.95) = 0.83	p = 0.595	
SF*FP*Group	F (10, 180) = 1.01	p = 0.439	
SF*Day1/Day10*Group	F (5, 90) = 0.34	p = 0.886	
FP*Day1/Day10*Group	F (2.00, 36.00) = 4.12	p = 0.024	06.69 %
SF*FP*Day1/Day10*Group	F (9.66, 173.95) = 0.65	p = 0.763	

Table 15: *Main effects, interactions and effect sizes resulting from the 4-way ANOVA on the mean percentage of correct responses measure which compares the different lesion groups' (Group) first day of reversal performance and last day of reversal performance (Day1/Day10) according to schedule fraction (SF) and foreperiod (FP).*

day1/day10*group), and three significant main effects (SF, FP and day1/day10). There was no significant main effect of Group. Neither the 2-way interactions nor the foreperiod and day1/day10 main effects need be considered because they are superseded by the 3-way interaction. It can be seen from ***Table 15*** that foreperiod is the strongest influence on performance with an effect size of 33.95%, whilst schedule fraction is the next (9.93%). The effect sizes for the day1/day10 main effect and the interactions are all small. The 3-way interaction (FP*day1/day10*group) is of obvious interest, and is graphed out and described below:

FP * Day1/Day10 * Group

Although its effect size is small (6.69%), this interaction is interesting because it compares the performance of the different groups between the first and last days of the reversal stage ***(Figure 28)***. It can be seen from the interaction bar chart that, overall, as foreperiod increases, the mean percentage of correct responses decreases. It can also be seen that as foreperiod increases, the differences between the groups and between Day 1 and Day 10 become more apparent: at

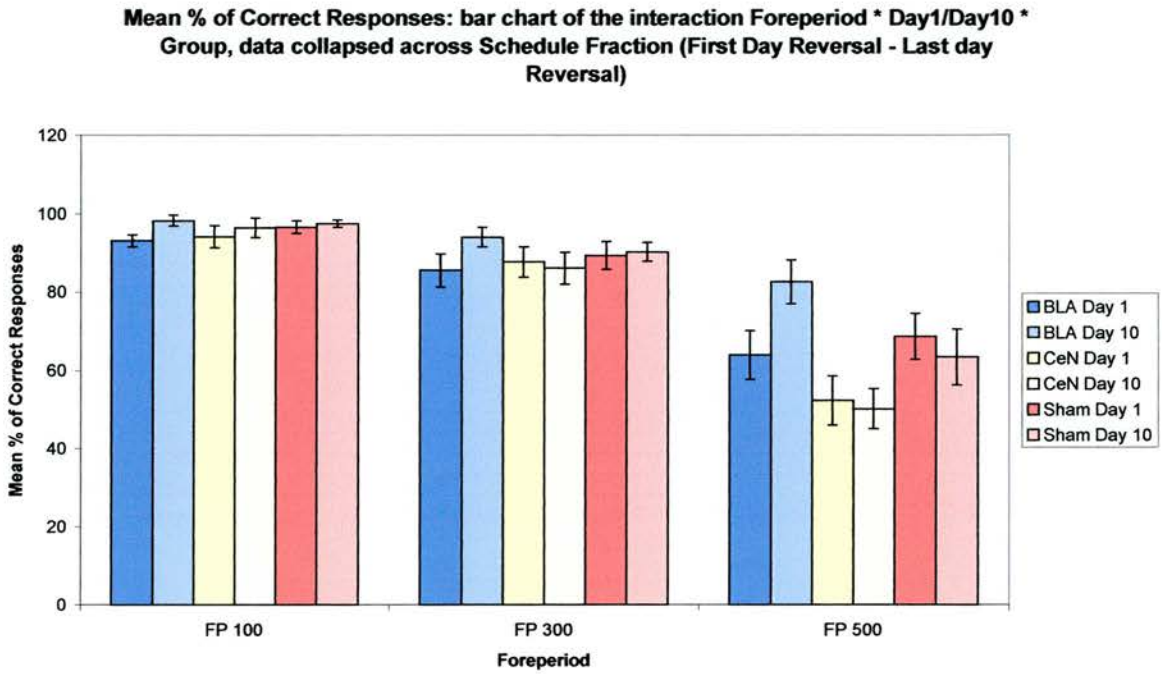


Figure 28: *Barchart of the interaction FP*day1/day10*group, data collapsed across schedule fraction, showing the mean (±se) percentage of correct responses made on the first day of reversal and the last day of reversal at foreperiods 100, 300, and 500. Colour of bar indicates group, solid colouring indicates performance on the first day of reversal and hatching indicates performance on the last day of reversal.*

foreperiod 100 there is very little difference in the mean percentage of correct responses between the three groups or between Day 1 and Day 10, except perhaps for the BLA-lesioned group, which shows a slight increase for Day 10. At foreperiod 300 the rats make fewer correct responses overall, but again there is very little difference between the three groups or between Day 1 and Day 10 except in the BLA group, which again shows a (somewhat larger) increase for Day 10. At foreperiod 500 the rats make the fewest correct responses overall, but differences in performance between the three groups and between Day 1 and Day 10 are apparent: there are differences between the groups for Day 1, with the Sham group making the largest mean percentage of correct responses, followed by the BLA-lesioned group and then the CeN-lesioned group. The BLA-lesioned group shows a large increase in the mean percentage of correct responses for Day 10 compared to Day 1, whereas the Sham-lesioned group shows a small decrease, and the CeN-lesioned group shows very little difference between the two stages. Boxplots were constructed in order to see whether the increase in mean percentage of correct responses for Day 10 at foreperiod 500 shown by the BLA-lesioned group could be explained

by outlying or extreme values in either the Day 1 or Day 10 data sets. None were found, though one outlying value was found in the CeN-lesioned group data for Day 1 and one outlying value was found in the Sham-lesioned group data for Day 10.

4.2.4.4 Last day Postoperative – Last day Reversal ANOVA

Main effect / interaction	F value	P value	Effect size
SF	F (5.00, 85.00) = 19.88	p = 0.000	11.40 %
FP	F (1.79, 30.45) = 81.42	p = 0.000	61.13 %
Last/Last	F (1, 17) = 0.71	p = 0.410	
Group	F (2, 17) = 1.18	p = 0.332	
SF*FP	F (8.78, 149.20) = 0.39	p = 0.935	
SF*Last/Last	F (4.90, 83.30) = 4.39	p = 0.001	04.41 %
SF*Group	F 10.00, 85.00) = 2.20	p = 0.025	02.53 %
FP*Last/Last	F (2.00, 34.00) = 7.45	p = 0.002	03.35 %
FP*Group	F (3.58, 30.45) = 2.39	p = 0.078	
Last/Last*Group	F (2, 17) = 1.91	p = 0.178	
SF*FP*Last/Last	F (10, 170) = 1.16	p = 0.322	
SF*FP*Group	F (17.55, 149.20) = 1.29	p = 0.203	
SF*Last/Last*Group	F (8.80, 83.30) = 0.41	p = 0.937	
FP*Last/Last*Group SF*FP*	F (4, 34) = 0.08	p = 0.988	
Last/Last*Group	F (20, 170) = 0.95	p = 0.520	

Table 16: *Main effects, interactions and effect sizes resulting from the 4-way ANOVA on the mean percentage of correct responses measure which compares the different lesion groups' (Group) last day of reversal performance and last day of reversal performance (Last/Last) according to schedule fraction (SF) and foreperiod (FP).*

This ANOVA produced no significant 4-way or 3-way interactions, three significant 2-way interactions (SF*last/last, SF*group and FP*last/last) and two significant main effects (SF and FP), neither of which need be considered since they are superseded by the 2-way interactions. There was no signicant main effect of Group. None of the significant interactions are of particular interest: the data for the interaction SF*last/last are collapsed across foreperiod and group, and merely informs that there are differences in performance between the last day of the postoperative stage and the last day of the reversal stage according to schedule fraction – in other words that the rats' performance has not returned to what it was pre-reversal. The same applies to the interaction FP*last/last. However, the interaction SF*group is slightly more interesting in that it informs one that the groups perform differently according to schedule

fraction, even though the data is collapsed across foreperiod and across the postoperative / reversal stages, and is therefore given in **Appendix G**.

4.2.5 Determination of extent of lesions (results)

As in the previous experiment, it can be seen from the schematic representations that there is considerable variation in the extent of both the BLA and the CeN lesions. It therefore was thought sensible to again compare size of lesion with performance since it could be argued that such variation within the groups might obscure potential lesion effects. The percentage volume of lesion within the desired structures only and the total percentage volume of lesion in each rat therefore were estimated from the schematic representations as detailed in the Determination of lesion extent section for Experiment A and are given in ***Tables 24 and 25*** below.

BLA-lesioned rats	% volume of lesion within desired structures only	Total % volume of lesion (within desired structures and structures adjacent to them)
01/003	87.5	45.6
01/031	75	39.5
01/036	73.0	45.3
01/032	61.8	34.0
01/033	57.2	32.0
01/001	54.6	30.0
01/035	53.9	25.6

Table 17: *% volume of lesion within desired structures only and total % volume of lesion within desired structures and structures adjacent to them for the BLA-lesioned rats.*

CeN-lesioned rats	% volume of lesion within desired structures only	Total % volume of lesion (within desired structures and structures adjacent to them)
01/023	76.6	37.5
01/027	50	20.7
01/029	36.0	35.4
01/013	31.2	17.8
01/018	30.0	18.5
01/019	18.8	10.4

Table 18: *% volume of lesion within desired structures only and total % volume of lesion within desired structures and structures adjacent to them for the CeN-lesioned rats.*

Separate graphs for each of the three foreperiods on the main measure of Correct Responses were then drawn showing the postoperative performance of the rats with the smallest and largest BLA lesions against the averaged postoperative performance of all of the Sham-lesioned rats

(Figure 29.d-f). As in the previous experiment, it was thought likely that any data point from the BLA-lesioned rats lying outwith the range of +/-2 standard deviations of the averaged Sham-lesioned rat data would belong to the rat with the larger rather than the smaller lesion. For comparative purposes, separate graphs for each of the three foreperiods were also drawn showing the preoperative performance of those same smallest lesion and largest lesion rats against the averaged preoperative performance of all of the Sham-lesioned rats *(Figure 29.a-c)*. Likewise, graphs were drawn showing the postoperative and preoperative performance of the rats with the smallest and largest CeN lesions against the averaged postoperative and preoperative performance of all the Sham-lesioned rats *(Figure 30.a-f)*.

Of the BLA-lesioned rats, it was found that Rat 01/003 had the largest lesion both in terms of the percentage volume of lesion within the desired structures only (87.5%), and in terms of the total percentage volume of lesion (45.6%), whilst Rat 01/035 had the smallest 'desired' lesion volume (53.9%) and 'total' lesion volume (25.6%) *(Table 17)*. It can be seen from *Figure 29.d-f* that only the rat with the largest BLA lesion, Rat 01/003, performed outwith the range of +/-2 standard deviations of the averaged Sham-lesioned rat data, making fewer correct responses at both FP100 SF2/3 and FP300 SF3/3. This could be interpreted as a lesion effect, but the fact that, preoperatively, Rat 01/003 also performed outwith the range of +/-2 standard deviations of the preoperative averaged Sham-lesioned rat data, making fewer correct responses at FP100 SF3/3 and FP300 SF1/2 *(Figure 29.a-c)*, suggests that this particular rat might just perform below average at shorter foreperiods regardless of the lesion.

Of the CeN-lesioned rats, it was found that Rat 01/023 had the largest lesion both in terms of the percentage volume of lesion within the desired structures only (76.6%), and in terms of the total percentage volume of lesion (37.5%), whilst Rat 01/019 had the smallest 'desired' lesion volume (18.8%) and 'total' lesion volume (10.4%) *(Table 18)*. It can be seen from *(Figure 30.d-f)* that whilst the rat with the largest lesion, Rat 01/023, performed outwith the range of +/-2 standard deviations of the averaged Sham-lesioned rat data, making fewer correct responses at FP100 SF2/3, the rat with the smallest lesion, Rat 01/019, also did so, making fewer correct responses at FP300 SF2/3, 3/3 and 2/2. There does not, therefore, appear

to be any relationship between extent of lesion and the degree to which performance of the lesioned rats falls outwith the range of +/-2 standard deviations of the averaged Sham-lesioned rat data. Preoperatively, Rat 01/023 also performed outwith the range of +/-2 standard deviations of the averaged Sham-lesioned rat data, making more correct responses at FP500 SF1/2 and 1/1 ***(Figure 30.a-c)***, again raising the possibility that any deviation from the averaged Sham-lesioned rat performance might be due to individual differences rather than to the effects of the lesion.

Given the difficulty in ascribing differences in performance between the rats with the largest and smallest lesions to the extent of the lesion itself, and given the small number of rats in each group, it was decided not to carry out formal correlational analysis.

Figure 29.a-f: *Graphs showing the averaged (+/-2 standard deviations) preoperative and postoperative performance of the rat with the smallest lesion of the BLA (01/035) and the rat with the largest lesion of the BLA (01/003), at each of the three foreperiods (100, 300, and 500 msecs) on the main measure of mean percentage of correct responses. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward.*

Figure 29.a: *preoperative performance at foreperiod 100*
Figure 29.b: *preoperative performance at foreperiod 300*
Figure 29.c: *preoperative performance at foreperiod 500*
Figure 29.d: *postoperative performance at foreperiod 100*
Figure 29.e: *postoperative performance at foreperiod 300*
Figure 29.f: *postoperative performance at foreperiod 500*

Figure 30.a-f: *Graphs showing the averaged (+/-2 standard deviations) preoperative and postoperative performance of the rat with the smallest lesion of the CeN (01/019) and the rat with the largest lesion of the CeN (01/023), at each of the three foreperiods (100, 300, and 500 msecs) on the main measure of mean percentage of correct responses. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward.*

Figure 30.a: *preoperative performance at foreperiod 100*
Figure 30.b: *preoperative performance at foreperiod 300*
Figure 30.c: *preoperative performance at foreperiod 500*
Figure 30.d: *postoperative performance at foreperiod 100*
Figure 30.e: *postoperative performance at foreperiod 300*
Figure 30.f: *postoperative performance at foreperiod 500*

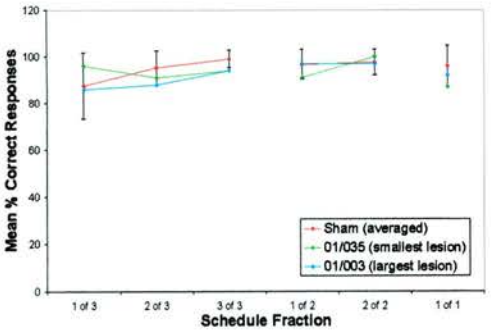


Figure 29.a: preoperative mean % of correct responses at foreperiod 100

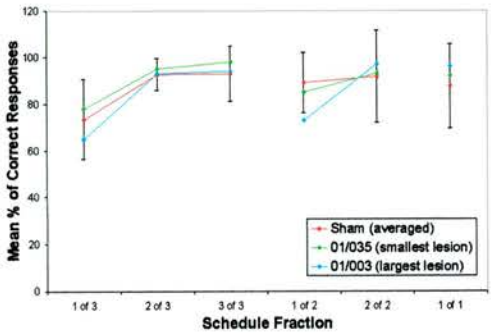


Figure 29.b: preoperative mean % of correct responses at foreperiod 300

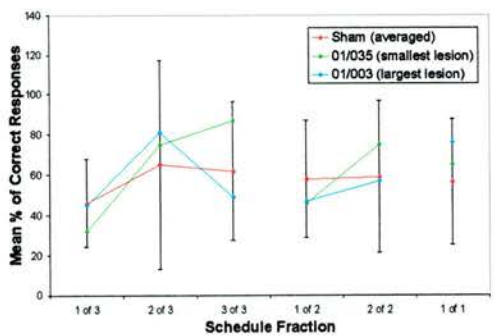


Figure 29.c: preoperative mean % of correct responses at foreperiod 500

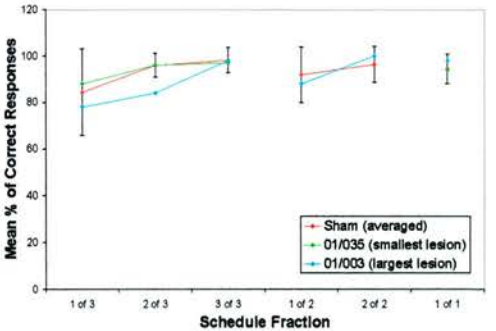


Figure 29.d: postoperative mean % of correct responses at foreperiod 100

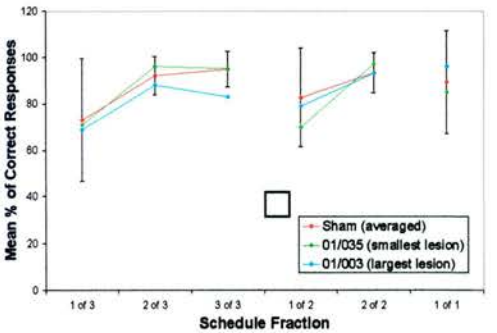


Figure 29.e: postoperative mean % of correct responses at foreperiod 300

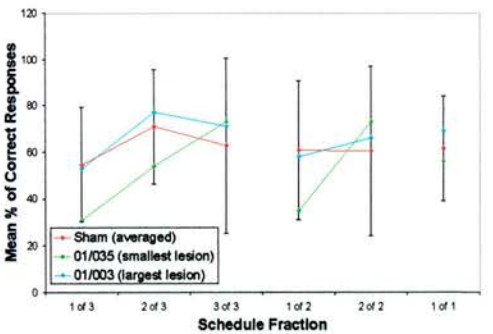


Figure 29.f: postoperative mean % of correct responses at foreperiod 500

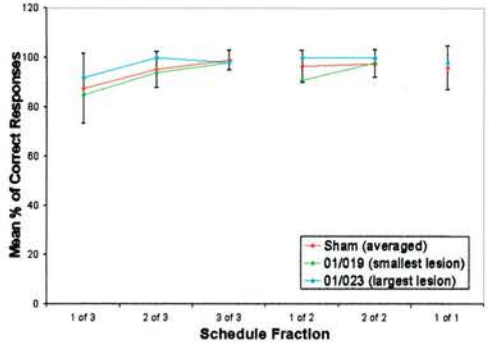


Figure 30.a: preoperative mean % of correct responses at foreperiod 100

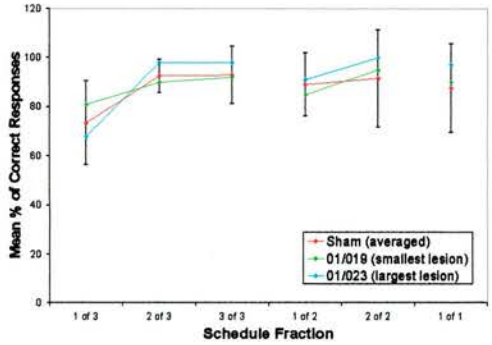


Figure 30.b: preoperative mean % of correct responses at foreperiod 500

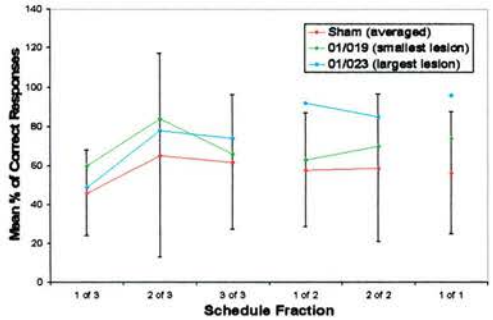


Figure 30.c: preoperative mean % of correct responses at foreperiod 500

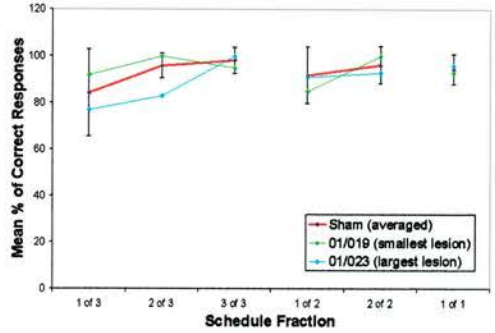


Figure 30.d: postoperative mean % of correct responses at foreperiod 100

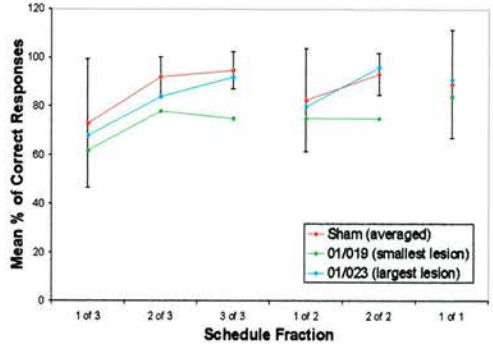


Figure 30.e: postoperative mean % of correct responses at foreperiod 300

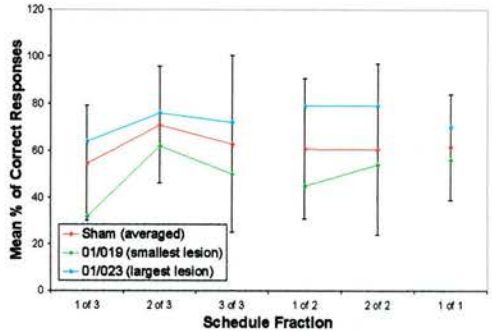


Figure 30.f: postoperative mean % of correct responses at foreperiod 500

4.3 Discussion of results

The aims of this experiment were fourfold. The first aim was to find out if the preoperative results of the previous experiment could be replicated; this is discussed in **Appendix E**. Then, since the size of the BLA lesion group in the previous experiment was very small (only four rats), the next aim was to determine whether a larger group size would clarify the effect that BLA lesions have on performance of the SFC task. The third aim was to determine if CeN lesions had any effect on the SFC task, and the final aim was to see whether reversing the schedule fraction cues would have any effect on performance. As in the previous experiment, two kinds of analysis were undertaken – ANOVA and planned comparisons.

4.3.1 Postoperative performance

In the first experiment postoperative performance was analysed by running three sets of ANOVAs: the first set compared the postoperative performance of the sham rats and the BLA-lesioned rats on all the measures, the second set compared the preoperative performance of the sham rats with their postoperative performance, and the third set compared the preoperative performance of the BLA-lesioned rats with their postoperative performance. None of these sets of ANOVAs showed any difference in the performance of sham and BLA-lesioned rats on any of the dependent measures.

In this experiment 4-way ANOVAs were carried out on the various measures in order to discover whether lesions of the BLA and/or the CeN as opposed to sham lesions had any effect on performance of the SFC task ***(Table 19)***. No significant 4-way interactions were produced for any of the measures, and only two significant 3-way interactions, one for the Correct Responses measure (SF*FP*pre/post), and one for the Post Response Pause measure (SF*pre/post*group). This latter result was interesting in that it showed that the groups performed differently on the various schedule fractions before and after surgery. However, the effect size was very small (1.91%) and the graph of the interaction ***(Figure 25)*** revealed differences in the performance of the sham rats of equal magnitude to those found in the

performance of the BLA- and CeN-lesioned rats. The significant 3-way interaction for the Correct Responses measure revealed differences in pre- and postoperative performance according to both schedule fraction and foreperiod, but did not differentiate between the sham and lesioned rats **(Appendix F: *Figure 37*)**. The effect size was again very small (0.54%).

Effect	Correct Responses	Reaction Time	Movement Time	Post Response Pause
SF	✓	✓	✓	✓
FP	✓	✓	✓	
Pre/Post	✓		✓	✓
GROUP				
SF*FP	✓	✓	✓	
SF*Pre/Post			✓	
SF*Group	✓			
FP*Pre/Post				
FP*Group				
Pre/Post*Group	✓			✓
SF*FP*Pre/Post	✓			
SF*FP*Group				
SF*Pre/Post*Group				✓
FP*Pre/Post*Group				
SF*FP*Pre/Post*Group				

Table 19: *Summary table of the significant main and interaction effects resulting from the 4-way ANOVAs comparing the different lesion group's (Group) preoperative and postoperative performance (pre/post) according to schedule fraction (SF) and foreperiod (FP) on the various measures. ✓ denotes that the effect is significant*

The ANOVAs also produced significant 2-way interactions for pre/post*group for the Correct Responses ***(Figure 24)*** and Post Response Pause measures, but the effect sizes were very small (0.78% and 1.02% respectively), and much of the interaction seems to be due to differences between the groups in the preoperative stage. A significant interaction was produced on the Correct Responses measure for SF*group, but the effect size is very small and, of course, cannot be attributed to differences in postoperative performance rather than preoperative performance. The only other significant 2-way interaction found was that between schedule fraction and foreperiod, as would be expected. As in the preoperative stage, it was produced on all of the measures except Post Response Pause. Likewise, significant main effects of schedule

fraction and of foreperiod were found for all of the measures barring Post Response Pause, for which only a main effect of schedule fraction was found. The effect sizes for the schedule fraction and foreperiod interaction and for the main effects were very similar, though necessarily somewhat smaller, for all the measures except Reaction Time. For this measure, the postoperative effect sizes for the main effects of schedule fraction and foreperiod were substantially smaller (40.58% and 22.82 % compared to 53.59% and 33.28%) as was the effect size for the interaction effect (6.84% compared to 13.11%). No main effect of Group was found on any of the measures.

Overall, it would appear that no real effect on performance was brought about by lesioning either the BLA or the CeN. The graph for the only significant 3-way interaction involving Group (SF*pre/post*group on the Post Response Measure, see ***Figure 25***) suggests that performance differed pre- and postoperatively for the sham rats as well as the lesioned rats, whilst graphs for other significant interactions suggest that any differences between the sham and lesioned rats lay in their preoperative performance ***(Figures 24 and 25)***. Moreover, all the interaction effect sizes were very small. It would be very difficult, therefore, to attribute any differences in performance to the effect of the lesions rather than to the effects of surgery per se, or to the passage of time. This conclusion is supported by the fact that there does not appear to be any relationship between performance and extent of lesion in the BLA- and CeN-lesioned rats.

However, in the results section and discussion for the previous experiment, it was noted that some of the results from the planned comparisons suggested that BLA lesions might have had an effect on performance despite the ANOVAs showing no difference in the performance of sham and BLA-lesioned rats on any of the measures. This is also the case for this experiment. On both the Movement Time and Post Response Pause measures all of the planned comparisons were significant for the preoperative Combined group and for the postoperative Sham group, strongly suggesting that the different schedule fractions did have an effect on performance, and that the rats were able not only to discriminate between rewarded and unrewarded schedule fractions but also to use the different cue light intensities to ascertain how close they were to

reward. The BLA lesion group and the CeN lesion group, however, were, on the whole, able to discriminate between rewarded and unrewarded schedule fractions on these measures, but were not able to use the different cue light intensities to ascertain how close they were to reward. This suggests that whilst the BLA- and CeN-lesioned rats' performance was influenced by the cue lights in their capacity as discriminative stimuli, it was no longer influenced by the cue lights in their capacity as conditioned reinforcers. With regard to these measures, at least, the results of the planned comparisons appear to confirm the idea that this form of analysis is better suited than ANOVA to picking out more subtle differences in behaviour on the SFC task.

However, as in the previous experiment, several problems came to light with regard to the planned comparisons. First, the direction of significance of some of the planned comparisons was not the same for the postoperative Sham group as for the preoperative Combined (sham and lesion) group on some of the measures. Although this comparison is not a fair one, in that the sizes of the groups are different, it was decided to err on the side of caution and ignore these planned comparisons when trying to interpret the results. However, the existence of these anomalous results must raise doubts about the validity of the planned comparisons as a whole. Second, the results of the planned comparisons for all of the postoperative groups on the Correct Responses measure were very similar. Although this suggests that there was no effect of lesion on performance, the pattern of results is of concern in that they showed that the rats did not perform differently on any of the different schedule fractions, never mind on rewarded and unrewarded schedule fractions, but that they did use the different cue light intensities to ascertain how close they were to achieving reward, a rather contradictory state of affairs. Since the second sub-hypothesis is premised on the main and first sub-hypothesis being true, this pattern again raises doubts about the validity of using planned comparisons to analyse the SFC task.

Although the small numbers of rats in each of the postoperative groups, and the partiality of the lesions in the case of the CeN rats, might contribute to the problems outlined above, it seems unlikely that they are solely responsible. Another contributory factor might be that three of the pairs of comparisons selected to test a given hypothesis were also used to test a

second hypothesis - for example, the paired schedule fractions 1/3 and 2/3 were used to test both the main hypothesis, that the different schedule fractions have a different effect on performance, and the second sub-hypothesis, that the rats used the different cue light intensities to ascertain how close they were to achieving reward. This would result in the direction of significance being 'wrong' for either the main hypothesis or for the second sub-hypothesis within the proposed scenario in which the lesions do not prevent the rats distinguishing between the different schedule fractions but do affect the rats' ability to ascertain how close they are to receiving reward. Whilst, in practice, this was mitigated to a certain extent by using the 'best of three' algorithm to judge whether the results of the planned comparisons supported a given hypothesis, the need to exclude some of the planned comparisons from the analysis forestalled this strategy on occasion.

Overall, although the results of the planned comparisons for some of the measures in both this and the previous experiment imply that the lesioned rats were unable to use the cue lights to ascertain how close they were to reward, the difficulties discussed above somewhat limit their usefulness in analysing the SFC task.

4.3.2 Reversal performance

For the Reversal stage, the schedule fraction cues were reversed for each rat. This meant, for instance, that whereas a bright cue light might have previously indicated three trials before reward, it now indicated only one trial, and that whereas no cue light might have indicated one trial before reward, it now indicated three trials. The intensity of the cue light indicating two trials before reward remained the same after reversal. Four sets of ANOVAs were carried out on the reversal data in order to ascertain whether lesions of the BLA or the CeN affected the rats' ability to either carry out the task or to learn the new meanings of the cues. However, although these sets of ANOVAs were run for all the measures, only the Correct Responses measure, as the primary measure of performance, was analysed fully.

The main set of ANOVAs compared performance during the ten days of the Reversal stage with performance during the Postoperative stage (Postoperative – Reversal). The results of

the ANOVA carried out on the Correct Responses measure revealed that there were differences between Postoperative and Reversal performance but gave very little indication of any real difference between the three groups. The FP*post/reverseerse*group interaction did reveal a difference between the groups during the Reversal stage, and the interaction graph ***(Figure 26)*** suggested that this difference might lie at foreperiod 500 in that the CeN group showed an increase in the mean percentage of correct responses in the Reversal stage at this foreperiod whereas the BLA and Sham groups showed a decrease. However, the small effect size for this interaction (0.86%) combined with the presence of outlying values in the CeN data set for the Reversal stage prevents much importance being placed on its significance. Overall, the rats performed somewhat better in the Reversal stage compared to the Postoperative stage. The graph for the SF*post/reverseerse interaction **(Appendix G: *Figure 40)*** shows that the mean percentage of correct responses was slightly higher during the Reversal stage for work schedules 3 and 2, but, interestingly, that it was slightly lower for work schedule 1 – in fact, the mean percentage of correct responses for schedule fraction 1/1 was the same as for the first schedule fractions of work schedules 3 and 2 (i.e. schedule fractions 1/3 and 1/2). This suggests either that the rats were starting from the same baseline on each work schedule because they were not fully aware of the new meaning of the cue lights in the Reversal stage, or that they were using the cue lights purely as discriminative stimuli signalling a reward or no-reward outcome.

The other three sets of ANOVAs were intended to provide a 'snapshot' of what might be happening at different times during the Reversal stage. The results of the Last day Postoperative – First day Reversal ANOVA carried out on the Correct Responses measure confirmed that there were differences between Postoperative and Reversal performance but again gave very little indication of any real difference between the three groups. A 2-way interaction (last/first*group) yielded by the ANOVA did suggest that the groups performed differently during the Postoperative and Reversal stages – the Sham group made slightly more correct responses than the BLA lesion and CeN lesion groups during the Postoperative stage whereas the CeN lesion group made slightly more correct responses than the Sham and BLA

lesion groups during the Reversal stage. However, boxplots showed outlying and extreme values in both the Postoperative Sham group and the Reversal CeN lesion group, suggesting that the difference between the groups was not necessarily an effect of lesion but might be due to a few rats performing particularly waywardly. The graph for the 3-way interaction SF*FP*last/first 1 **(Appendix G: *Figure 41)*** showed that the rats performed better during the Reversal stage than during the Postoperative stage on the shorter foreperiods, though it is difficult to determine whether this was because they had not yet managed to learn the new meanings of the cues or because they were no longer paying any attention to the cues at all. At foreperiod 500, the rats again started every work schedule from the same baseline, as described above, again suggesting that either the rats were no longer sure what the cue lights were signalling or that they were using the cue lights as purely discriminative stimuli signalling a reward or no-reward outcome.

The results of the First day Reversal – Last day reversal ANOVA carried out on the Correct Responses measure showed that there were differences between the groups in their performance on the first day of Reversal and on the last day (Day 10) of Reversal, but only according to foreperiod. The effect size for the interaction (FP*day1/day10*group) was relatively large compared to the other interaction effect sizes obtained in this experiment, but was still only 6.69%, and most of the interaction effect would seem to lie in the longest foreperiod 500. The rats made fewer correct responses overall at this foreperiod compared to the other foreperiods, but whilst the mean percentage of correct responses decreased between the first and the last days of Reversal for the CeN lesion group and the Sham group, it increased for the BLA group ***(Figure 28)***. It could be argued (very tentatively) that this increase was due to the BLA-lesioned rats no longer being able to utilise the cue lights as effectively to tell them which work schedule they were on, or perhaps more probably, given the results of the postoperative planned comparisons, to tell them how far they had progressed towards reward. In consequence, the BLA-lesioned rats were less likely to become impatient and withdraw from the nose-poke hole too early, and therefore made more correct responses.

The results of the final ANOVA carried out on the Correct Responses measure, Last day Postoperative – Last day Reversal, showed only that there were differences in performance on the last day of the Postoperative stage and the last day of the Reversal stage, regardless of group, demonstrating that the rats' performance had not returned to what it was pre-Reversal. Interestingly, though, given the suggestion made above that the rats' returning to the same baseline level of performance for the first schedule fraction of each work schedule might indicate either that they were not sure about the meaning of the cue lights or that they were using the cue lights purely as discriminative stimuli signalling a reward or no-reward outcome, it can be seen from the graph for the SF*group interaction ***(Appendix G: *Figure 42*)*** that the BLA-lesioned rats similarly returned to a baseline level of performance for the first schedule fraction of each work schedule whereas the other rats did not. Although the data for this interaction are collapsed across both the Postoperative and Reversal stages, it could be that the BLA-lesioned rats did not make use of the cue lights in their capacity as conditioned reinforcers in order to ascertain how close they were to achieving reward in either stage, but made use of them purely as discriminative stimuli indicating the availability of reward.

Overall, although the results produced by the four sets of ANOVAs carried out on the Reversal data suggest that there were differences in performance between the Postoperative and Reversal stages, they give little indication of any differences in performance between the three groups. Those few interactions that did appear to indicate differences between the groups were characterised by very small effect sizes or by extreme or outlying values in the data sets. However, the graphs of some of the interactions show that the rats performed slightly better on the whole during the Reversal stage compared to the Postoperative stage, whilst the pattern of correct responses made suggests that this might be either because they had not yet learnt the new meanings of the cues, or because they were using the cues purely as discriminative stimuli indicating the availability or not of reward on any particular response. There is some evidence to suggest that the latter might be particularly the case with the BLA-lesioned rats, which would tie in with some of the results of the planned comparisons that also suggested that BLA-lesioned rats were able to use the cue lights in their capacity as discriminative stimuli indicating the

availability of reward, but not in their capacity as conditioned reinforcers indicating progress to reward. There is little evidence to show that the CeN lesions had any effect on the rats' performance of the SFC task during either the Postoperative stage or the Reversal stage. This is hardly surprising given the unilaterality and small extent of the CeN lesions in themselves, but large areas of the BLA were also inadvertently lesioned in many of the rats belonging to the CeN lesion group, and the lack of effect on performance could be seen as support for the theory that the BLA is not necessary to the performance of the SFC task.

4.4 General discussion

In the introduction to the previous experiment it was suggested that the BLA is critical for the acquisition of positive incentive value by formerly neutral stimuli, and also that Pavlovian conditioned stimuli can exert a motivational influence on instrumental behaviour. It was therefore hypothesised that since the SFC task appears to involve Pavlovian cues exerting a motivational impact on instrumental performance, BLA-lesioned rats would be impaired in their performance of the task. This proved not to be wholly the case – ANOVA showed no difference between postoperative sham-lesioned and BLA-lesioned rats in performance of the SFC task but the results of the planned comparisons intimated that the BLA lesions might have had an effect on performance, at least on some of the measures. In this experiment, ANOVA also showed no difference in the postoperative performance of sham-lesioned and BLA- and CeN-lesioned rats on the SFC task, but again the results of the planned comparisons suggest that the (BLA) lesions might have had an effect on performance of the SFC task on some of the measures. For both SFC experiments, there is some suggestion that the BLA-lesioned rats were able to use the different cue lights to discriminate between rewarded and unrewarded schedule fractions, but they were not able to use the changing cue light intensities to ascertain how close they were to reward. However, it is recognised that the evidence for this is somewhat nebulous. The CeN lesions were so poor that no significant effect on performance was found. Despite the similarity between the results for the previous and this experiment, ANOVA showed that there were slight

differences in preoperative performance between the two, which no doubt can be attributed to the fact that the two different groups of rats were of different sizes and were trained for different lengths of time using different numbers of foreperiods. However, such factors do not sufficiently explain the difference in preoperative performance on the Reaction Time measure.

Moreover, such apparently slight differences in preoperative performance between the two experiments had a fairly considerable influence on which hypotheses were supported (or not) by the results of the planned comparisons, especially with respect to the Post Response Pause measure. There were also differences in the postoperative performance results for the previous and this experiment, some of which can probably be attributed to the fact that three sets of 3-way ANOVAs were used to analyse each measure in the first experiment, whereas a single 4-way ANOVA was used to analyse each measure in the second experiment. Other differences in postoperative performance can probably be attributed to the varying sizes of the lesions in the two experiments and, again, to the different group sizes and numbers of foreperiod etc. Of greater concern was the lack of consistency in the direction of significance between the planned comparison results for the preoperative Combined group and for the postoperative Sham group. No doubt much of the inconsistency can be attributed to the usual culprit of group size, but nevertheless it would appear that the use of planned comparisons is not a very robust method of analysing the SFC task. However, since the planned comparisons appear to be able to pull out more subtle differences in performance, it is rather difficult wholly to dispense with their use when analysing the SFC task. This in turn begs the question of whether the SFC task is a good procedure with which to study cost of reward. This issue will be considered at greater length below.

The results of this and the previous experiment raise several issues about the design of the SFC task that might have an effect on performance. The first involves the number of times each cue light is presented to the rat during training. As detailed in the Experimental procedure section for Experiment A, the SFC task involves the presentation of three different work schedules to the rat. Work schedule 1 requires just one correct response of nose-poke followed by hopper flap opening, work schedule 2 requires two correct responses and work schedule 3

requires 3 correct responses. The response required at each step of the different work schedules (i.e. for each schedule fraction) is signalled to the rat by the intensity of the cue light: 1/3 is signalled by a bright light, 2/3 and 1/2 are signalled by a dim light and 3/3, 2/2 and 1/1 are signalled by no light (or vice versa). Each session continues until the rat has achieved 120 correct responses, made up of 20 responses for each of the six schedule fractions. Within a session, therefore, the bright light is presented 20 times, the dim light is presented 40 times and no light is presented 60 times ***(Table 20)***

	Schedule Fraction					
	1/3	**2/3**	**3/3**	**1/2**	**2/2**	**1/1**
Light intensity	bright	dim	none	dim	none	none
Number of responses	20	20	20	20	20	20
Number of rewards			20		20	20

Table 20: *Showing the total number of presentations of the different schedule fraction cue intensities within each session: number of times bright light is presented = 20, number of times dim light is presented = 40, number of times no light is presented = 60*

It could be that any effects seen on the different performance measures are due to the rats having been presented with the bright light cue far less often than the other cue lights, and therefore having learnt the association between the cue and the response required by that schedule fraction less well. This would seem unlikely given the amount of training that the rats receive, but it is still a possibility. The planned comparison between schedule fractions 2/3 and 1/2 goes some way towards addressing this problem, since both schedule fractions are presented 20 times within the session, both are signalled by a dim light and both are unrewarded. If performance is not equivalent for these schedule fractions, it would suggest that the rats' behaviour is not merely due to the number of times that they has been presented with a given schedule fraction, but if performance is equivalent for these schedule fractions then the possibility still remains. In the first experiment, the preoperative results of the planned comparison between the means for 2/3 and 1/2 show a significant difference for the Correct Responses and Movement Time measures, but not for the Reaction Time and Post Response Pause measures. In the second experiment, the preoperative results of the planned comparison

between the means for 2/3 and 1/2 show a significant difference for all of the measures (Correct Responses, Reaction Time, Movement time and Post Response Pause). There would therefore seem to be some evidence to support the idea that the rats' behaviour is influenced by factors other than the number of times that they have been presented with the different schedule fractions.

However, it could also be argued that the very fact that there are differences between the means for 2/3 and 1/2 on the Correct Responses measure supports the idea that any differences in performance are due to the rats having been presented with the bright light cue far less often than with the other cue lights. As explained in the the Experimental procedure section for Experiment A, a rat that makes an anticipatory error on a given schedule fraction is 'timed out' and then re-presented with the cue for that same schedule fraction, and this continues until the rat completes the schedule fraction correctly. Therefore, as the mean percentage of anticipatory errors increases, the mean percentage of correct responses decreases. In both the experiments, the highest mean percentage of anticipatory errors and the lowest mean percentage of correct responses made by the rats occurred on schedule fraction 1/3. The most likely explanation of this pattern of results is that the distance from reward increases the likelihood of the rats making anticipatory errors out of impatience, but there is also a possibility that it is due to the rats having insufficiently learned the connection between completing the work schedule correctly and the distant reward. Ideally, this possibility would be investigated by comparing performance after 60, 120, 180 etc. presentations of schedule fraction 1/3 and of 1/1, 2/2 and 3/3, and by comparing performance after 40, 80, 120 etc. presentations of schedule fractions 1/2 and 2/3 and of 1/1, 2/2 and 3/3.

Another issue concerns the fact that the light intensities for schedule fractions 1/2 and 2/3 remained the same during the Reversal task as during the Preoperative and Postoperative stages, i.e. dim, and so the rats did not have to relearn the cues signalling these schedule fractions ***(Table 21)***. This issue could be easily solved by switching the light intensities as in the table below ***(Table 22)*** rather than reversing them:

Stage	Schedule Fractions					
	1/3	2/3	3/3	1/2	2/2	1/1
Preoperative and Postoperative	bright	dim	none	dim	none	none
Reversal	none	dim	bright	dim	bright	bright

Table 21: *Showing the cue light intensities for the different schedule fractions during the Preoperative and Postoperative stages and the Reversal stage.*

Stage	Schedule Fractions					
	1/3	2/3	3/3	1/2	2/2	1/1
Preoperative and Postoperative	bright	dim	none	dim	none	none
Reversal	none	bright	dim	bright	dim	dim

Table 22: *Showing the cue light intensities for the different schedule fractions during the Preoperative and Postoperative stages and possible cue light intensities for the Reversal stage.*

Moreover, if the light intensities for the different cues also did not progress from bright through dim to none or vice versa in the Preoperative and Postoperative stages, but were allocated as below ***(Table 23)***, for example, then there could be no suggestion that any differences in performance on the different schedule fractions were due to a stimulus generalisation effect:

Stage	Schedule Fractions					
	1/3	2/3	3/3	1/2	2/2	1/1
Preoperative and Postoperative	none	bright	dim	bright	dim	dim
Reversal	dim	none	bright	none	bright	bright

Table 23: *Showing possible cue light intensities for the different schedule fractions during the Preoperative,Postoperative and Reversal stages.*

A third issue lies in the use of different foreperiods. In the results section and the discussion to the first experiment it was suggested that the rats performed differently on the shorter and longer foreperiods for some of the measures. It was also suggested that longer foreperiods might have a 'flattening' effect on performance of the SFC task. Although neither appears to have been the case in the second experiment, perhaps because there were only three foreperiods rather than five, or because training did not continue for as long as it did in the first experiment, it would be interesting to investigate further, perhaps by using four different foreperiods of 200, 400, 600 and 800 milliseconds. Moreover, foreperiod obviously has a major impact on

performance for some of the measures, and it would be of interest to try and tease out the relative contributions of motor readiness and motivation to performance of the SFC task.

The SFC task is a fairly novel procedure, and as such little is known about the mechanisms underlying its performance. In the introduction and discussion to the previous experiment, it was suggested that the cue lights signalling the onset of each work schedule might act as discriminative stimuli, and the changing intensities of cue light within each work schedule as conditioned reinforcers that have a motivational influence on performance. As detailed previously, recent studies using discriminative stimuli have found that amygdala lesions (Malkova, Gaffan et al. 1997) and BLA lesions (Burns, Everitt et al. 1999) have no effect on performance, whilst other studies have found that BLA lesions do affect the acquisition of the new response with conditioned reinforcement (Cador, Robbins et al. 1989; Burns, Robbins et al. 1993). Thus the results of both the previous and this experiment could be explained by the theory that whilst the BLA-lesioned rats' performance was influenced by the cue lights in their capacity as discriminative stimuli, it was no longer influenced by the cue lights in their capacity as conditioned reinforcers. However, even if the above theory is accepted, it could be reasonably expected, perhaps, that the BLA lesions would have a larger effect on those aspects of performance that are governed by the cue lights in their capacity as conditioned reinforcers, namely performance within the work schedules. That they didn't might be due to the 'carry over' motivational influence of the discriminatory stimuli signalling the onset of each work schedule. Another interesting possibility is that the schedule fraction cues in the SFC task are not primarily appetitive, as has been assumed up to now, or at least not all of them are. It could be argued that the cues for schedule fractions 1/3, 2/3 and 1/2 are not appetitive, but in fact neutral or even slightly aversive in that they signal the absence of reward on that particular correct response, and that it is this which causes the rats to perform less well. However, even if this should be the case, there is some evidence to suggest that lesioning the BLA would not affect this pattern of response: although it is extremely unlikely that these schedule fraction cues are as aversive as those employed in the conditioned suppression procedure, Killcross et al (1997) have shown that BLA-lesioned rats exhibit normal suppression

of responding on a lever for reward during the presentation of a conditioned stimulus that has previously been associated with punishment. Interestingly, though, Killcross et al showed in the same experiment that CeN-lesioned rats do not exhibit normal suppression of responding on this task.

In their discussion Burns et al (1993) proposed that their instrumental conditional visuospatial discrimination task exemplifies visual habit learning as described by Mishkin (1984), which the latter claimed to be a form of stimulus-response learning rather than stimulus-reward associative learning. Burns et al suggested that their BLA-lesioned rats showed only transient deficits in performance on an instrumental conditional visuospatial discrimination task because they were able to compensate for the attenuation of their ability to use the conditioned stimulus as a conditioned reinforcer to energise performance by an increased reliance on the stimulus-response associations that underlie the task. In the discussion to the previous experiment, it was observed that it is the CeN rather than the BLA which is thought to be involved in stimulus-response learning (Everitt, Cardinal et al. 2000). Also, Hall et al (2001) had found that lesions of the CeN but not of the BLA abolished the Pavlovian-to-instrumental transfer effect, by which Pavlovian cues influence instrumental responding. Stimulus-response association is also thought to underlie the Pavlovian-to-instrumental transfer effect (Everitt, Cardinal et al. 2000), and this procedure bears some similarities with the SFC task. Given the lack of effect of BLA lesions on performance of the SFC task, it was thought worthwhile to run a group of CeN-lesioned rats alongside the BLA-lesioned rats when replicating the experiment in the hope that a dissociation in the effects of BLA and CeN lesions on performance might provide evidence supporting the idea that stimulus-response associations also underlie the SFC task. Unfortunately, the CeN lesions were both unilateral and very partial, and it was not possible to discern any effect of lesion on performance of the SFC task at all.

However, in the discussion to the previous experiment, it was also argued that perhaps the lack of impairment in the BLA-lesioned rats' performance in the SFC task was not due to the same reason advanced by Burns et al (1993), namely that conditional discrimination tasks exemplify visual habit learning and as such are sub-served by stimulus-response rather

than stimulus-reward learning, since performance in both Burns et al's task and in the Pavlovian-to-instrumental transfer procedure (Hall, Parkinson et al. 2001) was impaired by lesions of the NAcc, whereas performance in the SFC task was not (Bowman and Brown 1998). Given this discrepancy, it would be sensible to undertake further research into the mechanisms underlying the SFC task.

It would also seem sensible to consider whether the SFC task is, in fact, a particularly good way to assess perception of 'cost-of-reward' when compared to other cost/benefit procedures such as the fixed ratio schedules or T-mazes employed by Salamone and his colleagues (Salamone, Cousins et al. 1994; Salamone, Cousins et al. 1994; Salamone, Cousins et al. 1996; Salamone, Cousins et al. 1997). However, the value of the SFC task lies not only in its usefulness as a measure of the perception of 'cost-of-reward', but also in its usefulness as a measure of the perception of 'progress-to-reward'. As such, it is of interest to consider what other systems or structures in the brain might support associative information about 'progress-to-reward'. As stated in the introduction, the SFC task was adapted from a very similar procedure designed by Bowman et al (1996) to study motivational processes underlying 'progress-to-reward' in monkeys. The original procedure has since been used by Richmond and his colleagues to investigate the role of perirhinal cortex in attaching motivational significance to visual cues (Liu, Murray et al. 2000; Liu and Richmond 2000). In monkeys, perirhinal cortex has strong connections with inferior temporal cortex (Saleem and Tanaka 1996), a higher-order neocortical field involved in visual information processing (Mishkin 1982), and with both the ventral striatum (Baizer, Desimone et al. 1993) and the amygdala (Aggleton, Burton et al. 1980; Stefanacci, Suzuki et al. 1996; Shi and Cassell 1999). Richmond and his colleagues found that neurons in monkey perirhinal cortex carry signals related to the motivational significance of the visual cues used in their task, with some neurons responding to the first schedule fraction within any work schedule, and others to the last schedule fraction within any work schedule, and still other neurons responding selectively to a particular schedule fraction within a specific work schedule (Liu and Richmond 2000). They then went on to lesion the rhinal cortex in monkeys, and found that although the performance of the lesioned animals differed according to whether a

schedule fraction was rewarded or unrewarded, it no longer differed between the unrewarded schedule fractions. Moreover, when a new set of visual cues that signalled progress through the work schedules was introduced, the lesioned monkeys appeared unable to predict the amount of work needed to obtain the reward. Richmond and his colleagues concluded that the rhinal cortex is critical to the attachment of motivational significance to visual cues, and therefore to the neural circuitry involved in translating motivation to action (Liu, Murray et al. 2000).

Interestingly, however, Baxter et al (1999) have recently tested monkeys with rhinal cortex lesions on the same task used by Malkova et al (1997), which involved learning visual discrimination problems for auditory secondary reinforcement. As described earlier, Malkova et al found that monkeys with selective neurotoxic lesions of the amygdala were unimpaired on the task, but Baxter et al found a small though statistically significant impairment. The emphasis is on the smallness of the impairment - it might be that rhinal cortex participates in stimulus-response learning (Baxter et al (1999) and Murray and Bussey (1999) suggest to the extent that it is required to identify the stimulus to be associated with reward), but is not critical to discrimination learning. Instead, Baxter and Murray (2000) suggest that disconnection of inferior temporal cortex from mediodorsal thalamus and ventromedial PFC would produce a severe impairment in discrimination learning for auditory secondary reinforcement, and presumably in other discrimination tasks as well. They base this suggestion on the observation that the original lesions made by Gaffan and Harrison (1987), which caused severe impairment in discrimination learning for auditory secondary reinforcement, also indirectly damaged inferior temporal cortex, and directly and indirectly damaged entorhinal and perirhinal cortex, thereby disconnecting widespread areas of inferior temporal cortex from mediodorsal thalamus and ventromedial PFC. Support for this idea is provided by Gaffan et al's finding (1993) that lesions of both mediodorsal thalamus and ventromedial PFC, crossed with aspirative lesions of the amygdala, produced severe impairments in discrimination learning set performance.

Given the above findings, it would appear that the next logical step with regard to the SFC task would be to investigate both the effect of rhinal cortex lesions and the effect of disconnecting inferior temporal cortex from mediodorsal thalamus and ventromedial PFC on

performance. Another line of investigation lies in the direct connection between the BLA and orbitofrontal cortex (Kolb 1984; McDonald 1991; Amaral, Price et al. 1992). Orbitofrontal cortex (OFC) is an area of PFC that is associated with response integration and decision-making (Damasio 1994; Duncan, Emslie et al. 1996; Rolls 1996; Bechara, Damasio et al. 1997). As discussed in the introduction to the first experiment, BLA-lesioned rats are unable to adjust their behaviour in the presence of a CS that signals a previously desired but now devalued reward (Hatfield, Han et al. 1996). This has also been shown to be the case in rats with neurotoxic OFC lesions (Gallagher, McMahan et al. 1999), and has lead Gallagher to suggest that interconnections between the BLA and the OFC provide an important substrate for encoding the relationship between an event and the value of its expected outcome (Gallagher 2000). Likewise, Baxter et al (2000) have shown that rhesus monkeys with 'disconnection' lesions of the amygdala in one hemisphere and of OFC in the other hemisphere, combined with forebrain commissurotomy, are unable to adjust their choice behaviour when a reward outcome is devalued, suggesting that interaction between these areas is required for decision-making based on expected outcomes. This theory is supported by the findings of Schoenbaum et al (1998) who recorded from neurons in the BLA and the OFC of rats learning an olfactory discrimination task. They found that a substantial proportion of neurons sampled in both regions developed neural activity that differed reliably between trials with a negative outcome and trials with a positive outcome, thereby seeming to reflect an expectation about the impending consequences of making a particular response. Interestingly, this differential neural activity manifested itself before the rats had developed a behavioural strategy to avoid the aversive outcome. The above findings are of interest because, in many respects, the concept of 'progress-to-reward' resembles that of 'expectation of reward', and the SFC task could equally well be said to be measuring the latter rather than the former. It would therefore appear worthwhile to investigate the effect of OFC lesions on performance of the SFC task. All in all, the SFC task is potentially a very valuable tool in the study of reward-related learning, and could be utilised to study both 'progress-to-reward' and 'expectation of reward' but perhaps the most important first step to take is to establish what mechanisms underlie its performance.

Appendix A: Montague et al's model

Most early artificial neural networks were trained using supervised learning paradigms such as the error back propagation method (Rumelhart, Hinton et al. 1986). Supervised learning paradigms consist of matching a given set of input patterns with a set of target output patterns. The output unit's actual activity is compared with its target output in order to generate an error vector, which then is used to inform the output unit as to how it should change its weighting and thus its activity so as to achieve the target output. The error back propagation process makes similar information available to all the units in the network. Supervised learning is therefore dependent on the system already having a representation of the desired outcome. Montague et al's (Montague, Dayan et al. 1996) model, however, employs a more recent training paradigm called reinforcement learning (Myers 1991), whose principles are believed to be more closely compatible with how real neural systems work. Rather than trying to match a standard of correctness, a reinforcement learning system relies on evaluative feedback. This informs the network if its performance has improved or not, and sometimes by how much, but no direction is given as to how to correct the error. In this way reinforcement learning provides a measure of the "goodness of behaviour". The network tries to maximise this "goodness of behaviour" by actively generating different alternatives and comparing the result with previous results, and then selecting the better alternative. The use of evaluative feedback means that a system's behaviour can be adjusted without any knowledge of what the correct behaviour would be. A major advantage of reinforcement learning systems over supervised learning systems is that learning takes place on a generate-and-test basis, as it does in biological systems (Thorndike's "Law of Effect", which stipulates that if an action is followed by a satisfactory state of affairs or an improvement in that state, then it is likely to be reinforced (Thorndike 1911). But biological systems are very often presented with a multitude of stimuli that could lead to a particular outcome, and have to decide which is the most likely predictor of that outcome. Moreover, any action taken can have delayed as well as immediate consequences. This has been called the 'temporal credit assignment problem' (Barto 1994). Reinforcement learning systems like

Montague's model have attempted to solve this problem by first learning to anticipate reinforcing events, and then using this knowledge to adjust the behaviour of the system to maximise the frequency and / or magnitude of the reinforcing events encountered over time.

2.1.2 Montague et al's (Montague, Dayan et al. 1996) model

The model attempts to show how an animal learns to associate the presentation of a reward with a sensory stimulus coming from the environment. It is made up of three layers: (1) the cortical layer, which represents sensory input, (2) an intermediate layer, which receives weighted input from the cortical layer, and which represents what is probably happening in the limbic system, and (3) the VTA, where the cell bodies of dopamine neurons are located. The model assumes that the computational role of the system (in this case dopamine neurons in the VTA) is to predict a discounted sum of all future rewards, 'V(t)'. V(t) is discounted in that rewards which arrive sooner are registered as being more important than rewards which arrive later.

Each learning trial is divided into a series of timesteps (t) - say 20 in all, and a stimulus is presented as a series of cortical representations, one for each timestep, which last from the onset of the stimulus to its offset. At timestep 1 a cortical representation of a sensory stimulus travels to the intermediate layer. On its journey a weight is applied to it. This weight characterises the strength of the cortical representation's influence on a dopamine neuron in the VTA at that timestep (t), and is adjustable – this point will be returned to later. The intermediate layer assesses the cortical representation and its weighting at that timestep (t) and sends on a signal, V(t) to the dopamine neuron in the VTA. V(t) is made up of the cortical representation (V) x the weighting at that timestep (t) and indicates the amount of reward which currently is associated with the sensory cue. Then another cortical representation at the next timestep, 2, travels to the intermediate layer, with a weight being applied to it en route. The intermediate layer again assesses the cortical representation and its weighting at the second timestep (t) and sends on the V(t) signal to the dopamine neuron in the VTA. At the same time the dopamine neuron in the

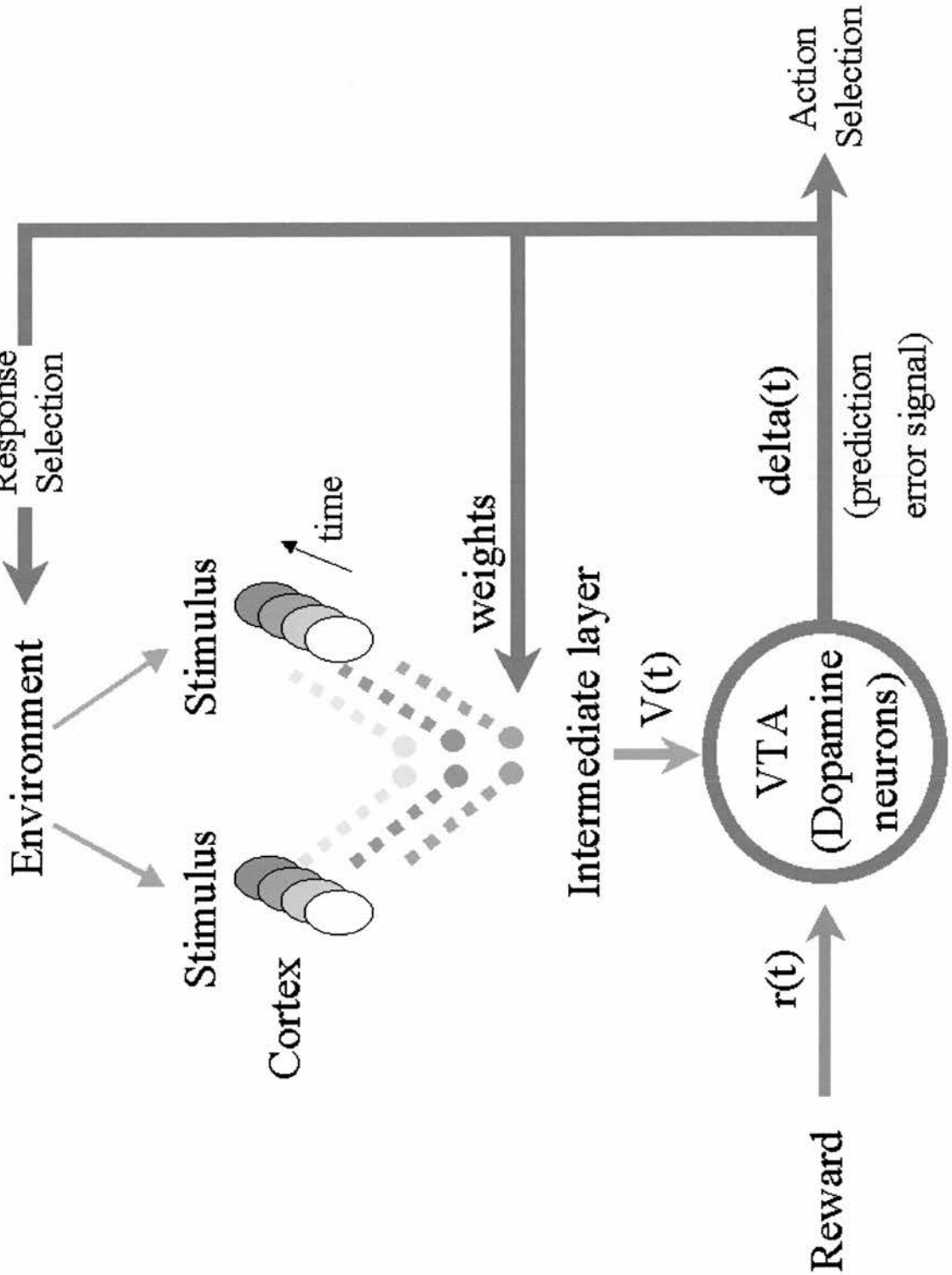


Figure 31: *Adaptation of Montague et al's (1996) Model (taken from Schultz et al 1997). V(t) is the discounted sum of all future rewards, r(t) is reward at time t, and delta(t) = r(t) + (V(t1) – V(t2)). See Montague et al (1996) and Schultz et al (1997) for further details.*

VTA is receiving a reward signal, r(t), where r = reward, and t = a given timestep. The dopamine neuron in the VTA then sends out the temporal difference or prediction error signal,

delta(t), which is composed of the reward signal r(t) plus the difference between V(t) at timestep 1 and V(t) at timestep 2, i.e. delta(t) = r(t) + (V(t1) - V(t2)). The VTA output, delta(t), is sent to various structures including the ventral striatum and the PFC, and importantly, feeds back to the weight associated with the last cortical representation and causes it to be adjusted. Eventually, after several presentations of the stimulus followed by the reward, the adjustments in weights will cause estimations of V(t) to converge to the true V(t) and the system will have 'learnt' by successive approximation that the stimulus predicts the reward. This process can be looked at trial by trial: the first time that the stimulus is presented and followed by the reward, the animal doesn't associate them. So although the cortical representations at timesteps 1 and 2 travel to the intermediate layer, no weights are applied to them - the weights are set at 0 if it is assumed that they can vary between 0 and 1. Likewise, at this point, no reward is expected, so V(t) is also set at 0 if it is assumed that V(t) can also vary between 0 and 1. But the animal receives this wonderful unexpected reward, so the reward signal is at maximum - 1, if it is again assumed that it can vary between 0 and 1. The dopamine neuron then adds together r(t) and V(t) to obtain and transmit the prediction error signal delta(t). Delta(t) feeds back to the weights and causes them to adjust so that the next time the sensory cue occurs, the reward is half-expected and so V(t) has a value of say 0.5. (V(t) can be regarded as a 'surprise' signal, which reflects the degree to which the current sensory state differs from the previous one). Since presentation of the reward is now half-expected, its impact is diminished, and r(t) has a value of say 0.5. Likewise, the prediction error signal sent out by the dopamine neuron is also diminished, and this again feeds back to the weight associated with the cortical representation of the stimulus, increasing it, but by a lesser amount than the first time. When the stimulus is presented for the third time it is even more expected that the reward will follow, so the value of V(t) is even greater, say 0.75, and the value of r(t) is smaller, say 0.25. Eventually, after several presentations of the stimulus followed by the reward, the adjustments in weights will cause estimations of V(t) to converge to the true V(t) and the system will have 'learnt' by successive approximation that the stimulus predicts the reward.

Implementations of Montague et al's model

Schultz et al (Schultz, Dayan et al. 1997) have used Montague et al's (Montague, Dayan et al. 1996) model to make several predictions about the activity of dopaminergic neurons in different learning situations, and these have been explored by further implementations of Montague et al's model carried out by the author and Eric Bowman. However, it should be borne in mind that these predictions have not yet been tested electro-physiologically.

1) Prediction of reward:

Implementation 1 shows the stimulus being presented between timesteps 12 and 17 and the reward following at timestep 20. For the first few trials, activity only occurs when the reward is presented at timestep 20, but with successive trials this activity shifts forward in time until it occurs at the beginning of the stimulus presentation (at timestep 12) and not at all when the reward is presented. This pattern closely resembles Schultz et al's neurophysiological data, although they did not observe the transition in the timing of the dopamine response as conditioning occurs.

2) Prediction of blocking:

The model predicts that if two stimuli are presented serially and followed by the reward, then the response shifts to the onset of the earliest stimulus. The authors claim that this result can explain blocking in real behavioural paradigms (Schultz 1997, p.1596) (Schultz, Dayan et al. 1997). However, this claim seems somewhat premature, given that the model is presented with both stimuli simultaneously from the start, rather than being 'preconditioned' with one stimulus before the other stimulus is introduced. However, in a further implementation (***Implementation 2***) of the model, 'preconditioning' with one stimulus before the other is introduced leads to the same results: during the first 50 trials stimulus A is presented alone between timesteps 12 and 17 with the reward following at timestep 20. Activity initially occurs when the reward is presented at timestep 20, but then shifts forward in time to the stimulus presentation. From trials 50 to 100 both stimulus A and stimulus B are presented simultaneously between timesteps 12 and 17 with the reward following at timestep 20. Activity continues to

occur at the time of presentation of the stimuli rather than the reward. Stimulus B is then presented alone between timesteps 12 and 17, but with no reward following at timestep 20. No activity occurs at all. Finally stimulus A is presented alone between timesteps 12 and 17, again with no reward following at timestep 20. Activity occurs at the time of presentation, but there is inhibition (blue colour) occurring at the time that the reward should have occurred, indicating that expectancy of reward has been disappointed.

3) Prediction of serial conditioning:

The model also predicts that associations will be made between conditioned stimuli during serial conditioning (***Implementation 3***): during the first 50 trials stimulus A is presented alone between timesteps 5 and 10 with the reward following at timestep 20. There is no shift in activity from presentation of the reward to presentation of the stimulus, suggesting that no conditioning is occurring - the stimulus is too distant in time from the reward. From trials 50 to 100 stimulus A is presented between timesteps 5 and 10 and stimulus B is presented between timesteps 12 and 17, with the reward following at timestep 20. Activity over these trials shifts forward in time through the presentation of stimulus B to the presentation of stimulus A. When stimulus A is then presented alone between timesteps 5 and 10, with no reward following, there is inhibition (blue colour) at the time that the second stimulus B should have occurred rather than at the time that the reward should have occurred. However, when stimulus B is presented alone between timesteps 12 and 17 with no reward following, then there is inhibition at the time that the reward should have occurred. The same pattern of activity is seen in ***Implementation 4***, in which stimulus B is presented alone between timesteps 12 and 17 for the first 50 trials, with the reward following at timestep 20: there is a shift in activity from presentation of the reward to presentation of the stimulus. From trials 50 to 100 stimulus A is presented between timesteps 5 and 10 and stimulus B is presented between timesteps 12 and 17, with the reward following at timestep 20. Activity over these trials shifts forward in time from the presentation of stimulus B to the presentation of stimulus A. When stimulus A is then presented alone between timesteps 5 and 10, with no reward following, there is inhibition (blue colour) at the time that the second stimulus B should have occurred rather than at the time that the reward should have occurred.

However, when stimulus B is presented alone between timesteps 12 and 17 with no reward following, then there is inhibition at the time that the reward should have occurred. This strongly suggests that the earlier stimulus has become associated with the second stimulus, which has in turn become associated with the reward, i.e. serial conditioning is occurring. These results go some way to answering the question outlined previously about whether second order conditioning is due to a growth in the association between the first and second stimuli, rather than in the association between the first stimulus and a general representation of the reward activated by the presentation of the second stimulus. The pattern of activity seen in ***Implementations 3 and 4*** after presentation of stimulus A alone and not followed by reward also has implications for the blocking paradigm, in which animals are purported to ignore (i.e. not become conditioned to) the existence of a second stimulus after learning that a first stimulus leads to a reward: the inhibition seen at the time that the second stimulus should have occurred suggests that even though behavioural blocking may occur, the dopamine neurons have access to representations of the 'blocked' stimulus.

Figure 32, Implementations 1-4

All implementations of Montague et al's (Montague, Dayan et al. 1996) model were suggested by the author and carried out by Eric Bowman, School of Psychology, University of St Andrews.
The x axis represents the number of timesteps (20) making up a trial and the y axis represents the number of trials which took place. How dense the red is indicates the degree of dopamine activity occurring relative to baseline, and how dense the blue is indicates the amount of inhibition of dopamine activity occurring relative to baseline.

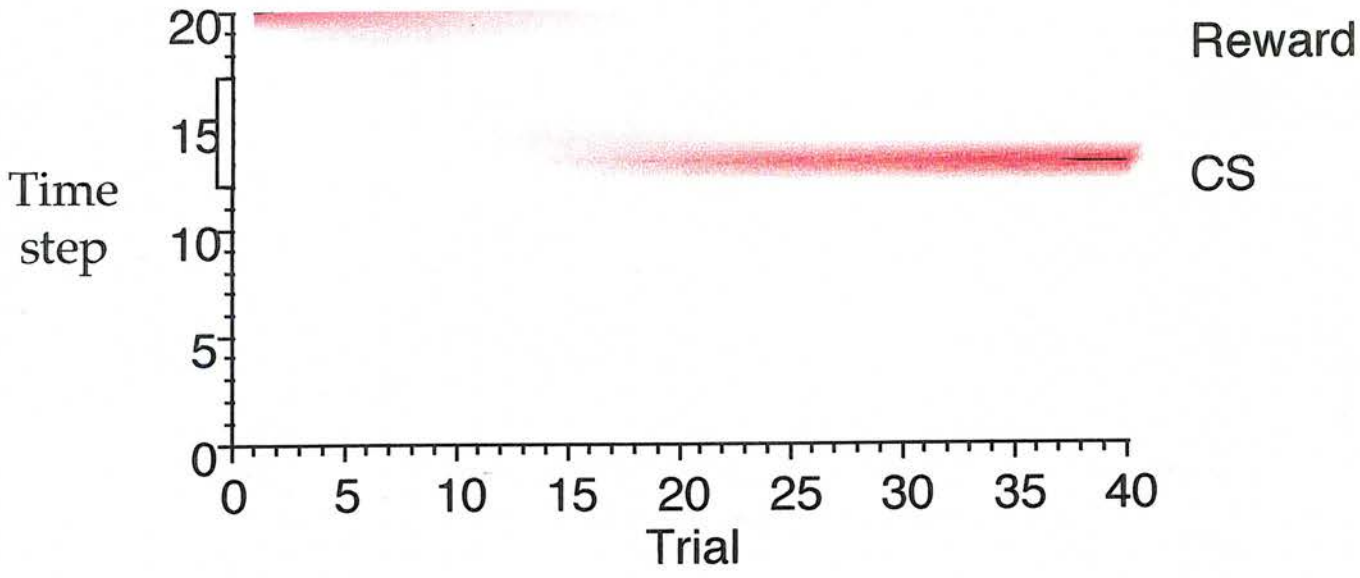
Implementation 1 (prediction of reward)

Implementation 2 ("blocking")

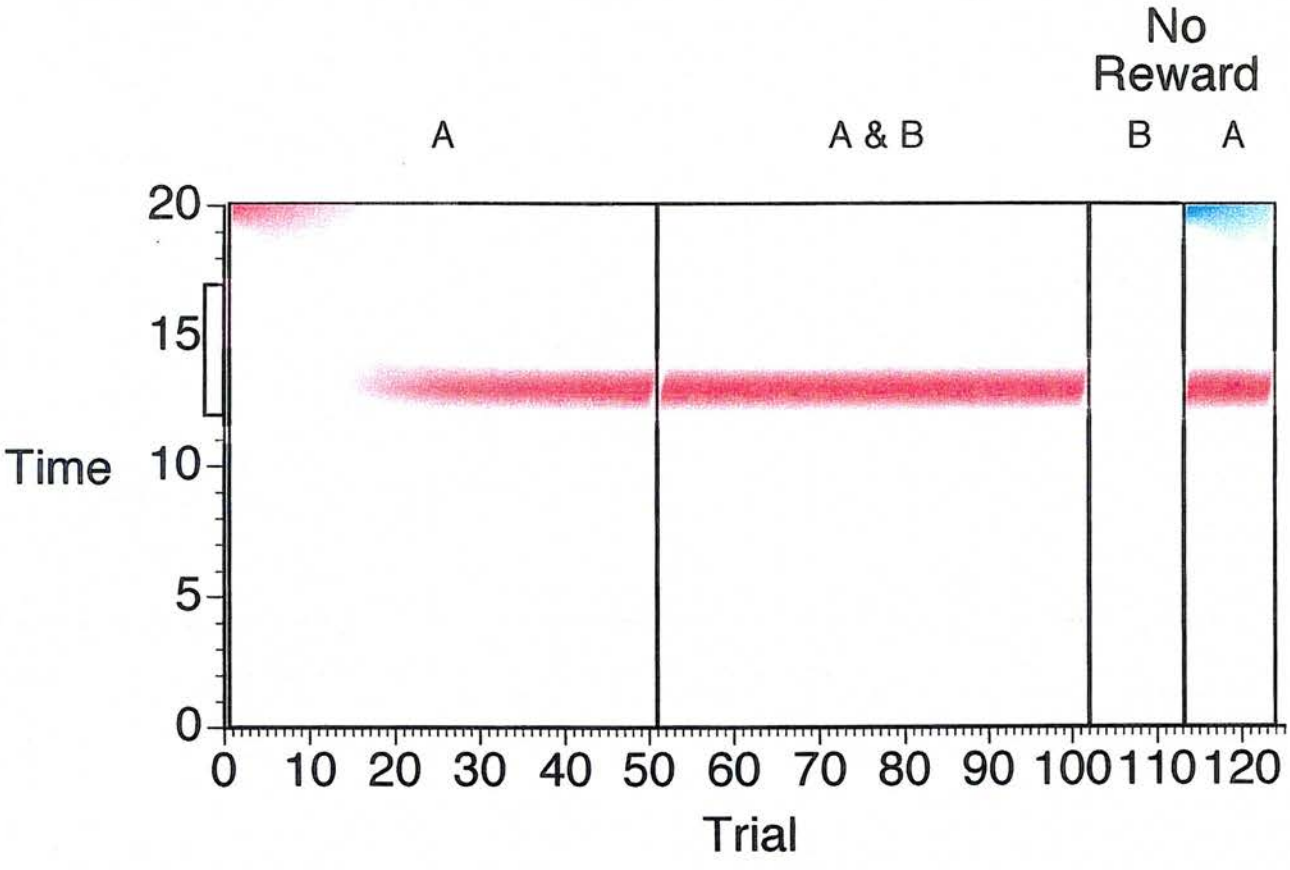
Implementation 3 ("serial conditioning")

Implementation 4: ("serial conditioning")

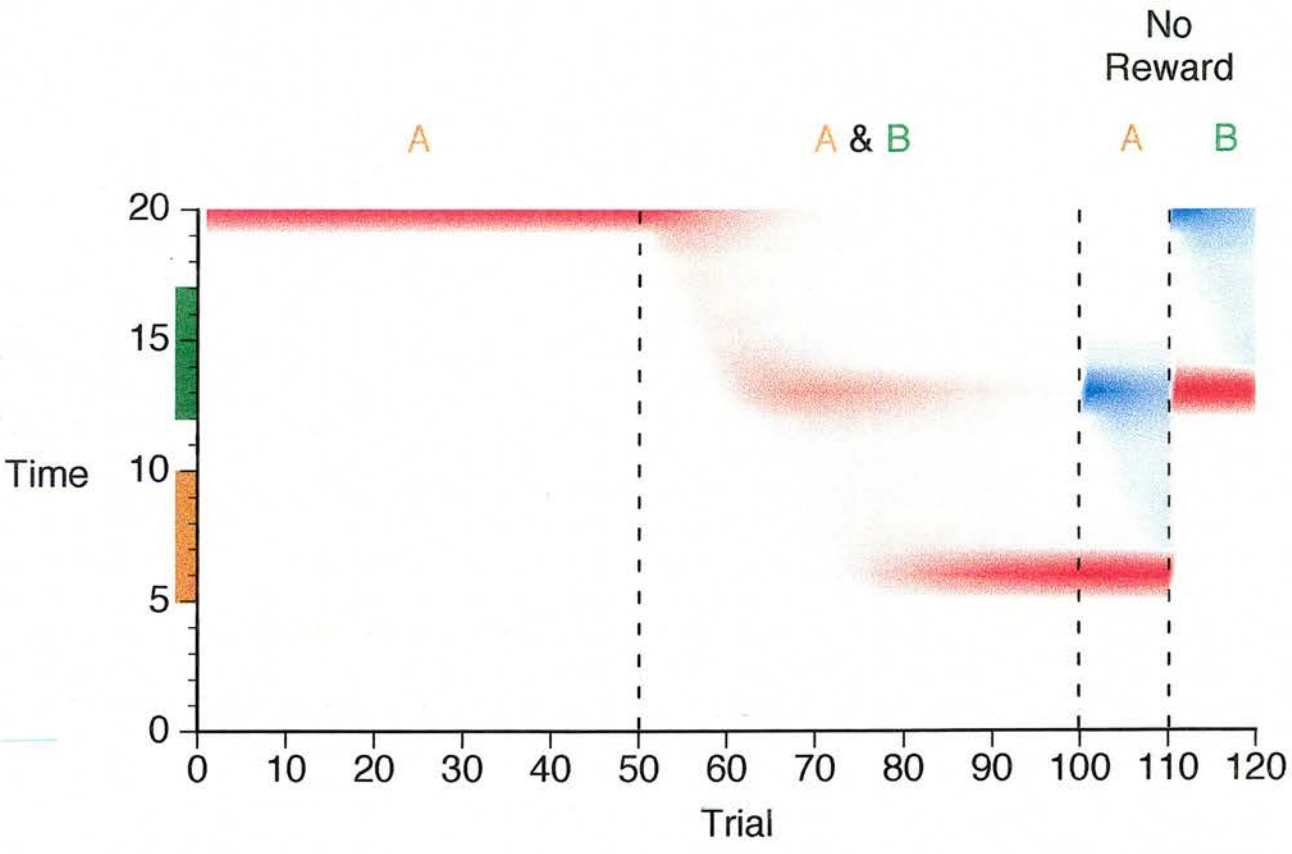
Implementation 1



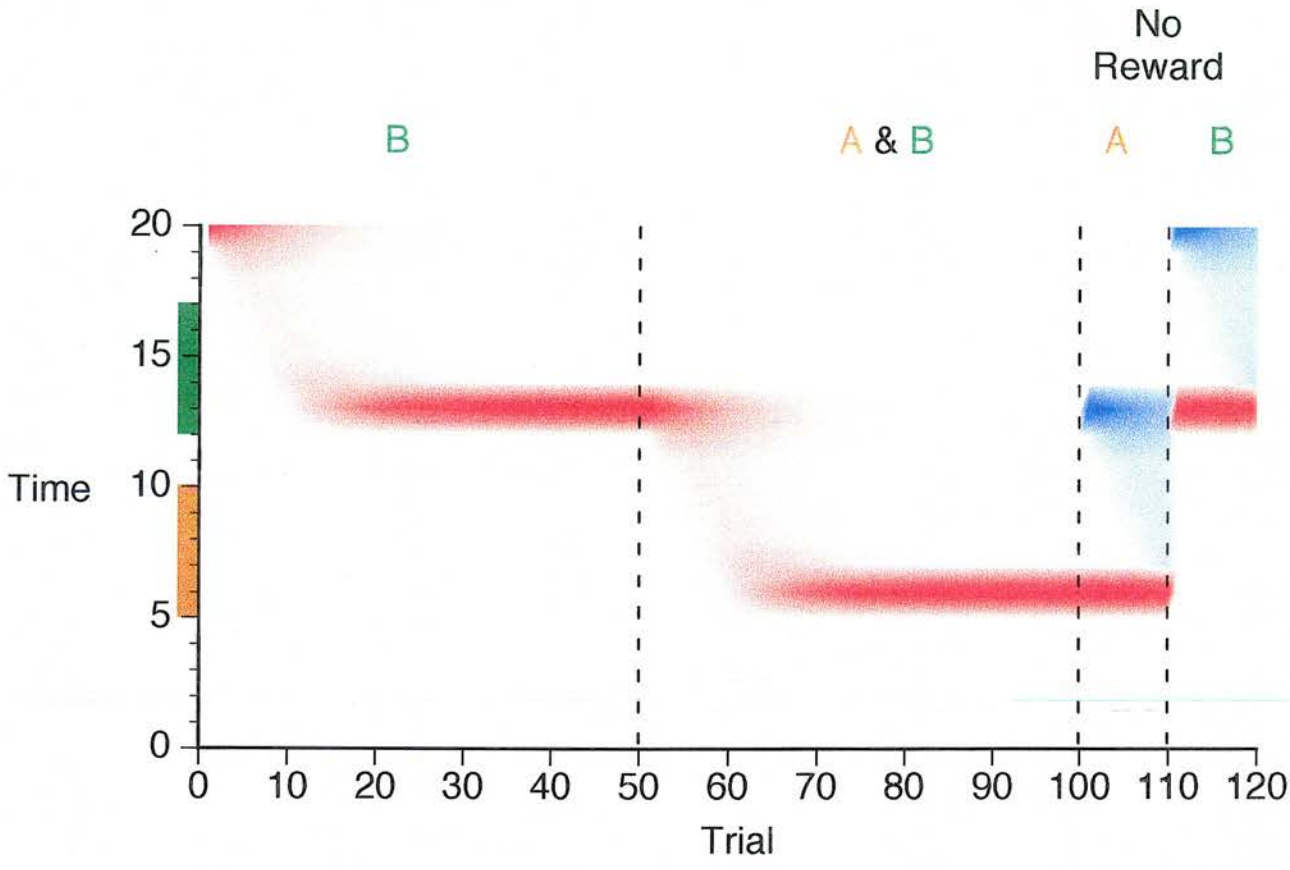
Implementation 2



Implementation 3



Implementation 4



Appendix B: Mauchly's Test of Sphericity

In both SFC task experiments, homogeneity of covariance was tested using Mauchly's Test of Sphericity. Most of the tests (given in the tables below) were significant, and the Huynh-Feldt correction was therefore used in all cases to lessen the risk of a true Type I error, i.e. that the null hypothesis will be rejected when it is in fact true.

The SFC task: Experiment A

Analysis of preoperative performance

	Correct Responses	Reaction Time	Movement Time	Post Response Pause
Schedule Fraction	sig.	sig.	sig.	sig.
Foreperiod	sig.	sig.	not sig.	sig.

Table 24: *Results of Mauchly's Test of Sphericity on the preoperative ANOVAs for the various measures*

Analysis of postoperative performance

ANOVA set	Measure	Schedule Fraction	Foreperiod
Postoperative Sham - Postoperative Lesion	Correct Responses	not sig.	sig.
	Reaction Time	sig.	sig.
	Movement Time	sig.	not sig.
	Post Response Pause	sig.	not sig.
Preoperative Sham - Postoperative Sham	Correct Responses	sig.	sig.
	Reaction Time	sig.	sig.
	Movement Time	sig.	not sig.
	Post Response Pause	sig.	sig.
Preoperative Lesion - Postoperative Lesion	Correct Responses	sig.	sig.
	Reaction Time	sig.	sig.
	Movement Time	sig.	not sig.
	Post Response Pause	sig.	not sig.

Table 25: *Results of Mauchly's Test of Sphericity on the different measures for the 3 sets of ANOVAs used to analyse postoperative performance for the various measures*

The SFC task: Experiment B

Analysis of preoperative performance

	Correct Responses	Reaction Time	Movement Time	Post Response Pause
Schedule Fraction	not sig.	sig.	sig.	sig.
Foreperiod	sig.	sig.	sig.	not sig.
Schedule Fraction*Foreperiod	sig.	sig.	sig.	sig.

Table 26: *Results of Mauchly's Test of Sphericity on the preoperative ANOVAs for the various measures*

Analysis of preoperative/postoperative performance

	Correct Responses	Reaction Time	Movement Time	Post Response Pause
SF	sig.	sig.	sig.	sig.
FP	sig.	sig.	not sig.	not sig.
SF*FP	sig.	sig.	sig.	sig.
SF*Pre/Post	sig.	sig.	sig.	sig.
FP*Pre/Post	sig.	not sig.	sig.	not sig.
SF*FP*Pre/Post	not sig.	sig.	sig.	sig.

Table 27: *Results of Mauchly's Test of Sphericity on the preoperative / postoperative ANOVAs for the various measures*

Analysis of reversal performance

Effect	Correct Responses			
	Postop – Reversal	Last day Postop – First day Reversal	First day Reversal – Last day Reversal	Last day Postop – Last day Reversal
SF	not sig.	not sig.	not sig.	not sig.
FP	sig.	sig.	sig.	sig.
SF*FP	sig.	not sig.	not sig.	sig.
SF*Post/Rev	sig.	not sig.	not sig.	not sig.
FP*Post/Rev	not sig.	not sig.	not sig.	not sig.
SF*FP*Post/Rev	sig.	sig.	not sig.	not sig.

Table 28: *Results of Mauchly's Test of Sphericity on the postoperative / reversal ANOVAs used to analyse reversal performance for the measure of mean percentage of correct responses.*

Appendix C: Simple main effects

The SFC task: Experiment A

Analysis of preoperative performance

Correct Responses

Factor	F value	P value
Foreperiod		
FP 100	F (20, 200) = 09.50	$p < 0.01$
FP 200	F (20, 200) = 10.31	$p < 0.01$
FP 300	F (20, 200) = 12.18	$p < 0.01$
FP 400	F (20, 200) = 08.79	$p < 0.01$
FP 500	F (20, 200) = 06.28	$p < 0.01$
Schedule Fraction		
SF 1/3	F (20, 200) = 24.45	$p < 0.01$
SF 2/3	F (20, 200) = 17.09	$p < 0.01$
SF 3/3	F (20, 200) = 64.53	$p < 0.01$
SF 1/2	F (20, 200) = 26.81	$p < 0.01$
SF 2/2	F (20, 200) = 52.45	$p < 0.01$
SF 1/1	F (20, 200) = 42.63	$p < 0.01$

Table 29: *Simple main effects of changing levels of schedule fraction at different foreperiods and vice versa*

Reaction Time

Factor	F value	P value
Foreperiod		
FP 100	F (20, 200) = 14.06	$p < 0.01$
FP 200	F (20, 200) = 11.80	$p < 0.01$
FP 300	F (20, 200) = 18.65	$p < 0.01$
FP 400	F (20, 200) = 19.60	$p < 0.01$
FP 500	F (20, 200) = 15.22	$p < 0.01$
Schedule Fraction		
SF 1/3	F (20, 200) = 36.76	$p < 0.01$
SF 2/3	F (20, 200) = 106.50	$p < 0.01$
SF 3/3	F (20, 200) = 80.81	$p < 0.01$
SF 1/2	F (20, 200) = 69.94	$p < 0.01$
SF 2/2	F (20, 200) = 87.34	$p < 0.01$
SF 1/1	F (20, 200) = 75.79	$p < 0.01$

Table 30: *Simple main effects of changing levels of schedule fraction at different foreperiods and vice versa*

Movement Time

Factor	F value	P value
Foreperiod		
FP 100	F (20, 200) = 52.91	p < 0.01
FP 200	F (20, 200) = 53.96	p < 0.01
FP 300	F (20, 200) = 49.45	p < 0.01
FP 400	F (20, 200) = 33.89	p < 0.01
FP 500	F (20, 200) = 27.56	p < 0.01
Schedule Fraction		
SF 1/3	F (20, 200) = 9.02	p < 0.01
SF 2/3	F (20, 200) = 0.43	p > 0.05
SF 3/3	F (20, 200) = 0.10	p > 0.05
SF 1/2	F (20, 200) = 1.03	p > 0.05
SF 2/2	F (20, 200) = 0.06	p > 0.05
SF 1/1	F (20, 200) = 0.38	p > 0.05

Table 31: *Simple main effects of changing levels of schedule fraction at different foreperiods and vice versa*

The SFC task: Experiment B

Analysis of preoperative performance

Correct Responses

Factor	F value	P value
Foreperiod		
FP 100	F (6.03, 114.66) = 03.47	p < 0.01
FP 300	F (6.03, 114.66) = 15.93	p < 0.01
FP 500	F (6.03, 114.66) = 20.43	p < 0.01
Schedule Fraction		
SF 1/3	F (6.03, 114.66) = 102.21	p < 0.01
SF 2/3	F (6.03, 114.66) = 37.78	p < 0.01
SF 3/3	F (6.03, 114.66) = 68.19	p < 0.01
SF 1/2	F (6.03, 114.66) = 72.59	p < 0.01
SF 2/2	F (6.03, 114.66) = 61.49	p < 0.01
SF 1/1	F (6.03, 114.66) = 59.33	p < 0.01

Table 32: *Simple main effects of changing levels of schedule fraction at different foreperiods and vice versa*

Reaction Time

Factor	F value	P value
Foreperiod		
FP 100	F (5.71, 108.43) = 6.56	p < 0.01
FP 300	F (5.71, 108.43) = 3.46	p < 0.01
FP 500	F (5.71, 108.43) = 4.95	p < 0.01
Schedule Fraction		
SF 1/3	F (5.71, 108.43) = 5.31	p < 0.01
SF 2/3	F (5.71, 108.43) = 7.26	p < 0.01
SF 3/3	F (5.71, 108.43) = 2.40	p < 0.05
SF 1/2	F (5.71, 108.43) = 3.24	p < 0.01
SF 2/2	F (5.71, 108.43) = 5.31	p < 0.01
SF 1/1	F (5.71, 108.43) = 2.51	p < 0.05

Table 33: *Simple main effects of changing levels of schedule fraction at different foreperiods and vice versa*

Movement Time

Factor	F value	P value
Foreperiod		
FP 100	F (4.79, 91.06) = 88.58	p < 0.01
FP 300	F (4.79, 91.06) = 60.12	p < 0.01
FP 500	F (4.79, 91.06) = 50.15	p < 0.01
Schedule Fraction		
SF 1/3	F (4.79, 91.06) = 14.72	p < 0.01
SF 2/3	F (4.79, 91.06) = 1.86	p > 0.05
SF 3/3	F (4.79, 91.06) = 0.04	p > 0.05
SF 1/2	F (4.79, 91.06) = 2.25	p > 0.05
SF 2/2	F (4.79, 91.06) = 0.47	p > 0.05
SF 1/1	F (4.79, 91.06) = 0.98	p > 0.05

Table 34: *Simple main effects of changing levels of schedule fraction at different foreperiods and vice versa*

Post Response Pause

Foreperiod	F value	P value
FP 100	F (2.39, 45.45) = 32.50	p < 0.01
FP 300	F (2.39, 45.45) = 41.44	p < 0.01
FP 500	F (2.39, 45.45) = 28.15	p < 0.01

Table 35: *Simple main effects of changing levels of schedule fraction at different foreperiods and vice versa*

Appendix D: Practice Lesions

The SFC task, Experiment A: BLA lesions

Co-ordinates, neurotoxins and resulting lesions reported by several papers e.g. Cador et al (1989), Everitt et al (1989) and Meil and See (1997) were examined in an effort to establish good lesion parameters for the BLA:

Cador et al (1989) and Everitt, Cador and Robbins (1989):

Co-ordinates:	AP:	- 0.9	+ 0.1
	L:	+/- 4.5	+/- 4.5
	DV:	- 8.0	- 8.0

Co-ordinates taken from bregma, DV taken from skull surface, incisor bar set at +5mm. 1 site per hemisphere, 0.3µl of (0.12 M) NMDA in phosphate buffer (pH 7.4) per site.

Observations: neither the smallest nor the largest lesions reported in Cador et al (1989) appear to be limited to the BLA, but remove some of CeN as well. The largest lesions reported in Everitt et al (1989) appear to be well localised to the BLA, although there is some infringement of CeN, whilst the smallest lesions appear to remove only the more lateral areas of BLA.

Meil and See (1997):

Co-ordinates:	AP:	- 2.2
	L:	+/- 4.4
	DV:	- 8.0

Co-ordinates taken from bregma, DV taken from skull surface. 1 site per hemisphere, 0.6µl of (0.12 M) NMDA in artificial cerebrospinal fluid (pH 4.0) per site. 3 minutes for each infusion, followed by 3 minutes in situ.

Observations: the smallest lesions reported by Meil and See (1997) appear to be limited to the BLA, but the largest lesions take out much of the region below BLA, down to piriform cortex. The lesions resulting from the parameters described above were not wholly satisfactory, and eventually it was decided to take the co-ordinates directly from the Paxinos and Watson (1998) atlas:

Paxinos and Watson co-ordinates:

Practice rat: 99/099

Co-ordinates: AP: - 2.3
L: +/- 5.0
DV: - 8.8

Co-ordinates taken from bregma, DV taken from skull surface, incisor bar set at -3.3mm. 1 site per hemisphere, 0.3µl of (0.12 M) NMDA in phosphate buffer (pH 7.4) per site. 4 minutes for each infusion, followed by 2 minutes in situ.

Results: bilateral lesions were obtained, but they were very large, removing the entire amygdaloid complex and surrounding areas including piriform cortex. It was decided to use the Paxinos and Watson co-ordinates for the actual experiment, but to reduce the volume of the excitotoxin from 0.3µl to 0.1µl of (0.12 M) NMDA per site.

The SFC task, Experiment B: BLA and CeN lesions

As discussed above, in the first SFC task experiment only four rats sustained reasonable lesions of the BLA. However, these lesions were, on the whole, rather too large and did not extend very far back within the BLA. For the second SFC task experiment, it was decided to try and improve the positioning and extent of the lesions by again examining the results obtained by other researchers in the field. Those obtained by Parkinson et al (2000) appeared to be well localised to the BLA, with very little infringement of CeN. It was therefore decided to carry out a practice surgery using the lesion parameters reported in the Parkinson et al (2000) paper. Likewise, co-ordinates, neurotoxins and resulting lesions reported by several researchers (e.g. Holland et al (2000), and Parkinson et al (2000)) were examined in an effort to establish good lesion parameters for the CeN. Practice surgeries were carried out on four sets of parameters, with one set taken directly from the Paxinos and Watson (1998) atlas, another set supplied by Patrick Pallier (personal communication) and the final two sets taken from Holland et al (2000) and from Parkinson et al (2000). The practice surgeries for both BLA and CeN lesions, and subsequent practice surgeries undertaken to refine them, are reported below:

BLA Practice lesions

Practice 1

Lesion parameters were taken from Parkinson et al (2000), except that 0.2µl of (0.09M) quinolinic acid per site was used rather than 0.3µl:

Practice rat:	00/790		
Co-ordinates:	AP:	- 2.3	- 3.0
	L:	+/- 4.6	+/- 4.6
	DV:	- 7.3	- 7.3

Co-ordinates taken from bregma, DV taken from dura, incisor bar set at -3.3mm. 2 sites per hemisphere, 0.2µl of (0.09M) quinolinic acid per site. 6 minutes for each infusion, followed by 2 minutes in situ.

Histology: the lesions were far too large. It was thought that this might be due to a mistake in making up the neurotoxin, resulting in a higher concentration than 0.09M, and so another batch was made up for a second practice. The same co-ordinates and volumes were used:

Practice 2

Practice rats: 00/726 00/733

Co-ordinates as for Practice 1, 0.2µl of (0.09M) quinolinic acid per site. 6 minutes for each infusion, followed by 2 minutes in situ.

Histology: again, the lesions were much too large on both sides for both rats, suggesting that it was not the concentration of the neurotoxin that was at fault. It was therefore decided to reduce the volume of neurotoxin from 0.2µl to 0.1µl of (0.09M) quinolinic acid per site. Infusion took place over 2 minutes and the needle was left in situ for a further 3 minutes:

Practice 3

Practice rats: 00/837 00/838

Co-ordinates as for Practice 1, 0.1µl of (0.09M) quinolinic acid per site. 2 minutes for each infusion, followed by 3 minutes in situ.Histology: the lesions were found to be still much too large in both rats. It was therefore decided to reduce the concentration of the neurotoxin from 0.09M to 0.06M, but to increase the volume back up to 0.2µl per site:

Practice 4

Practice rats: 00/839 00/841

Co-ordinates as for Practice 1, 0.2µl of (0.06M) quinolinic acid per site. 2 minutes for each infusion, followed by 3 minutes in situ.

Histology: the lesions were found to be too large. It was decided to retain the concentration of the neurotoxin at 0.06M and to decrease the volume to 0.1µl of (0.06M) quinolinic acid per site:

Practice 5

Practice rats: 00/833 00/835

Co-ordinates as for Practice 1, 0.1µl of (0.06M) quinolinic acid per site. 2 minutes for each infusion, followed by 3 minutes in situ.

Histology: the lesions were still somewhat too large.

It was decided to vary the co-ordinates slightly from those used by Parkinson et al (2000) to see if this would produce any improvement in the location of the lesions. The rats used in this practice surgery were taken from the same cohort used for the second SFC task experiment in order to minimise variation, and therefore were only two in number. For this reason, slightly different co-ordinates were plotted from skull surface for each hemisphere. 2 sites were decided on in the right hemisphere, with the more anterior site being located slightly higher. However, in the left hemisphere it was decided to try and target the entire depth of the BLA by having two lesions in the same vertical axis for the anterior and posterior AP and L co-ordinates, thus giving 4 sites in all.

Practice 6

Practice rats: 01/024 01/028

Left side co-ordinates:	AP:	- 2.3	- 3.0
	L:	+ 4.8	+ 5.0
	DV (lower):	- 8.6	- 8.6
	DV (upper):	- 7.7	- 7.7

Co-ordinates taken from skull surface, with the skull level. 4 sites per hemisphere, 0.07µl of (0.06M) quinolinic acid per lower site, and 0.03µl per upper site. 2 minutes for each infusion,

followed by 3 minutes in situ for the lower sites, and 1 min for each infusion followed by 3 minutes in situ for the upper sites.

Right side co-ordinates:	AP:	- 2.3	- 3.0
	L:	- 4.8	- 5.0
	DV:	- 8.2	- 8.6

Co-ordinates taken from skull surface, with the skull level. 2 sites per hemisphere, 0.1µl of (0.06M) quinolinic acid per site. 2 minutes for each infusion, followed by 3 minutes in situ.

Histology: both sets of co-ordinates produced reasonable lesions, with BLA removed but CeN spared ***(Figure 21: E1 (Rat 01/024 – left side) E2 (Rat 01/028 – right side))***. It was therefore decided to use the left side parameters for the BLA lesions in the second SFC task experiment.

CeN Practice lesions

Four different sets of co-ordinates were tried:

Practice A1

Co-ordinates were taken from the Paxinos and Watson (1998) atlas.

Practice rat:	00/789		
Co-ordinates:	AP:	- 2.4	- 3.2
	L:	+/- 3.9	+/- 4.3
	DV:	- 8.2	- 7.9

Co-ordinates taken from bregma, DV taken from skull surface, incisor bar set at -3.3 mm. 2 sites per hemisphere, 0.1µl of (0.06M) ibotenic acid per site. 3 minutes for each infusion, followed by 3 minutes in situ.

Histology: the lesions were far too large.

Practice B1

Lesion parameters were suggested by Patrick Pallier (personal communication) who adapted them from co-ordinates taken from the Pellegrino and Cushman (1979) atlas. This atlas differs from that of Paxinos and Watson (1998) in that rats are placed in the stereotaxic frame with the incisor bar set at 5.0mm above the interaural line, in the orientation of De Groot.

Practice rats:	00/785	00/786	
Co-ordinates:	AP:	+ 0.2	- 0.6
	L:	+/- 3.8	+/- 4.4
	DV:	- 8.7	- 7.9

Co-ordinates taken from bregma, DV taken from skull surface, incisor bar set at +5.0 mm. 2 sites per hemisphere, 0.1µl of (0.06M) ibotenic acid per site. 3 minutes for each infusion, followed by 3 minutes in situ.

Histology: the lesions were too large.

Practice C1

Lesion parameters were taken from Holland et al (2000).

Practice rats:	00/782	00/783
Co-ordinates:	AP:	- 2.3
	L:	+/- 4.2
	DV:	- 7.7

Co-ordinates taken from bregma, DV taken from skull surface, incisor bar set at -3.3mm. 1 site per hemisphere, 0.2µl of (0.06M) ibotenic acid per site. 6 minutes for each infusion, followed by 4 minutes in situ.

Histology: the lesions were too large

Practice D1

Lesion parameters were taken from Parkinson et al (2000), except that 0.1 µl of ibotenic acid per site was used rather than 0.2 µl.

Practice rat:	00/788	
Co-ordinates:	AP:	- 2.2 - 2.7
	L:	+/- 4.0 +/- 4.0
	DV:	- 7.8 - 7.8

Co-ordinates taken from bregma, DV taken from dura, incisor bar set at -3.3mm. 2 sites per hemisphere, 0.1µl of (0.06M) ibotenic acid per site. 3 minutes for each infusion, followed by 3 minutes in situ.

Histology: the lesions were too large.

Overall, the lesions produced were far too large, making it difficult to ascertain how accurate the different sets of co-ordinates actually were. It was decided, therefore, to reduce the concentration of the neurotoxin from 0.06 M to 0.045 M.

Practice A2

Lesion parameters were taken from the Paxinos and Watson (1998) atlas.

Practice rats:	00/791	00/723	
Co-ordinates:	AP:	- 2.4	- 3.2
	L:	+/- 3.9	+/- 4.3
	DV:	- 8.2	- 7.9

Co-ordinates taken from bregma, DV taken from skull surface, incisor bar set at -3.3 mm. 2 sites per hemisphere, 0.1μl of (0.045M) ibotenic acid per site. 3 minutes for each infusion, followed by 3 minutes in situ.

Histology: the lesions were still too large.

Practice B2

Lesion parameters were suggested by Patrick Pallier (see above)

Practice rats:	00/792	00/793	
Co-ordinates:	AP:	+ 0.2	- 0.6
	L:	+/- 3.8	+/- 4.4
	DV:	- 8.2	- 7.9

Co-ordinates taken from bregma, DV taken from skull surface, incisor bar set at +5.0 mm. 2 sites per hemisphere, 0.1μl of (0.045M) ibotenic acid per site. 3 minutes for each infusion, followed by 3 minutes in situ.

Histology: the lesions were still too large.

Practice A3

Lesion parameters were taken from the Paxinos and Watson (1998) atlas.

Practice rats:	00/830	00/832	00/834
Co-ordinates:	AP:	-2.4	- 3.2
	L:	+/- 3.9	+/- 4.3
	DV:	- 8.2	- 7.9

Co-ordinates taken from bregma, DV taken from skull surface, incisor bar set at -3.3mm. 4 sites per hemisphere, 0.1 μl of (0.04 M) ibotenic acid per site. 3 minutes for the infusion, and 3 minutes in situ.

Histology: the lesions were still too large.

It was decided to further reduce the concentration of the neurotoxin from 0.04M to 0.03M.

Practice A4

Practice rats:	00/831	00/836
Co-ordinates:	AP:	-2.4	- 3.2
	L:	+/- 3.9	+/- 4.3
	DV:	- 8.2	- 7.9

Co-ordinates taken from bregma with the skull level, DV taken from dura. 2 sites per hemisphere, 0.1 µl of (0.03 M) ibotenic acid per site. 2 minutes for the infusion, and 3 minutes in situ.

Histology: the lesions produced by this set of co-ordinates and this concentration of neurotoxin were reasonable, with CeN removed but BLA spared ***(Figure 23: A1 (Rat 00/831 – left side) A2 (Rat 00/836 – right side))***. It was therefore decided to use these parameters for the CeN lesions in the second SFC task experiment.

Second SFC task experiment:

BLA lesions

Co-ordinates:	AP:	- 2.3	- 3.1
	L:	+/- 4.8	+/- 5.0
	DV (lower):	- 8.6	- 8.6
	DV (upper):	- 7.7	- 7.7

Co-ordinates taken from skull surface, with the skull level. 4 sites per hemisphere, 0.07 µl of (0.06 M) quinolinic acid per lower site, and 0.03 µl per upper site. 2 minutes for the infusion, and 3 minutes in situ for the lower sites, and 1 min for the infusion and 3 minutes in situ for the upper sites.

CeN lesions

Co-ordinates:	AP:	-2.4	- 3.2
	L:	+/- 3.9	+/- 4.3
	DV:	- 8.2	- 7.9

Co-ordinates taken from bregma with the skull level, DV taken from dura. 2 sites per hemisphere, 0.1 µl of (0.03 M) ibotenic acid per site. 2 minutes for the infusion, and 3 minutes in situ.

Appendix E: The SFC task, Experiment B, preoperative performance

The preoperative data from all of the rats was combined, regardless of the experimental group to which they would later be assigned in order to increase numbers and therefore analytical power.

Results of ANOVAs on preoperative performance

Graphs of mean values for each of the various performance measures (mean percentage of correct responses, mean reaction time, mean movement time and mean post response pause) were drawn up and described, and the data for each measure subjected to two-way within-subject ANOVA, with schedule fraction and foreperiod as the factors. The Mauchly sphericity test was used to test for homogeneity of covariance, and effect sizes (given in brackets when referred to in text) were calculated for every significant main effect and interaction in order to assess the impact of each on performance. Simple main effects were also calculated since all the ANOVAs were within-subjects, thus making the use of post-hoc tests inadvisable **(Appendix C)**.

Correct Responses

Comparison of the graph showing the mean percentage of correct responses made during this experiment ***(Figure 33)*** with the graph for the previous experiment ***(Figure 14)*** reveals that they are very similar. In both figures it can be seen that the mean percentage of correct responses made by the rats varies across the different schedule fractions both within and between the work schedules. It can also be seen that the mean percentage of correct response varies (more dramatically) according to foreperiod, with more correct responses being made overall at shorter foreperiods. In this experiment, as in the previous, main effects were found for schedule fraction ($F(5.00, 95.00) = 37.30$, $P = 0.001$) and for foreperiod ($F(1.180, 22.41) = 103.24$, $P = 0.001$). Effect sizes calculated

for both main effects confirmed that foreperiod (79.65%) has a more dramatic influence on behaviour than does schedule fraction (17.96%), as was also the case in the previous experiment.

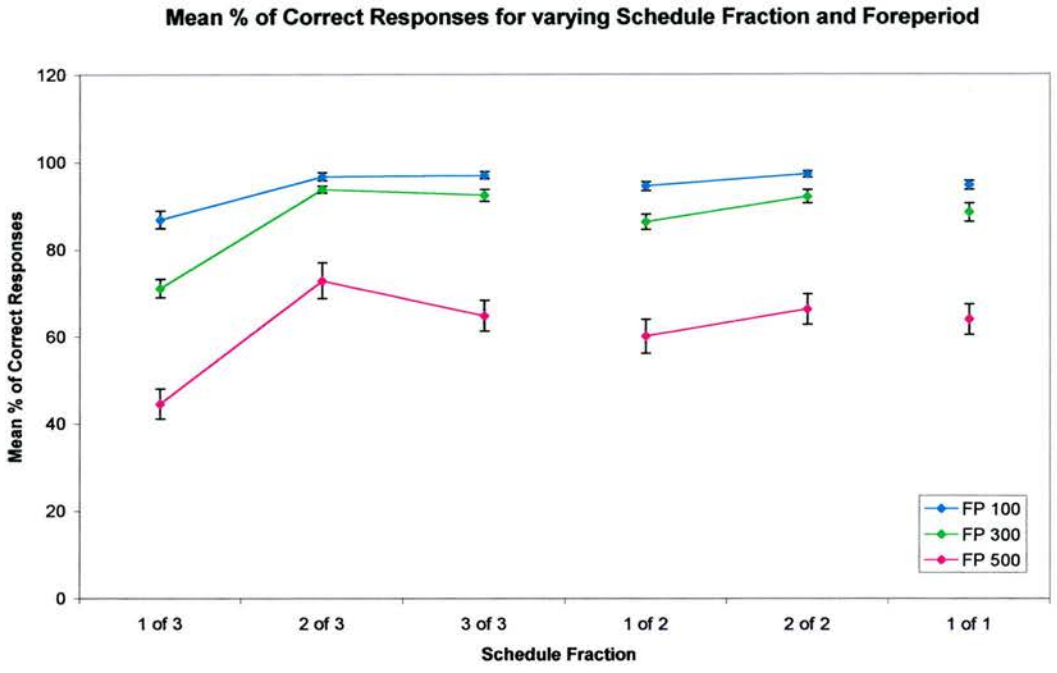


Figure 33: *Graph showing the mean (±se) percentage of correct responses made preoperatively. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Colour of line indicates length of foreperiod (100, 300, and 500 msecs).*

Overall, however, the percentage of correct responses achieved by the rats in this experiment is higher than that achieved by the rats in the previous experiment. Indeed, at foreperiod 100, the percentage of correct responses seems to have nearly plateaued at close to 100% for all of the schedule fractions. An interaction effect was also found between schedule fraction and foreperiod ($F(6.03, 114.66) = 3.86$, $P = 0.001$). The effect size for the interaction is small (02.38%), again as in the previous experiment, but simple main effects were calculated in an attempt to pinpoint it more accurately **(Appendix C)**. However, all of the simple main effects proved significant ($p < 0.01$), making it impossible to do so. In the previous experiment, the interaction effect for this measureappeared to come from two different patterns of response which differed according to foreperiod: at the shorter foreperiods (100, 200 and 300) the rats made more correct responses the

closer they came to achieving reward within each work schedule, whereas at the longer foreperiods (400 and 500) they made more correct responses at the second schedule fraction (2/3) of work schedule 3 and fewer at the third (rewarded) schedule fraction (3/3). In this experiment, the interaction effect would appear to be largely due to the ceiling effect at foreperiod 100, but there is some suggestion from ***Figure 33*** that two different patterns of response *may* also be present: despite the ceiling effect, the pattern of response for foreperiod 100 in this experiment most closely resembles that for foreperiod 100 in the previous experiment, whereas the patterns of response for foreperiods 300 and 500 resemble those for foreperiods 400 and 500 in the previous experiment.

Reaction Time

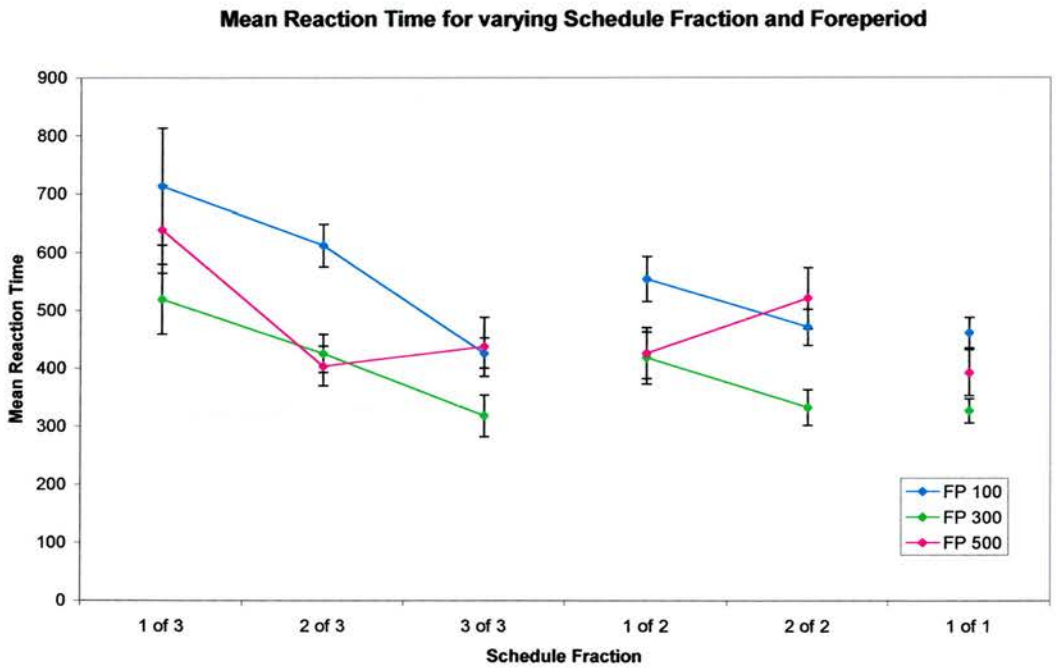


Figure 34: *Graph showing mean (±se) preoperative reaction time. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Colour of line indicates length of foreperiod (100, 300, and 500 msecs*

Comparison of the graph showing mean reaction time for this experiment ***(Figure 34)*** with that for the previous experiment ***(Figure 15)*** reveals large differences in the pattern of responses. In the previous experiment, the main effect of foreperiod manifested itself as a decrease in reaction time as

foreperiod increased. The interaction between schedule fraction and foreperiod appeared to have its origin within work schedule 3: whereas there is an increase in reaction time on the second schedule fraction (2/3) of the work schedule before a decrease on the third rewarded schedule fraction (3/3) for the three shortest foreperiods, there is a steady decrease in reaction time throughout the work schedule for the two longest foreperiods. In this experiment, however, although the main effect of schedule fraction ($F(2.40, 45.66) = 7.99$, $P = 0.001$) indicates that there is a difference in mean reaction time between the different schedule fractions, there appears to be no logical pattern to it – at foreperiods 100 and 300 reaction time decreases as the rats work through work schedules 3 and 2 towards reward, but at foreperiod 500 reaction it increases. Likewise, the main effect of foreperiod ($F(1.41, 26.80) = 10.81$, $P = 0.001$) indicates that there is a difference in mean reaction time between the different foreperiods, but whereas reaction time is decreased at foreperiod 300 compared to foreperiod 100, reaction time at foreperiod 500 falls between the two. The rats' performance at foreperiod 500 therefore appears to be very much out of kilter with their performance at the other two foreperiods, and this is where the interaction effect ($F(5.71, 108.43) = 2.58$, $P = 0.024$) would appear to reside. The effect sizes for the main effects and interaction in the two experiments are also very different – in the previous experiment, foreperiod most dramatically influenced performance, whereas in this experiment schedule fraction has more influence than schedule fraction (53.59% compared to 33.28%). Unsurprisingly, the interaction effect between schedule fraction and foreperiod is also larger in this experiment (13.11%) than in the previous. Simple main effects were calculated **(Appendix C)** but all proved significant ($p < 0.05$), indicating that changing levels of schedule fraction at every foreperiod produced significant differences in performance, and vice versa. Boxplots were also constructed for the 18 different combinations of schedule fraction and foreperiod in an attempt to understand why the pattern of response should differ so much from that of the previous experiment. At foreperiod 500, there were 2 outliers at schedule fraction 1/3 and 1 outlier at 1/2 but no outliers or extreme values at the other schedule fractions. These anomalies, however, do not account for the difference in the pattern of response.

Movement Time

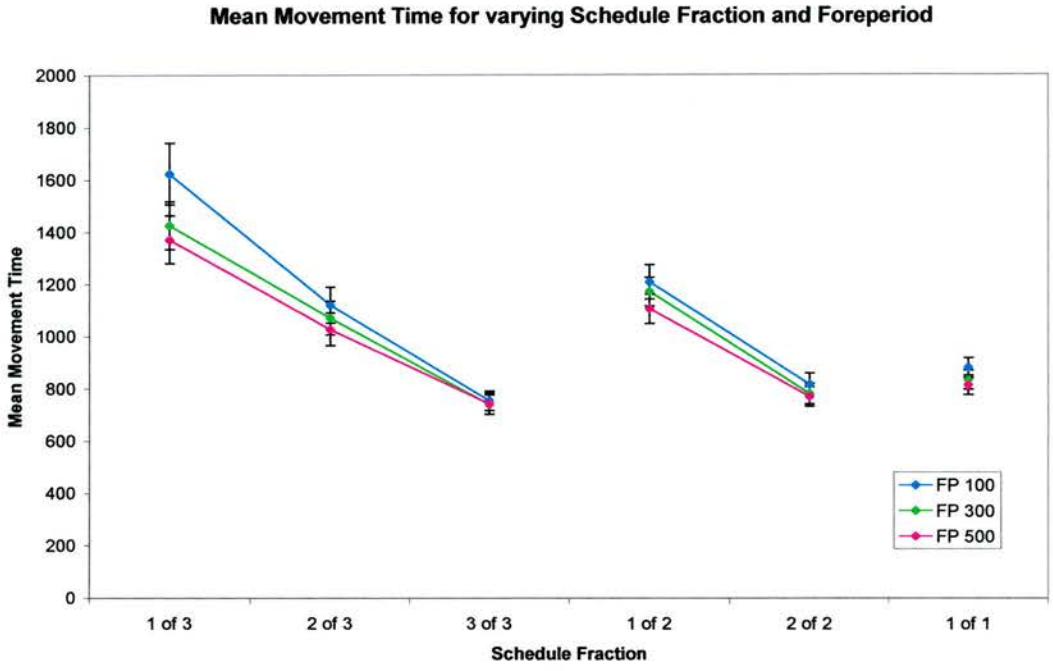


***Figure 35**: Graph showing mean (±se) preoperative movement time. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Colour of line indicates length of foreperiod (100, 300, and 500 msecs).*

It can be seen from ***Figure 35*** (which is very similar to ***Figure 15***, showing mean movement time for the previous experiment) that movement time decreases as the rats work their way through a given work schedule towards reward. ***Figure 35*** also shows a slight decrease in mean movement time as foreperiod increases, especially on the first schedule fraction of work schedule 3 (1/3). Subsequent performance of ANOVA showed main effects of schedule fraction ($F(1.90, 36.16) = 34.59$, $P = 0.001$) and of foreperiod ($F(1.70, 32.38) = 18.57$, $P = 0.001$) and an interaction effect between schedule fraction and foreperiod ($F(4.79, 91.06) = 3.55$, $P = 0.006$). The effect size for the main effect of schedule fraction is 96%, indicating that schedule fraction has an extremely strong influence on performance, whereas the effect size for the main effect of foreperiod is very small (2.32%) and the interaction effect size is even smaller (1.67%). This latter would appear to lie within schedule fraction 1/3, and so simple main effects were calculated in order to verify whether

this was the case **(Appendix C)**. Although the simple main effects of changing levels of schedule fraction at all the foreperiods are very significant ($p < 0.01$), only the simple main effect of changing levels of foreperiod at schedule fraction 1/3 is significant ($p < 0.01$), thereby supporting the theory that the interaction lies within this schedule fraction.

Post Response Pause

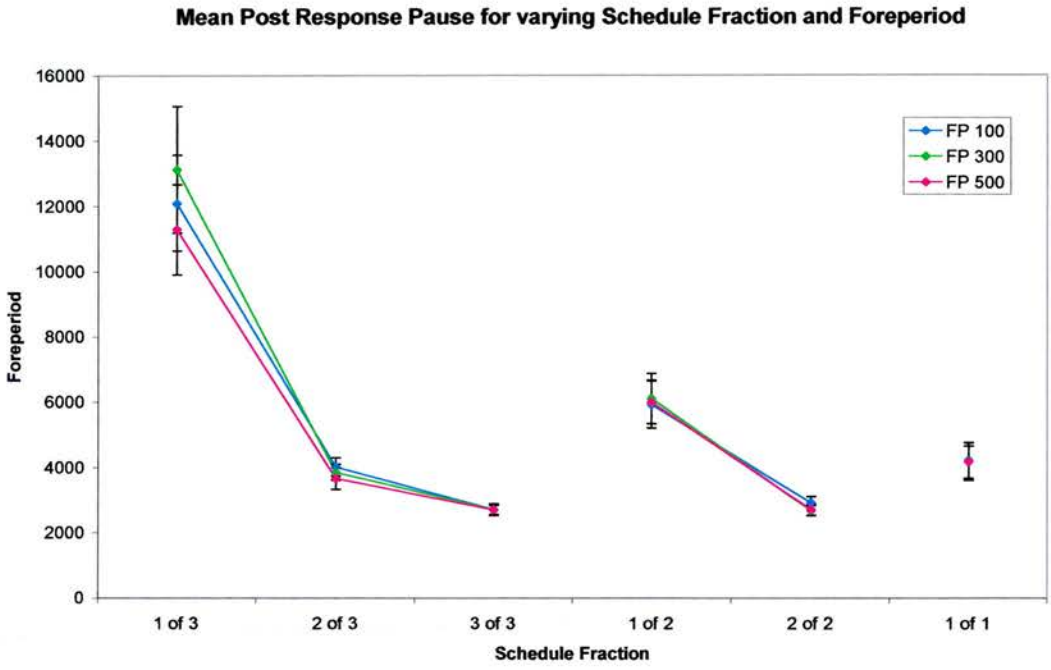


Figure 36: *Graph showing mean (±se) preoperative post response pause. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Colour of line indicates length of foreperiod (100, 300, and 500 msecs).*

Comparison of the graph for mean post response pause for this experiment ***(Figure 36)*** with that for the previous experiment ***(Figure 17)*** reveals that they are very similar. Both graphs show that mean post response pause decreases as the rat works its way through each work schedule towards reward. Both graphs also show that mean post response pause is shortest for the rewarded schedule fractions 3//3 and 2/2, which seems somewhat counter-intuitive given that mean post response pause includes the time taken to consume the reward. As in the previous SFC experiment, foreperiod does not appear to have any effect on mean post response pause. Subsequent performance of ANOVA gave a

very strong (99.06%) main effect for schedule fraction ($F(1.65, 31.37) = 28.40$, $P = 0.001$) but not for foreperiod, and no interaction effect was found between schedule fraction and foreperiod. Simple main effects of schedule fraction at every foreperiod were calculated, and all proved very significant ($p < 0.01$), indicating that there are significant differences in performance between the different levels of schedule fraction at all the foreperiods.

Summary of Preoperative ANOVA results

Measure	Schedule Fraction	Foreperiod	Schedule Fraction * Foreperiod
Correct Responses	< 0.001	< 0.001	< 0.01
Reaction Time	< 0.01	< 0.01	< 0.05
Movement Time	< 0.001	< 0.001	< 0.01
Post Response Pause	< 0.001	Not sig.	Not sig.

Table 36: *Summary of preoperative ANOVA results for all the measures*

A main effect of schedule fraction was found for all of the dependent measures, and a main effect of foreperiod was found for all of the dependent measures except post response pause. Significant interactions were found between schedule fraction and foreperiod for all of the dependent measures except post response pause. The ANOVA results for this experiment are therefore the same as those for the previous experiment, with some differences in the degree of significance. However, examination of the graphs showing performance on the various measures for the two experiments reveal differences, especially on the reaction time measure.

Results of planned comparisons on preoperative performance

As in Experiment A, planned comparisons were also performed on pairs of conditions from the main effect of schedule fraction for the preoperative Combined group in order to test various hypotheses as to how the rats interpret the schedule fraction cue lights. These hypotheses are given in detail in the Analysis of preoperative performance section for Experiment A.

Correct Responses (see *Table 12*)

Preoperative data (Combined (Sham and Lesion) group): The results of two of the planned comparisons for the main hypothesis (1/3 and 2/3, and 1/2 and 2/2) were significant, but the result of the third (2/3 and 3/3) was not. Overall, the results suggest that the rats did perform somewhat differently according to which schedule fraction they were on. The results of two of the planned comparisons for the first sub-hypothesis (1/2 and 2/2, and 1/3 and 3/3) were also significant but the result of the third (2/3 and 3/3) was not, suggesting that, overall, the rats performed differently on rewarded and unrewarded schedule fractions. All of the results of the planned comparisons for the second sub-hypothesis (1/3 and 2/3, 1/3 and 1/2, and 2/3 and 1/2) were significant, suggesting that the rats did use the different cue light intensities to ascertain how close they were to reward rather than as an indication of the availability of reward. The results of the planned comparisons for the preoperative data in this experiment are therefore identical to those obtained in the previous experiment (see ***Table 1***).

Reaction Time (see *Table 12*)

Preoperative data (Combined (Sham and Lesion) group): The results of two of the planned comparisons for the main hypothesis (1/3 and 2/3, and 2/3 and 3/3) were significant, but the result of the third (1/2 and 2/2) was not. Overall, these results suggest that the rats did perform somewhat differently according to which schedule fraction they were on. The results of two of the planned comparisons for the first sub-hypothesis were also significant (2/3 and 3/3, and 1/3 and 3/3) but the result of the third (1/2 and 2/2) was not, again suggesting that, overall, the rats performed differently on rewarded and unrewarded schedule fractions. Likewise, the results of two of the planned comparisons for the second sub-hypothesis (1/3 and 2/3, and 1/3 and 1/2) were significant but the result of the third (2/3 and 1/2) was not, suggesting that, overall, the rats did use the different cue light intensities to ascertain how close they were to reward rather than as an indication of the availability of reward. Similar results were obtained in the previous experiment with regard to the

main and first sub-hypothesis, though in that experiment the result of the planned comparison between 1/3 and 2/3 was not significant, whereas the results of the others were (see ***Table 1***). With regard to the second sub-hypothesis, however, the results are very different – unlike this experiment, none of the results of the planned comparisons for this sub-hypothesis proved significant, suggesting that the rats are did not use the cue lights as a means of ascertaining how close they were to reward but merely as an indication of the availability of reward.

Movement Time (see *Table 12*)

Preoperative data (Combined (Sham and Lesion) group): The results of all of the preoperative planned comparisons are significant for this measure, suggesting that not only did the rats perform differently according to which schedule fraction they were on, differentiating between rewarded and unrewarded schedule fractions, but that they also used the different cue light intensities to ascertain how close they were to reward rather than as an indication of the availability of reward. The results of the planned comparisons for the preoperative data in this experiment are therefore identical to those obtained in the previous experiment (see ***Table 1***).

Post Response Pause (see *Table 12*)

Preoperative data (Combined (Sham and Lesion) group): The results of all of the preoperative planned comparisons are significant for this measure, suggesting that not only did the rats perform differently according to which schedule fraction they were on, differentiating between rewarded and unrewarded schedule fractions, but that they also used the different cue light intensities to ascertain how close they were to reward rather than as an indication of the availability of reward. These results therefore vary from the preoperative data results obtained for this measure in the previous experiment, which suggested that the rats were only able to use the different cue light intensities as an indication of the availability of reward (see ***Table 1***).

Discussion of preoperative performance

Overall, preoperative performance in this experiment was very similar to preoperative performance in the previous one. The results of ANOVA carried out on the various measures showed that the rats performed better on work schedules requiring fewer correct responses than on work schedules requiring more correct responses, and that performance improved as they come closer to achieving reward within the work schedules. As in the first experiment, it would appear, therefore, that the cue lights signalling the onset of each work schedule had a motivational influence on performance. The results of the planned comparisons largely confirmed this finding – the rats not only performed differently on all of the measures according to whether a schedule fraction was rewarded or unrewarded, but they also performed differently according to which unrewarded schedule fraction they were on. This suggests that they were able both to use the cue lights at the outset of each work schedule as discriminative stimuli indicating how much work they had to do to obtain reward, and to use the changing intensities of the cue lights within the work schedules as a means of ascertaining how close they were to achieving reward.

Although the patterns of performance, and consequently the main and interaction effects and their effect sizes, were very similar for most of the measures in both the previous and this experiment, some important differences also emerged. Perhaps the most striking difference was performance on the Reaction Time measure. In the previous experiment, reaction time was shown to decrease overall as foreperiod increased and also to decrease within the work schedules as the rats worked towards obtaining the reward. In this experiment, however, reaction time did not decrease overall as foreperiod increased, and only decreased within the work schedules at foreperiods 100 and 300 – at foreperiod 500 reaction time increased. Performance at foreperiod 500 would therefore appear to be out of kilter with performance on the other foreperiods, but boxplots revealed no major abnormalities in the data set. This pattern of response appears inexplicable. Although main effects were still found for schedule fraction and foreperiod, and an interaction

effect was found between the two, the effect sizes were very different from those of the first experiment – the effect sizes for schedule fraction and foreperiod and for the interaction effect in this experiment were 53.59%, 33.28% and 13.11% respectively, compared to 16.04%, 81.78% and 2.17% in the previous experiment. The results of the planned comparisons on the Reaction Time measure were necessarily also somewhat different from those of the previous experiment: the comparison between schedule fractions 1/3 and 2/3 was not previously significant, whilst the comparison between 1/2 and 2/2 was significant, but in this experiment these results are reversed. Obviously, the difference in the preoperative pattern of results between the previous and this experiment on this measure necessitates any interpretation of the postoperative and reversal data being made with caution.

Another difference between the previous experiment and this was found in the Post Response Pause measure. In both experiments a main effect was found for schedule fraction, for which the effect size was very similar (98.35% for the previous experiment compared to 99.06% for this experiment), but no main effect for foreperiod or interaction effect. There were, however, slight differences in the actual pattern of response for the two experiments – for instance mean post response pause was slightly lower at SF 2/3 and slightly higher at SF 1/1 for this experiment compared to the previous one – which had a fairly major impact on the planned comparisons. In the previous experiment, the comparisons between schedule fractions 1/3 and 1/2 and between 2/3 and 1/2 were not significant, but in this experiment they both proved to be extremely significant, thereby supporting the idea that the rats use the cue lights not only as an indication of the availability of reward, but also as a means of ascertaining how close they are to achieving it. In the discussion of Preoperative performance in the previous experiment, it was suggested that the Post Response Pause measure could be described as the 'purest' measure of performance on the SFC task, but that it was perhaps not sensitive enough to reveal such subtle differences in performance. The results of the present experiment refute this.

A final difference in performance between this experiment and the previous one concerns the suggestion made in the Discussion of Preoperative Performance for the previous experiment that two different patterns of response are discernible on some of the measures. This does not appear to be the case in this experiment (except, possibly, in the Correct Responses measure), perhaps because there were only three foreperiods rather than five, or because training did not continue for as long as it did in the previous experiment.

Appendix F: Preoperative/postoperative interactions

Correct Responses

SF * FP * Pre/Post interaction

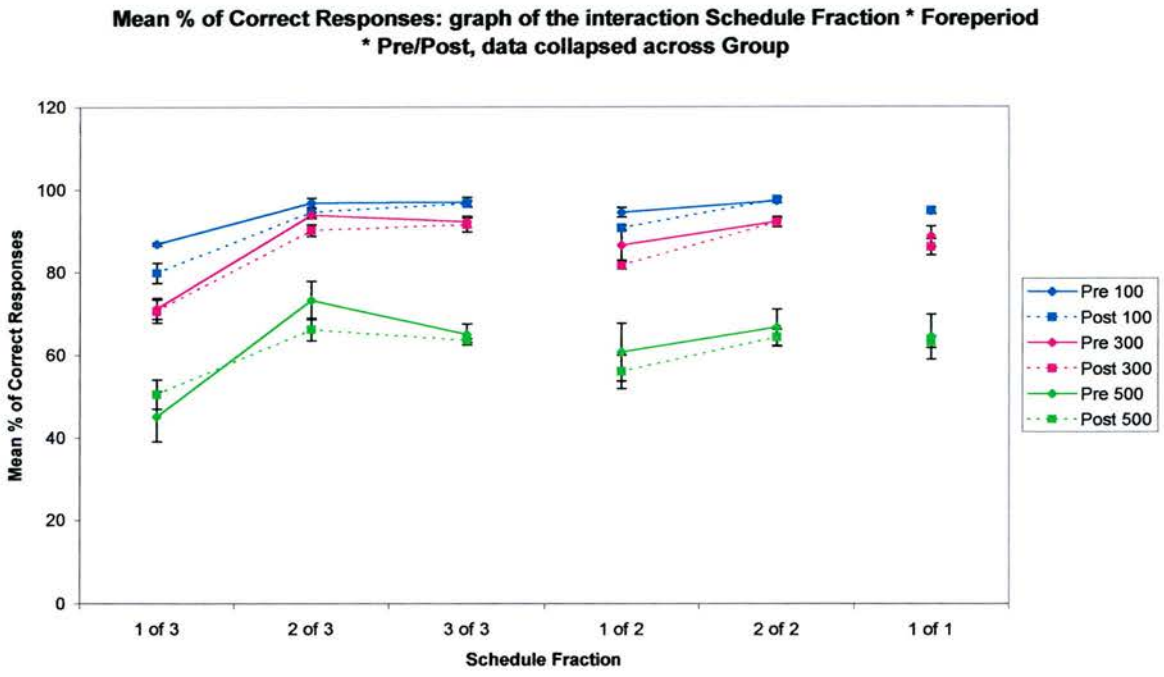


Figure 37: *Graph of the interaction SF*FP*pre/post, data collapsed across group, showing the mean (±se) percentage of correct responses made pre- and postoperatively. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Colour of line indicates length of foreperiod (100, 300, and 500 msecs); solid lines indicate preoperative performance and dotted lines indicate postoperative performance.*

The interaction graph (***Figure 37***) shows what appear to be main effects for schedule fraction, foreperiod and pre/post. The main effect of schedule fraction is not particularly strong (16.36%), but it can be seen that the mean percentage of correct responses made by the rats varies across the different schedule fractions both between and within the work schedules. The main effect of foreperiod is very strong (75.5%) and can be seen in the decrease in the mean percentage of correct responses as foreperiod increases. The main effect of pre/post is very weak (0.43%), but it can be

seen that postoperatively (apart from at schedule fraction 1/3) the rats made a smaller mean percentage of correct responses on the unrewarded schedule fractions whereas preoperative and postoperative performance on the rewarded schedule fractions is identical. The interaction effect between these three factors is very small (0.54 %) and somewhat unclear, but would appear to lie partly in the greater difference between the mean percentage of correct responses made at foreperiods 300 and 500 compared to foreperiods 100 and 300, partly in the two different patterns of response that emerge according to foreperiod (at foreperiods 100 and 300 the rats make more correct responses the closer they come to achieving reward within each work schedule, but at foreperiod 500 the rats make fewer correct responses on the third (rewarded) schedule fraction (3/3) compared to the second schedule fraction (2/3) of work schedule 3) and partly on the differences between preoperative and postoperative performance described above. The influences of schedule fraction and foreperiod on performance, and the interaction between them are, of course, very similar to what has been found in the preoperative data. Since the data is collapsed across group, the difference in pre- and postoperative performance must presumably be due to the fact that all the animals have undergone surgery, or to the passage of time, or both.

Reaction Time

SF * FP interaction

Comparison of the postoperative interaction graph (***Figure 38***) with the graph showing the preoperative data **(Appendix E: *Figure 34*)** reveals that they are very similar, though the effect sizes of the main effects and interaction for the postoperative data suggest that they have less influence on performance than preoperatively. Postoperatively, the interaction effect would again appear to lie within foreperiod 500: at foreperiods 100 and 300 reaction time decreases as the rats work through the work schedules towards reward, but at foreperiod 500 reaction time increases again slightly after schedule fraction 2/3 in work schedule 3, and remains the same in work

schedule 2. Moreover, the mean reaction time for foreperiod 500 falls largely and very unexpectedly between the mean reaction times for foreperiods 100 and 300 As with the preoperative data for this measure, the rats' performance at foreperiod 500 appears to be very much out of kilter with their performance at the other two foreperiods.

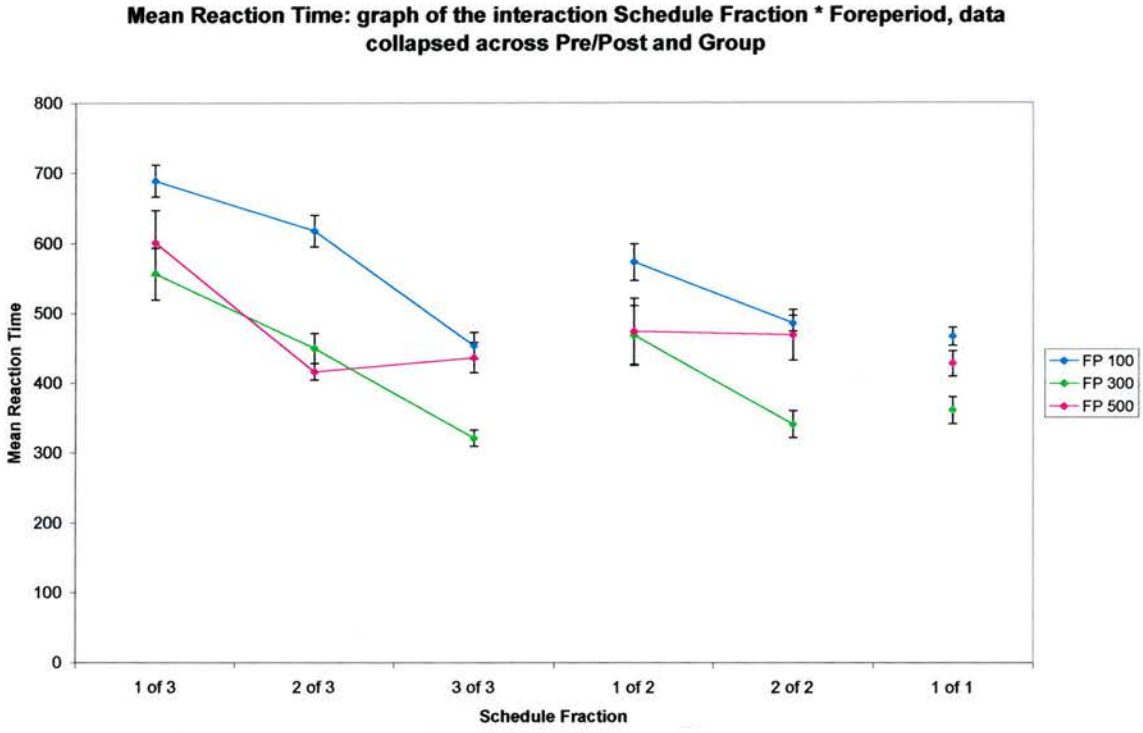


***Figure 38**: Graph of the interaction SF*FP, data collapsed across pre/post and group, showing mean (±se) reaction time. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Colour of line indicates length of foreperiod (100, 300, and 500 msecs).*

Movement time

SF * Pre/Post interaction

Figure 39 shows that mean movement time decreases as the rats works their way through a given work schedule towards reward. The interaction effect, which is fairly small (2.84%), would appear to be due to the rats moving more slowly on the unrewarded schedule fractions during the postoperative stage than during the preoperative stage. On the rewarded schedule fractions,

however, mean movement time is equivalent in both stages. Since the data is collapsed across group, the difference in pre- and postoperative performance must presumably be due to the fact that all the animals have undergone surgery, or to the passage of time, or both.

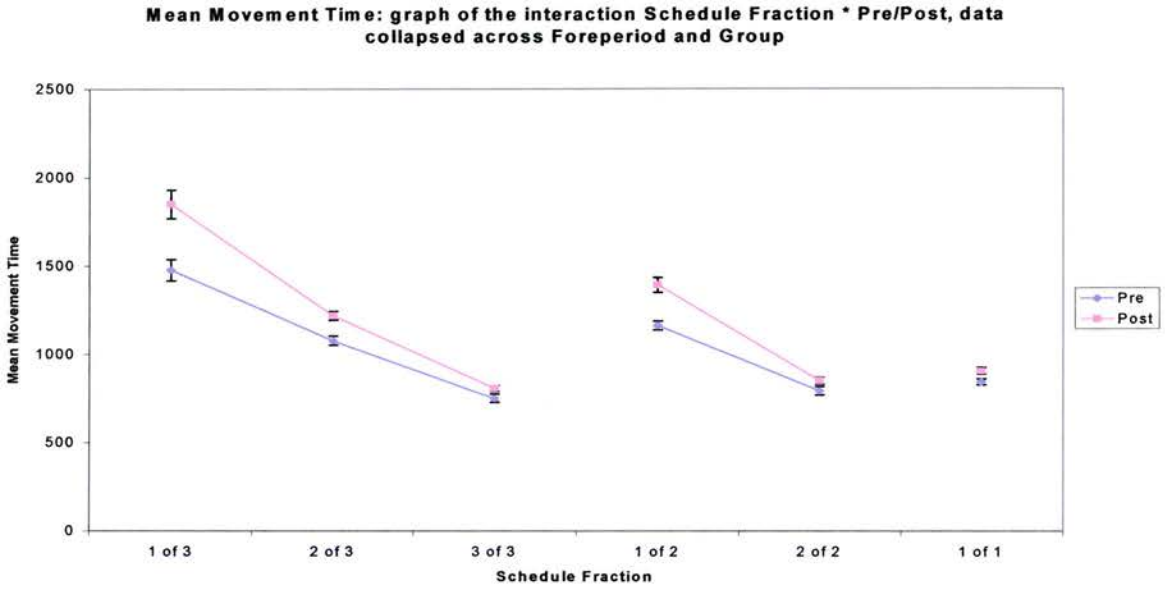


Figure 39: *Graph of the interaction SF*pre/post, data collapsed across foreperiod and group, showing mean (±se) movement time preoperatively and postoperatively. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Blue line indicates preoperative performance and pink line indicates postoperative performance.*

Appendix G: Reversal interactions

Postoperative – Reversal ANOVA

SF * Post/Reverse interaction

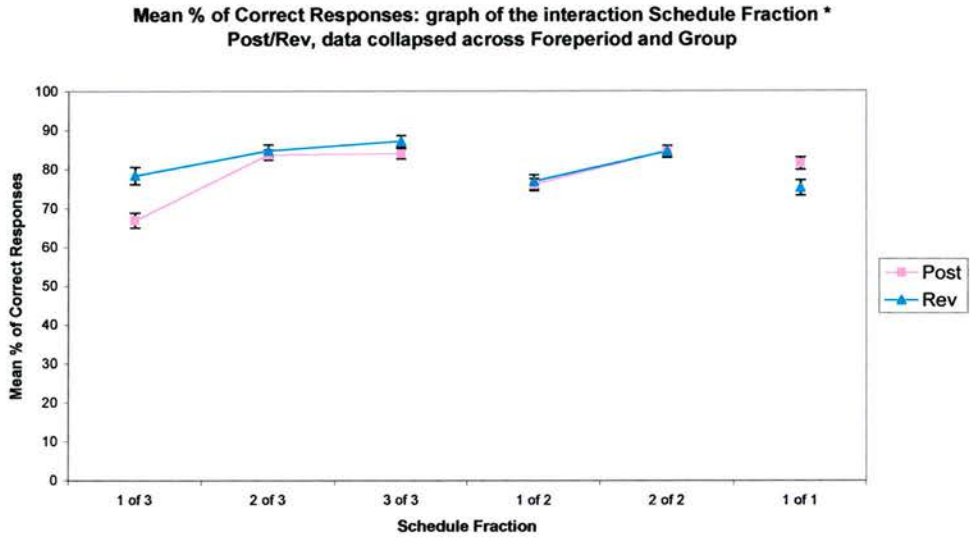


Figure 40: *Graph of the interaction SF*Post/rev, data collapsed across foreperiod and group, showing the mean (±se) percentage of correct responses made postoperatively and during reversal. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Pink line indicates postoperative performance and green line indicates reversal performance.*

Although the data in this interaction is collapsed across group as well as foreperiod, and is therefore of limited interest, it does confirm that there are differences between postoperative and reversal performance which vary according to schedule fraction. The effect size for this interaction is, however, small (2.45%). ***Figure 40*** shows that, during work schedule 3, the rats made a higher percentage of correct responses during the reversal stage than during the postoperative stage; moreover, during both stages the rats made more correct responses as they came closer to achieving reward, but this increase is much less sharp during the reversal stage, perhaps due to a ceiling effect. During work schedule 2 the rats also made more correct responses as they came closer to achieving reward, but there is no difference in performance between the postoperative and reversal stages. However, the rats made more correct responses at schedule fraction 1/1 during the postoperative

stage than during the reversal stage. It is of interest that during the reversal stage the rats made very nearly the same mean percentage of correct responses at the first schedule fractions of work schedule 3 (1/3), work schedule 2 (1/2) and at schedule fraction 1/1 - in other words they are starting from the same baseline on every work schedule. This suggests that the rats are not fully aware of the meaning of the cue lights during the reversal stage, and are therefore always returning to the same start point. The lower mean percentage of correct responses at the rewarded schedule fraction 1/1 during the reversal stage compared to the postoperative stage would seem to support this idea, in that it may result from the rats believing this cue to be signalling the unrewarded schedule fraction 1/3. However, the increase in mean percentage of correct responses within the work schedules as the rats approach reward suggests that the rats are to some extent aware of the new meaning of the cues, though it may reflect an increasing awareness of the new meaning of the cues as the number of sessions increases.

Last day Postoperative - First day Reversal

SF * FP * Last/First interaction

Although the effect size for this interaction is small (4.81%), this interaction is nevertheless of interest because it confirms that there is a difference in performance between the postoperative and reversal stages which varies according to both schedule fraction and foreperiod. The interaction graph (***Figure 41***) shows that as foreperiod increases, the overall mean percentage of correct responses decreases. At the two shorter foreperiods, 100 and 300, the mean percentage of correct responses increases through work schedule 3 as the rat approaches reward in the postoperative stage, but in the reversal stage the mean percentage of correct responses is much higher for the first schedule fraction of the work schedule and is maintained at about the same level through the work schedule. During work schedule 2 there is again an increase in the mean percentage of correct responses as the rat approaches reward during the postoperative stage, but a slight decrease in the mean percentage of correct responses during the reversal stage. However, at foreperiod 500, there is

very little difference in the rats' performance between the postoperative and reversal stages during work schedule 3, though the increase in the mean percentage of correct responses for the reversal stage is greater than for the postoperative stage during work schedule 2. At schedule fraction 1/1, the mean percentage of correct responses is slightly lower during the reversal stage compared to the postoperative stage for foreperiod 100, lower for foreperiods 300 and much lower for foreperiod 500. From the interaction graph, therefore, it would appear that at the shorter foreperiods the rats

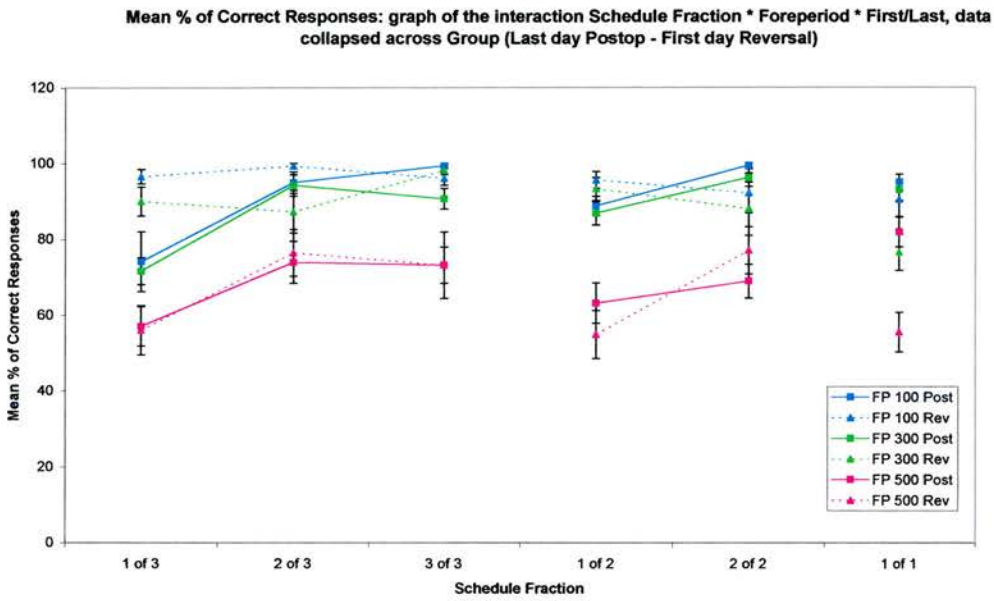


Figure 41: *Graph of the interaction SF*FP*Last/first, data collapsed across group, showing the mean (±se) percentage of correct responses made on the last day of postoperative performance and the first day of reversal performance. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward. Colour of line indicates length of foreperiod (100, 300, and 500 msecs); solid lines indicate postoperative performance and dotted lines indicate reversal performance.*

are performing better during the reversal stage than during the postoperative stage. It is difficult to determine whether this is because the rats are continued to think that cues that now signal unrewarded schedule fractions are still signalling rewarded schedule fractions, or because they were no longer attending to the cues at all; however, the lower mean percentage of correct responses during the reversal stage compared to the postoperative stage for all foreperiods at schedule fraction 1/1 could be taken as evidence that the former is the case. At foreperiod 500, the fact that the mean

percentage of correct responses is the same for the first schedule fraction of every work schedule, or, in other words, that the rats are starting from the same baseline on every work schedule, would suggest that the rats are no longer sure what the cues are signalling. On the other hand, the similarity of performance during the postoperative and reversal stages at this foreperiod would suggest that the rats have learnt, at least to some extent, the new meaning of the cues.

Last day Postoperative – Last day Reversal ANOVA

SF * Group interaction

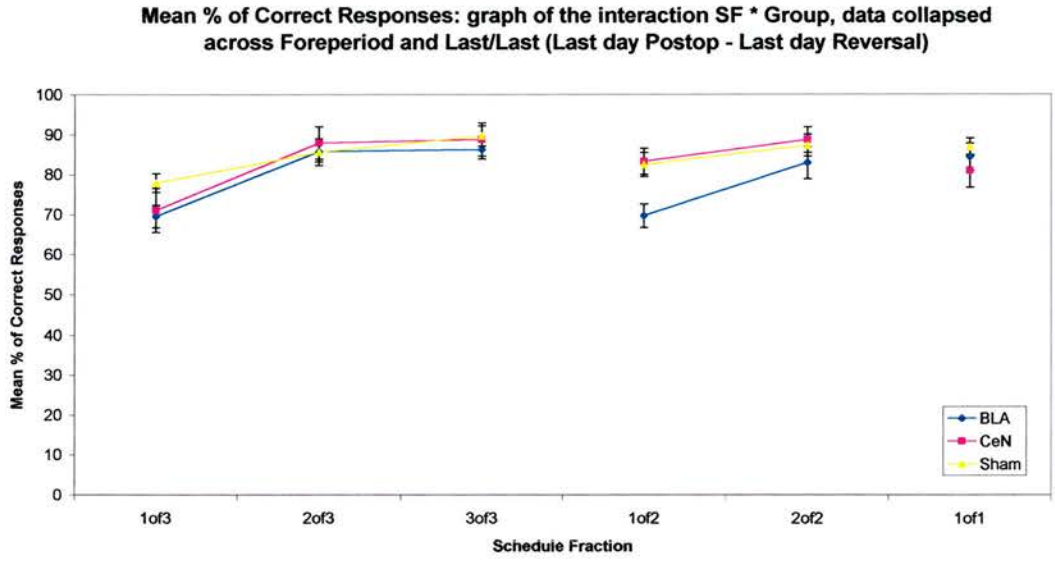


Figure 42: *Graph of the interaction SF*group, data collapsed across foreperiod and Last/last, showing the mean (±se) percentage of correct responses made on the last day of the postoperative stage and the last day of reversal by the three groups. Points connected by lines represent the different schedule fractions within work schedules requiring 3, 2, or 1 correct responses in order to achieve reward..*

The interaction graph (***Figure 42***) shows that, overall, the mean percentage of correct responses increases as the rats approach reward within each work schedule. In work schedule 3 this increase is more linear for the Sham-lesioned group than for the BLA- or CeN-lesioned groups, whilst the mean percentage of correct responses is lower at the first schedule fraction of work schedule 2 (SF 1/2) for the BLA-lesioned group compared to the other two groups. Boxplots were constructed in order to determine whether these differences in performance could be due to outlying or extreme

values in the data. The boxplots revealed an outlying value in the Sham-lesioned group data at schedule fraction 2/3, which perhaps explains the linearity of the Sham-lesioned group's graph line in work schedule 3, and an outlying value in the BLA-lesioned group data at schedule fraction 2/2. However, this latter does not explain why the mean percentage of correct responses should be lower at schedule fraction 1/2 for the BLA-lesioned group compared to the other two groups.

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