A PSYCHOPHARMACOLOGICAL INVESTIGATION OF RESPONSE PREPARATION IN THE RAT

Martin O'Neill
Supervised by Prof. Verity J. Brown

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Declaration

i) I, Martin O'Neill, hereby certify that this thesis, which is approximately 36,000 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.

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ii) I was admitted as a research student in September 2001 and as a candidate for the degree of PhD in September 2001; the higher study for which this a record was carried out in the University of St Andrews between 2001 and 2005.

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Abstract

Speed and accuracy of responding to environmental events is modulated by expectation and anticipation of the timing and location of stimuli, which in turn is determined by the prior likelihood of the stimuli. Neuronal systems that utilise the neurotransmitters dopamine and adenosine play a key role in motor control, with drugs affecting the dopamine system having a particular impact on motor preparation and initiation processes. However, neither these processes, nor the effect of adenosine upon them, are well understood. This thesis investigates the effects of manipulating dopamine (by administration of amphetamine, raclopride and SCH-23390) and adenosine (by administration of the adenosine A_{2A} antagonist, KW-6002), on motor preparation in the rat. Instrumental delayed responding tasks were developed in which temporal and spatial probability were manipulated and the effects of these manipulations and interactions with the drugs were analysed.

Reaction times were determined by both the length of a foreperiod (preparation time) as well as the prior likelihood of a stimulus (stimulus probability). Movement times and anticipatory responses were particularly strongly influenced by both spatial and temporal probability of stimuli, with preparation time having less effect. As expected, amphetamine and KW-6002 stimulated, while raclopride and SCH-23390 inhibited, motor activity. Amphetamine enhanced the effects of both preparation time and probability, whereas KW-6002 particularly enhanced the effect of probability. Although raclopride inhibited overall motor activity, the effect of increasing probability on responding was enhanced.

These findings implicate the dopamine system, particularly via the dopamine D_2 receptor, and the adenosine system, via the A_{2A} receptor, in the translation of stimulus likelihood into expectation and anticipation for the determination of motor output.

Introduction

1.1 Overview

Everyday, we are bombarded with stimuli, many of which require an adequate response from a selection of alternatives. Most of these stimuli are familiar to us but others are not. It is unsurprising that we are more capable of initiating responses that are highly compatible with familiar stimuli. Imagine for example waiting for traffic lights to change from red to amber to green before putting the car in gear, releasing the handbrake and accelerating. If a set of traffic lights were to change directly from red to green – a combination of stimuli rarely, if ever, previously encountered – the processes of action selection are delayed and it would take longer to put the required sequence of actions into effect.

To complicate matters further, stimuli exist in both time and space, so the temporal and spatial characteristics of stimuli must be encoded and represented within neuronal systems in order for efficient control of action. Take again the traffic light example. At some point we have all felt that a traffic light is taking longer than normal to change from red. Why do we feel this? Normally we have no idea of when the traffic light turned red but more importantly, we do not look at the clock on arrival at a red traffic light to compute the length of time the light has been red yet, intuitively, at some point we note an unusually long duration of the red light. There is an internal notion that the light should be changing any moment.

The first example suggests there are internal mechanisms that are primed to prepare responses to familiar stimuli and are disrupted by unfamiliar presentations of even familiar stimuli. The second example suggests the existence of some sort of internal timing mechanism, capable of appreciating the passage of time without reference to a timing device, such as a clock. The subject of this thesis is an exploration of the psychological and neuropharmacological characteristics of response selection in the context of dynamic spatial and temporal properties of stimuli.

Analysis of the functional effects of pathological conditions such as Parkinson's disease has provided some clues to the neural substrates that mediate such response selection processes. In turn, this has influenced the investigations in animals, involving both lesion and pharmacological manipulations. Of course the laboratory situation simplifies the complexities of response situations in

everyday life, but simplified reaction time tasks have been developed that enable investigation of the underlying cognitive processes and neuronal systems involved in action control. Also, interval timing studies have been developed to examine temporal processing directly. Nevertheless, these fields are largely segregated within the literature and it is the central aim of this thesis to address these issues and to identify empirically any similarities or differences in temporal probability processing and the processing of temporal durations. This introduction will review the available literature on reaction time and interval timing, dealing with:

1) Conceptual issues, including a brief history of the use of reaction time and the effects of:
   - Anticipation
   - Response compatibility
   - Attention
2) Neural circuitry that might underlie reaction time effects.
   (The circuitry being relevant in that it provides structural constraints on psychological/functional processes, notwithstanding the evidence which indicates that structural models have been revised as a consequence of increased functional understanding).

1.2 The use of reaction time to study mental processes; a brief history

The idea that reaction time analysis could be used as a tool for inferring timing of information processing originated in physiology and was later incorporated into psychology. It stems from ideas related to *automatic processing* and *reflexive action*, dating back to the end of the 19th century. Sechenov (cited in Posner 1986) was an early proponent of the study of *reflexive* behaviour, proposing that human thought should be viewed as the first two thirds of a reflexive arc, because he believed that motor activity was not critical to the definition of a reflex and that reflexes often terminated at their central phase, in thought. He proposed an invariant relationship not between an external stimulus and a response but between an external stimulus and an internal state, where the

endpoint is not necessarily initiation of a motor pattern. In the early 20th century Sherrington insightfully viewed a reflex action as a form of nervous system coordination, resulting in reflex independence because other reflex systems are indifferent to the specified reaction. Although literally inconceivable due to the extensive connections within the nervous system, Sherrington recognized that this independence of a reflex is an abstraction, a conception of a reflex whose pathway is separate from other reflexes. These ideas of invariance and separability are the underpinnings of automatic activation, proposed to be independent of attentional processing (Posner, 1986). Additionally, Evarts (1973) proposed that *speed* be taken into consideration as a basic criterion for reflex action.

The ideologies of reflexive action were extended by the classic works of Pavlov and Skinner who elegantly displayed basic associational processes and operant conditioning, respectively (Ferster and Skinner 1957; Pavlov 1966). However, the control mechanisms underlying such processes were at the time undefined. From these studies, it was not possible to define whether the responses observed were purely under external stimulus control or if another system was operating to mediate the subsequent response, that is, an internal mechanism (Posner, 1986).

The fusion of conditioning procedures with mental chronometric methods provided a crucial approach to the study of central nervous system activity allowing examination of internal versus external control mechanisms. The chronometric approach in itself has expanded dramatically over the past few decades providing extremely sensitive and powerful tools with which to study internal psychological processes (Posner, 1986). Chronometry is the "measure of time" which has been applied to tests of information processing capabilities. Mental chronometric studies have since been adopted in an attempt to examine these same criteria (in humans and experimental animals).

As described in Posner's *Chronometric Explorations of Mind* (1986), the roots of information processing theory extend back as far as Descartes. The empirical basis of information processing theory was provided by Helmholtz, whose neural conduction findings in frogs and humans were applied by Donders to the study of human mental processes. Donders (1969) developed the subtraction method, which assumed that a given reaction time is composed of a

succession of stages and that tasks could be developed with different stages which could be inserted or removed. The time required for each stage could then be calculated by subtraction of the reaction times of the two conditions.

Donders compared simple reaction times to choice reaction times. A simple reaction time is when there is only one possible response (a button press by the index finger, for example), following presentation of one possible stimulus (a tone, for example). A choice reaction time is when there are multiple possible responses to multiple different stimuli, such as a button press by the index finger in response to a light or a button press by the middle finger to a tone. Note that stimulus modality, as well as many other characteristics by which stimuli may vary such as intensity and location, have considerable implications for reaction times (see Luce 1986; Posner 1986). Indeed, the essence of this thesis is an investigation of reaction times in response to stimuli that vary in temporal and spatial characteristics. This example is simply to illustrate that a choice reaction time involves discriminating between two (or more) stimuli. Choice reaction times are longer than simple reaction times and by subtracting simple reaction times from choice reaction times, Donders suggested that the difference in reaction time reflected the additional mental processes required for 1) stimulus identification (identifying a stimulus from a choice of two) and 2) response selection (selection of the appropriate response from a choice of two).

Moreover, Donders applied an additional procedure, which he termed the c-procedure, which was similar to the choice reaction time procedure except that instead there was only one possible response to two (or more) stimuli. In this procedure, the response was to be made to only one stimulus and withheld for all others. Donders suggested that subtraction of the simple reaction times from the c-procedure reaction times provided a measure of the recognition time (stimulus identification) and subtraction of choice reaction times from c-procedure reaction times provided a measure of the time require to choose between responses (response selection).

However, the subtraction method was met with criticism, and the major points of critique are addressed by Luce (1986, Section 6.2). It was highlighted that in many situations there is no relationship between response time and the amount of information processing required by a task (see Posner 1986, p.15-16).

Fitts and Seeger (1953) suggested that stimulus-response relationships are defined by *compatibility*.

Activation of a given stimulus-response system shortens the time required for it to be reactivated when repeated, sometimes referred to as a repetition effect. This could be due to a conscious awareness in the form of expectancy (Requin et al. 1973). However, there is evidence to suggest that this repetition effect involves an involuntary, automatic component; more frequent events are processed more rapidly even when they are not expected in a given trial (Hinrichs and Craft 1971), suggesting that the repetition effect is to a degree independent of expectancy. Posner (1986; p.90) suggests that this effect is due to '...an automatic increase in the ability to reactivate an associative connection following activation.' Whether or not the repetition effect is due to conscious expectation or automatic processing appears to be contextual, depending on the given circumstance. This is demonstrated in delayed reaction time tasks.

When foreperiods of different lengths are randomly distributed, faster reaction times are observed as a function of lengthening foreperiod (Hohle 1965; Näätänen and Merisalo 1977). However, when foreperiods of different lengths are presented in different blocks (within-block constant foreperiods), faster reaction times are observed with shorter foreperiods (Hohle 1965; Näätänen and Merisalo 1977). Frith and Done (1986) suggest that the stimulus-response process obtains automatic-like status (fast reaction times) when stimulus-response compatibility is at a maximum. For example, a highly compatible stimulus-response, such as an internal response to a word at the level of semantic representation, provides an automatic, rapid response. Low stimulus-response compatibility, on the other hand, results in longer responses. It is proposed that this is due to the required extraction of necessary information from attention and memory sources to provide the system with the appropriate response (Posner 1986).

1.2.1 "Routes to action"

The notion of stimulus-response compatibility was extended by Frith and Done (1986), who described three possible "*routes to action*" in reaction time tasks. In the case of simple reaction time tasks where the response required is pre-cued, the "fast" route is undertaken. This represents a high state of stimulus-

response compatibility, as the pre-cue signal enables preparation of a response in advance. Also, the stimulus does not need to be identified. In a choice reaction time task, the route employed is also dependant on the compatibility of the stimulus-response relationship (Fitts and Seeger 1953). Where stimulus-response relations are high, a "direct" route is used whereas in cases of low stimulus-response compatibility, i.e., where there is an arbitrary association between stimulus and response, the "slow" route is utilised. A requirement of the "slow" route is reference to a matrix of learned associations between stimuli and responses before action initiation, which refers to the aforementioned extraction of necessary information from memory and possibly attentional resources. Frith and Done (1986) further extended this notion to the control of voluntary movement and willed action, a premise that was later discussed in terms of the consequences of neurodegenerative disorders that result in reaction time impairments (Jahanshahi and Frith 1998).

The proposition deems that voluntary movement is either under external, "stimulus intention" control or internal, "willed intention" control. It is the internal, willed action system that appears to be selectively impaired in Parkinson's disease, which Frith and colleagues suggest may adopt the slow and fast routes with the "stimulus intention" system employing the direct route (Frith and Done 1986; Jahanshahi and Frith 1998). Thus, the motor impairment observed in Parkinson's disease may be a manifestation of the inability to generate internally derived spontaneous action (Frith and Done 1986; Jahanshahi and Frith 1998; Robbins and Brown 1990). This notion is supported from human studies that examined internal versus externally guided responding (Briand et al. 1999; Brown and Marsden 1988; Flowers 1976). Also, experimental animal studies support these suggestions (see Robbins and Brown 1990). Brown and Robbins (1991) found that the ability to prepare a response to stimuli based on the previous likelihood of the stimuli (a subjective, internally driven process) was impaired in a Parkinsonian model in the rat.

Stimulus-response compatibility may be enhanced by previous exposure to a stimulus that is associated with a reinforced response. Novel stimuli require repeat exposure before the desired response (motor output pattern) is learned. Under the "*routes to action*" framework described by Frith and Done throughout training, repeated exposure to a stimulus requiring a particular response would

result in over learning which would mean eventually the fast or even direct route could be taken rather than the slow route.

1.2.2 Conditional probability and reaction time

In the study by Frith and Done (1986), they manipulated the temporal probabilities of stimuli and reported a negative correlation between increasing temporal probability and reaction time.

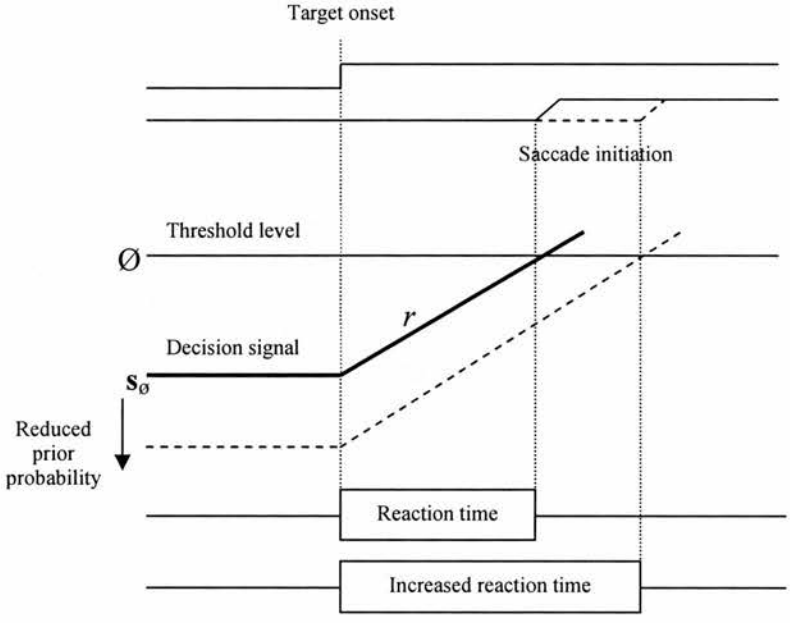

Figure 1.1 Model of the decision process for initiating saccades as proposed by Carpenter and Williams (1995). Following target onset, the putative decision signal ($s_ø$, bold line) rises linearly until it reaches a threshold level (Ø) whereupon a saccade is initiated. The rise (r) in this decision signal represents the decision time, or reaction time, to initiate a saccade. Thus, reaction time is the measure from target onset until saccade initiation (termed saccadic latency in Carpenter and Williams, 1995). In their model, the variation in the rate r from trial to trial has a normal distribution. The vertical position of the initial decision signal before target onset is defined by the prior probability of the target stimulus. A reduced prior probability of a stimulus results in a downward shift in the decision signal that causes the rise in the rate r to lengthen (dashed line) and a slower saccade (i.e., with a longer reaction time) is initiated. (Adapted from Carpenter and Williams 1995)

This delay-dependent speeding of reaction time had previously been described in humans (Näätänen 1970; Näätänen and Merisalo 1977; Requin and Granjon 1969) and subsequently in rats (Brown et al. 1996; Brown and Robbins 1991). In randomly distributed, variable foreperiod reaction time tasks, as the length of foreperiod duration preceding the imperative signal increases, reaction time decreases and anticipatory (prior to the signal) responding increases. This is presumed to reflect an increase in 'motor readiness' (Näätänen and Merisalo 1977), which is a term used to describe the preparation capacity of the motor system. This increase in 'motor readiness' is attributed to the rising conditional temporal probability associated with an increase in foreperiod, signifying the temporal location of the target. Carpenter and Williams (1995) manipulated the prior probability of a visual target to the left or right visual field and found that human saccadic reaction times were faster when the prior probability of the target location was greater. These findings suggest that stimulus-response compatibility is not simply determined by internal representations of complex stimuli, such as recognition of words (Posner, 1986), but also relies on the magnitude of previous exposure to simple (spatial or temporal) stimuli. Carpenter and Williams (1995) applied likelihood theory to their findings and formulated a model to describe the computational processes underlying the decision process that is necessary for the calculation of conditional probability (see also Carpenter 2004). This model is illustrated in Figure 1.1.

Likelihood theory suggests that the estimated likelihood (L) of a target being present relative to it not being present is modified on the basis of the outcome of each preceding reaction time trial (E) in which a target is present to form an updated likelihood (L'). Carpenter and Williams proposed that the presence of a target in their task causes a signal in a 'decision unit' to rise linearly at a rate *r* from its initial value $s_{ø}$ until it reached a fixed threshold Ø, at which point a saccade is initiated. Thus, if the initial value of $s_{ø}$ is lower than average (in low prior probability conditions) then the linear rise of r will reach the critical threshold value later and thus result in slower saccadic reaction times.

Therefore, together these studies have shown that the spatial and temporal conditional probability of a stimulus based on previous experience, are both relevant in the determination of response initiation and timing. However, a critical difference between varying spatial probability and varying temporal

probability of a stimulus is that probability of spatial location can be manipulated independently of probability at other spatial locations. Whereas varying temporal probability is confounded with elapsed time *per se*. Thus, whether the decrease in reaction time as a function of increasing foreperiod delay is due to the increasing temporal probability of the imperative signal or due to an increase in the time to prepare a response is not easily disentangled. Chapter 3 of this thesis directly assesses this issue with a task in which stimulus probability was held constant with varying preparation time durations. Frith and Done (1986) previously assessed the effect of constant probability with increasing foreperiod duration and found that the probability was reflected in reaction times.

Also, it has been suggested that the increase in anticipatory responses as foreperiod duration increases reflects a miscalculation of the temporal probability of the imperative signal, where the temporal probability summation reaches unity before the imperative signal and a motor program is initiated, resulting in an anticipatory response (Brown et al. 1996). There is a possible alternative explanation for this increased incidence of anticipatory responses as a function of increasing foreperiod duration that relates directly to the issue of temporal probability versus preparation time.

1.3 Temporal control of behaviour

It has been suggested that there is an 'internal clock'; a psychological function that monitors the passage of time (see Meck 1996 for review). This hypothetical 'internal clock' is of particular interest when considering performance in reaction time tasks because it might be that performance in operant tasks is not simply and solely under stimulus control (signal detection) but is also mediated by temporal information processing mechanisms (MacDonald and Meck 2004).

1.3.1 Scalar expectancy theory

Scalar expectancy theory is a mathematical model that describes the formal underpinnings of the information processing model of interval timing (Gibbon et al. 1997), particularly with respect to the cognitive processes involved in discriminating between two temporal durations (Meck 1996). Scalar expectancy theory assumes that when different time intervals are to be

discriminated, the variation in response rate distributions increases proportional with the time interval being estimated. In other words, as the time interval that is to be estimated increases then the likelihood of errors in time estimation increases, which is reflected in a greater variance of behavioural responses at longer time intervals with shorter and longer estimates of the actual duration in roughly equal proportion.

Figure 1.2 is a schematic illustration of the information processing model of interval timing processes. A *pacemaker* or *oscillatory process* autonomously emits pulses that are gated by a switch into an *accumulator* that integrates pulses in a linear fashion over time. The switch is activated by an external timing signal, indicating the beginning of a time period. This is the 'clock' component of the model. The output of the accumulator is then referenced to a distributed memory store where the expected duration of some salient event is coded, and compared by a *decision* module. The decision stage compares current time with remembered time. If the values from the accumulator and memory store are sufficiently close then a response is made. Scalar variance may enter at any stage of the information processing model (Gibbon et al. 1997). This is a basic description of the conceptual framework that has led to experimental testing of the validity of each component. The components of this model have been examined predominantly by interval timing tasks in the rat, such as the temporal bisection procedure (Church and Deluty 1977) and the peak interval procedure (Meck and Church 1984).

1.3.2 Interval timing tasks

In the temporal bisection procedure, rats are trained to respond on either of two levers by discriminating between two signals. They learn to respond on one lever following a signal of short duration and on the other lever following a signal of long duration (correct responses are rewarded). When performance stabilises, intermediate durations are introduced. Responses to the intermediate durations are not rewarded. The proportion of responses at the long lever is plotted as a function of signal duration. The psychophysical function is sigmoidal in shape and the measure of interest is the *indifference point*, which is the signal duration that is equally as likely to result in a response to either the short or long lever. Manipulations that influence temporal processing result in a shift of this

bisection point leftward or rightward, interpreted as an increase or a decrease in the perception of the passage of time, respectively. Such manipulations are discussed below.

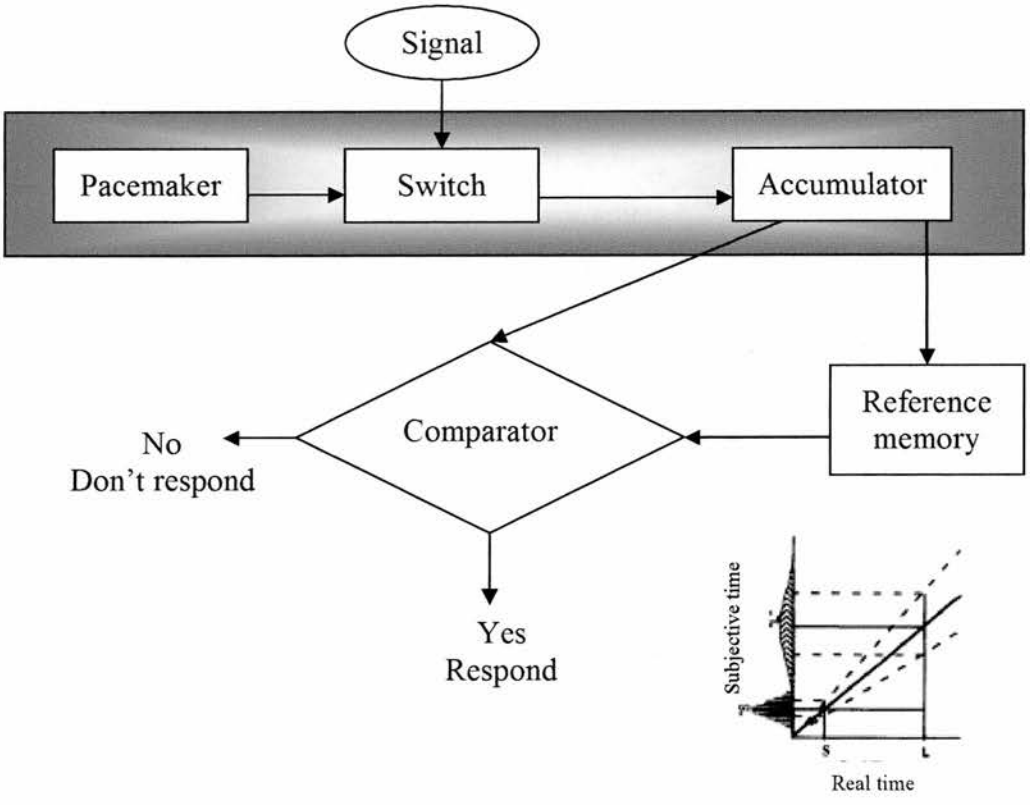

Figure 1.2 The information-processing, scalar timing model of temporal processing. The 'clock' components are highlighted. The information processing components of the scalar timing model generate subjective timing distributions, that are wider with longer time intervals as illustrated in the inset (S – short, L – long).

The peak interval procedure provides a measure of temporal discrimination with response rate. The task involves rewarding a response after a specific interval. The interval is signalled by a continuous tone or a light for the duration of the interval. The first lever press after the interval is rewarded. Randomly interspersed within rewarded trials are 'peak' trials where the signal is extended with no reward. When the data from the 'peak' trials is averaged, a Gaussian distribution of responding is observed around the time of reward, with the maximum rate of responding at the expected time of reinforcement which then tapers off roughly symmetrically after the time has passed. The peak *time* of the resulting psychophysical function is thought to be sensitive to manipulations

that influence temporal processing, with a leftward or rightward shift representative of an increase or decrease in the perception of the passage of time, respectively. The psychophysical function from the peak interval procedure displays nicely the properties of scalar expectancy theory, as the degree of variance from the mode of the 'peak' increases proportionally with the temporal duration that is to be estimated.

1.3.3 Can theories of interval timing explain behaviour in reaction time tasks?

In terms of operant tasks employing variable foreperiods, scalar expectancy theory might be capable of explaining the increase in anticipatory errors as a function of increasing foreperiod duration, because it assumes that more errors in time estimation should occur at longer time intervals. This implies that at longer foreperiods a proportionally greater inaccurate estimate of the amount of time elapsed should occur, which would result in an increase of the likelihood of a pre-emptive initiation of a motor program and an anticipatory response. This is an alternative explanation to the one provided by Brown et al. (1996) based on temporal probability summation.

However, there are two critical points regarding this explanation. First, scalar expectancy theory assumes a normal distribution of responses around an estimated time. Therefore, the amount of late responses would be expected to reflect the amount of anticipatory responses. This is not observed in delayed reaction time tasks, as anticipatory responses tend to be considerably greater in number than late responses. Second, when considering reaction times, scalar expectancy theory fails to account for the apparent *improvement* in performance. Indeed, it is not yet clear whether reaction time and anticipatory responding are mediated by the same psychological and neuronal mechanisms or are independent processes. There are suggestions that these are independent processes, as indicated in ablation studies that have identified differential effects on anticipatory responding and reaction time following lesions of subcortical structures of the basal ganglia. For instance, ablation of the subthalamic nucleus has been shown to increase anticipatory responding without affecting reaction times (Phillips and Brown 1999; 2000), whereas depletion of striatal dopamine

results in reaction time impairments, which has been observed in the absence of an effect on anticipatory responding (Brasted et al. 1998; Brown and Robbins 1991; Ward and Brown 1996).

To summarise, in operant tasks that incorporate randomly distributed foreperiods of variable length, anticipatory responding may be under the influence of the temporal probability of the imperative signal or may be characteristic of the amount of time elapsed at each foreperiod. Although Frith and Done (1986) compared the effects of stimulus temporal probability with time elapsed on reaction time, to date there has been no attempt to compare directly the effects of stimulus temporal probability with time elapsed on responding *per se*. This is surprising because the neuronal systems that have been identified as crucial for performance in operant reaction time tasks belong to the same systems that have been implicated in internal clock mechanisms; operant reaction time tasks and interval timing studies in the rat, which are largely segregated fields of investigation in the literature, identify striatal dopamine transmission and prefrontal cortical function in both signal detection and temporal processing.

The cortex and striatum are structures of the cortico-basal ganglia circuitry, which are implicated in reaction time responding and time estimation processes. These circuits, particularly the striatum, are also likely targets for the effects of amphetamine, KW-6002, raclopride and SCH-23390 – the drugs selected on this basis for investigation in this thesis. Moreover, amphetamine and raclopride have been implicated as potent effectors in operant tasks and interval timing studies. Thus, the next section of this introduction will provide an overview of cortico-basal ganglia functional anatomy, and the effects of perturbations of these circuits, on operant behaviour and time estimation processes. Also, the role of the dopamine system will be discussed and the rationale for investigation of the role of the adenosine system provided.

1.4 Cortico-basal ganglia circuitry

The term basal ganglia is used to refer to a group of functionally-related sub-cortical nuclei. Generally, the term is used to include the striatum (caudate nucleus, putamen and nucleus accumbens), globus pallidus, subthalamic nucleus and substantia nigra (even though the latter is in the midbrain and historically the term referred to forebrain nuclei). It is also common to find the expression 'basal

ganglia-thalamocortical loop' which refers to the wider network, into which input to the basal ganglia comes mainly to the striatum from virtually all of the cerebral cortex and projects topographically (see Voorn et al. 2004 and Figure 1.3), via the thalamus, back to frontal cortex.

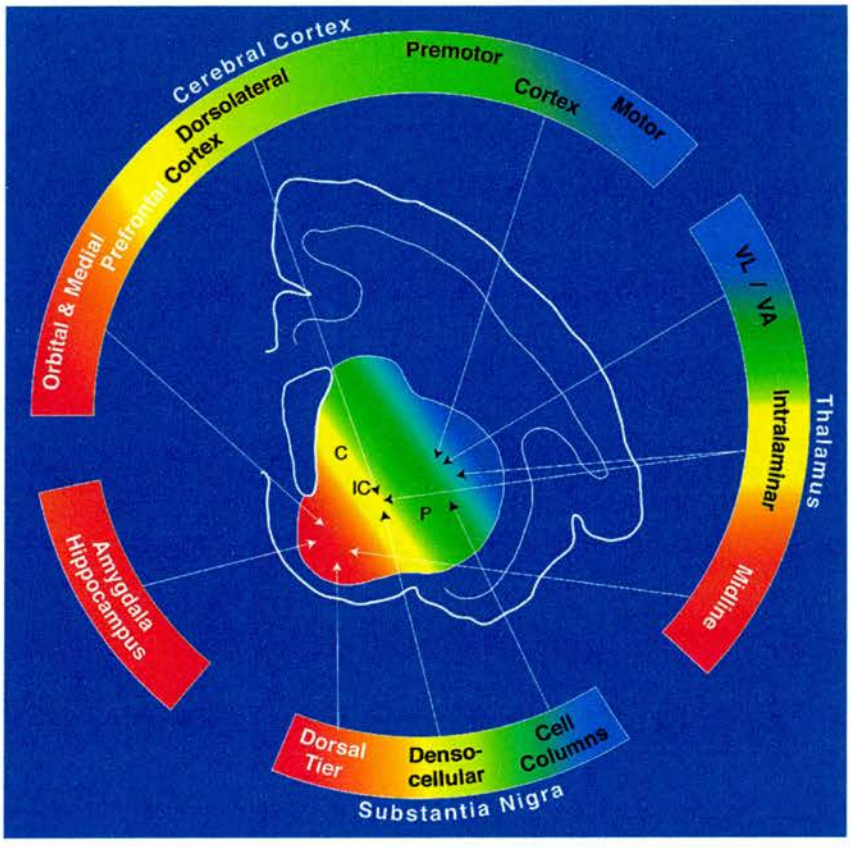

Figure 1.3 The topographically arranged striatal afferent projections in the primate from the cerebral cortex, thalamus, substantia nigra, amygdala and hippocampus. The colour coding corresponds to functionally related regions. The ventromedial striatal region shown in red corresponds to the nucleus accumbens and receives projections from orbital and medial prefrontal cortex, midline nuclei of the thalamus, amygdala and hippocampus. These regions are associated with limbic functions, such as the processing of motivationally salient cues. The yellow through to blue regions represent both the caudate nucleus and the putamen of the striatum. The dorsolateral motor striatal region shown in blue receives input from the motor areas of cortex as well as the ventrolateral and ventroanterior (VL/VA) thalamic nuclei. Between these two extremes is the central portion of the striatum (yellow/green), which receives input from the associative premotor and dorsolateral cortex and intralaminar thalamic nuclei and is thus referred to as the associative striatal region, thought to be involved in

the cognitive processing of stimulus information (Illustration kindly supplied by Suzanne Haber, see Haber 2003).

1.4.1 Pathways through the basal ganglia

One of the most influential ideas regarding the functional-anatomical organization of the basal ganglia is that of the basal ganglia-thalamocortical loops was provided by Albin and Delong (Albin et al. 1989; DeLong 1990). Although there have been and continue to be refinements of the originally proposed scheme (see Figure 1.4) (Gurney et al. 2004).

Essentially, cortical information is channelled through the components of the basal ganglia to the thalamus and back to frontal cortex via a "direct" (cortex - striatum – internal globus pallidus (GPi) / substantia nigra pars reticulata (SNr) – thalamus), an "indirect" (cortex - striatum – external globus pallidus (GPe) – subthalamic nucleus (STN) – GPi/SNr - thalamus) (Alexander and Crutcher, 1990) and a "hyperdirect" (cortex – STN – GPi / SNr) (Mink 1996; Nambu et al. 2002) pathway. Additionally, there is a further pathway that has as yet escaped being termed in the literature and could be regarded as an "intermediate-indirect" (striatum – GPe – GPi/SNr) (Shink et al. 1996; Smith et al. 1998) pathway.

The pathways are further defined by neurochemical and receptor characteristics. Cortical afferents release glutamate, which provides the main excitatory drive of striatal neurons. This cortical input is modulated by dopamine release from midbrain afferent projections (Centonze et al. 2001; Smith and Bolam 1990) and is also modulated by GABA and acetylcholine, which are released from interneurons that are intrinsic to the striatum (Kawaguchi 1997). Furthermore, the neuromodulator adenosine, which is secreted in the striatum by most constituent neurons as well as being formed in the extracellular space by degradation of ATP released from synaptic vesicles (Ribeiro et al. 2002), also appears to modulate information from the cortex to striatum. Additionally, GABAergic collateral projections from other medium spiny neurons may play a crucial role in modulation of striatal output (Plenz 2003).

1.4.2 Neurochemical interactions in the striatum

The striatum is considered the main input station of the basal ganglia and consists of mainly one type of neuron, the medium spiny neuron, which accounts

for more than 90% of the striatal population and receives about 10,000 cortical afferent inputs (Wilson 1995). Thus, a remarkable degree of convergence occurs at each medium spiny neuron. The remaining portion of neurons is made up of a variety of distinctive interneurons.

All medium spiny neurons are innervated by cortical projections that provide the main depolarizing driving force of striatal spiny cells. The cortical inputs make synaptic contacts principally on the heads of the distal dendritic spines of the medium spiny neurons and release glutamate as their neurotransmitter. The striatum also receives glutamatergic input from the thalamus (McFarland and Haber 2000). This is an interesting connection that enables the thalamus to modulate striatal activity but the possible functional significance has not been fully discussed in the literature and merits further investigation. The medium spiny neurons are classified into two subtypes, distinguished by their projection targets, neuropeptide content and dopamine receptor expression. These are the striatopallidal and striatonigral neurons, which constitute the indirect and direct pathways, respectively.

1.4.2.1 Dopamine D_1 and D_2 receptors

Dopamine receptors are abundant in the striatum and play a critical role in striatal output: it is via these that the midbrain dopamine projection modulates corticostriatal input. An example of a corticostriatal and dopaminergic synapse is shown in Figure 1.5. The distribution of the dopamine D_1 and D_2 receptors in the striatum is a contested issue in the literature. There are conflicting reports and ideas on the distribution and expression of the dopamine D_1 and D_2 receptor subtypes on the two different populations of striatal spiny neurons. Basically, there is the segregation argument (Bloch and Le Moine 1994; Gerfen et al. 1990; Gerfen and Keefe 1994; Le Moine et al. 1991), which proposes that D_1 receptors are exclusively expressed by striatonigral neurons of the indirect pathway and D_2 receptors are restricted to the striatopallidal neurons of the indirect pathway. Alternatively, Surmeier and colleagues have argued in favour of D_1 and D_2 receptor colocalisation on both populations of spiny neurons (see Surmeier et al. 1993; Surmeier et al. 1994).

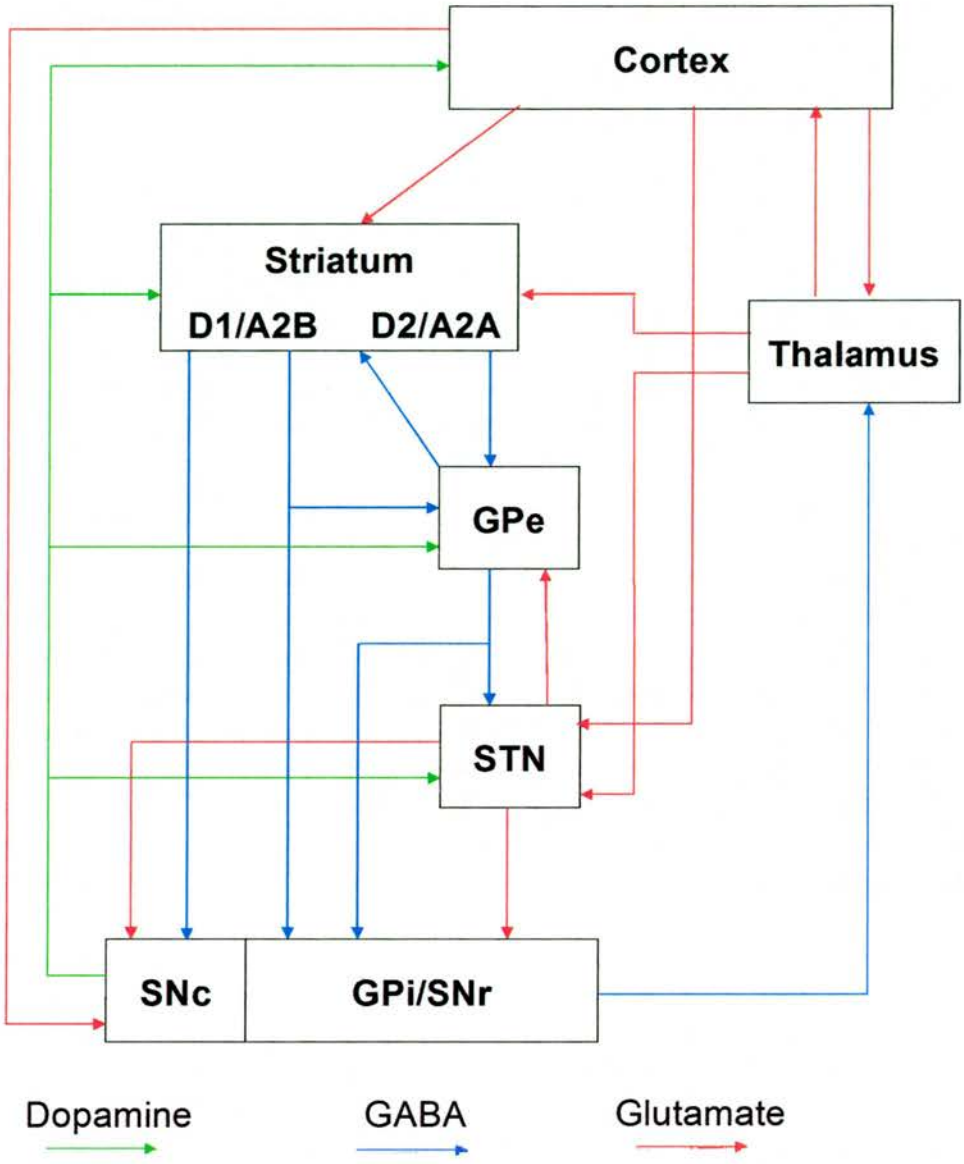

Figure 1.4 Box-arrow diagram illustrating the complexity of the connections between the nuclei of the basal ganglia and the afferent, descending glutamatergic connections from the cortex and thalamus (red connections) to the striatum and subthalamic nucleus and the ascending afferent dopaminergic midbrain projections (green connections). Abbreviations: D, dopamine receptors; A, adenosine receptors; GPe, external globus pallidus; GPi, internal globus pallidus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus. (Redrawn from Gurney et al. 2004)

Essentially, the proponents on each side of the debate emphasises the shortcomings of the methodological techniques employed by the opposition

resulting in premature conjecture (see Gerfen and Keefe, 1994; Bloch and Le Moine 1994; Surmeier et al., 1994). The methodological critique is clearly due to technical limitations at that time and this is accepted by both groups. It is likely that the inability of certain immunocytochemical and *in situ* hybridisation techniques to display colocalisation is due to a lack of sensitivity. The opposite may be the case with the PCR[1] technique, which is extremely sensitive and the extensive D_1/D_2 expression overlap observed with PCR could be due to this ultra-sensitivity. More recently, a study using more refined technologically advanced techniques has provided direct anatomical and physiological evidence in support of colocalisation (Aizman et al., 2000).

Aizman et al. (2000) combined confocal microscopy and immunolabelling to demonstrate the presence of both dopamine receptor classes on virtually all striatal neurons and absence from glial cells, *in vitro* and *in vivo*. In addition, emission fluorescence (a process by which release of a sodium-sensitive fluorescent probe (SBFI-AM) from neurons following drug application can be monitored and measured) showed that stimulation of both D_1 and D_2 receptors altered sodium levels in all striatal neurons examined. Stimulation of D_1 receptors caused a decrease in sodium influx and efflux – effects that were reversed by subsequent blocking of D_1 receptors. D_2 stimulation, on the other hand, increased sodium influx but did not affect sodium efflux- the influx effect was blocked by administration of a D_2 antagonist. Thus, although this study exquisitely shows that both classes of dopamine neurons are present on practically all striatal neurons it further characterises mechanisms, via differential effects on sodium influx/efflux, by which D_1 or D_2 specific ligands may exert their physiologically distinct effects. Thus, the authors reconcile the segregation and colocalisation argument by suggesting that intracellular rather than intercellular mechanisms are responsible for many of the '...synergistic and antagonistic actions exerted by activation of D_1 and D_2 subclasses of dopamine receptors on numerous signal-transduction pathways'. They further postulate that the co-localised versus the segregated views on dopamine receptor expression in

[1] PCR is a technique which allows amplification of a single strand of mRNA. Thus, although a single strand of mRNA coding for both D_1 and D_2 receptors was shown to be present on the majority of medium spiny striatal neurons, the functional relevance of a single strands presence is disputable. In particular, it is not known if a single strand would ultimately lead to protein synthesis and configuration of a functionally expressed receptor.

the striatum may be reconciled by the notion that D_2 receptors are expressed at higher levels than D_1 receptors on striatopallidal neurons (and vice-versa for striatonigral neurons).

Also, there is evidence from electrophysiological and microdialysis studies suggesting that the outcome of independent stimulation of either the D_1 or D_2 receptor pathways is not restricted to their respective trajectories, the direct and indirect pathways, respectively (see Kreiss et al. 1997 and Le Moine et al., 1997).

As well as postsynaptic dopamine receptors, the dopamine D_2 is located presynaptically. Electrophysiological experiments suggest the existence of D_2 receptor on presynaptic glutamatergic afferent terminals that, when activated, inhibit corticostriatal activity (Flores-Hernandez et al. 1997; Hsu et al. 1995), but see Calabresi et al. (1996) for a review of conflicting anatomical and physiological findings.

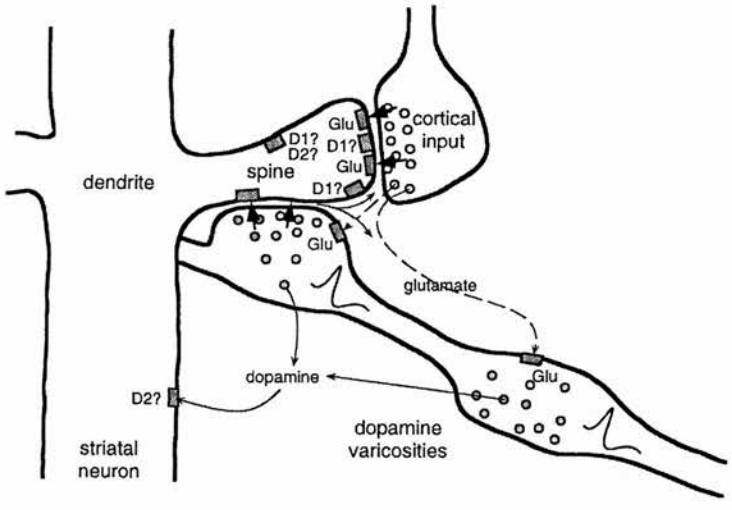

Figure 1.5 An example of a corticostriatal glutamate synapse on the head of a striatal dendritic spine and a midbrain dopamine afferent releasing dopamine at the spine of the dendritic shaft (Schultz 1998, used with permission).

In summary, dopamine released from nigrostriatal axonal terminals appears to exert its modulatory function on striatal function via D_1 and D_2 receptors located postsynaptically on medium spiny neurons. The predominant result of D_1 activation is regarded as excitation, whereas D_2 receptor activation, on the other hand, has an inhibitory effect. However, there is considerable controversy surrounding the physiological consequences of D_1 and D_2 receptor

activation (see Calabresi et al. 2000 for discussion). The inconsistencies in the anatomical and physiological findings regarding D_1 and D_2 receptors illustrate the complexities of the neurochemical and physiological interactions at the neuronal and network level of the basal ganglia. Dopamine receptors are expressed in other structures of the basal ganglia, such as the STN (Flores et al. 1999), and there are collateral projections between axons of the striatopallidal and striatonigral neurons (Wu et al. 2000; Yung et al. 1996), which may explain the complex interactions of these receptor populations.

1.4.2.2 Adenosine A_{2A} receptor

In addition to dopamine, adenosine is also present in the extracellular space in the striatum, where it is capable of modulating striatal activity via adenosine A_1 and A_2 receptors. Drugs that increase dopaminergic activity are classically considered to stimulate sensorimotor activity. On the other hand, drugs that inhibit adenosine activity, such as caffeine, stimulate sensorimotor activity. Thus, dopamine and adenosine have opposing effects, such that blocking the adenosine system has comparable effects to stimulating the dopamine system. A common feature of the adenosine and dopamine systems in the striatum is the distribution of the receptor subtypes.

The most generally accepted notion is that the dopamine D_1 receptor is co-localised with the adenosine A_1 receptor on the striatonigral neurons of the direct pathway, whereas the dopamine D_2 receptor is co-localised with the adenosine A_{2A} receptor on the striatopallidal neurons of the indirect pathway. However, the above discussion on the controversy surrounding the distribution of the dopamine receptor subtypes in the striatum warns that caution should be exercised when interpreting the available data on receptor distributions. Nonetheless, there is a growing body of evidence from different methodological approaches indicating co-localisation of the adenosine A_{2A} receptor and the dopamine D_2 receptor in the striatum. The adenosine A_{2A} receptor is most abundantly expressed in the striatum relative to any other brain region (Alexander and Reddington 1989; and see Svenningsson et al. 1999)

The A_{2A} and D_2 receptors are reported to have an antagonistic relationship. Binding studies have shown that stimulation of the A_{2A} receptor decreases the affinity of the dopamine D_2 receptor for dopamine agonist drugs (Ferre et al.

1999; Ferre et al. 1991). *In vivo* microdialysis studies have shown that selective ablation of the relay nuclei of the indirect pathway (GPe/STN) disrupted the effects of the adenosine A_{2A} antagonist, KW-6002, on neurotransmitter release in the output nuclei (Ochi et al. 2004). Also, a study by Le Moine et al. (1997) showed that network and neuronal interactions occur in the striatum between the dopamine D_1 and D_2 receptors, respectively, and the adenosine A_{2A} receptor (see also Fuxe et al. 1998). An electrophysiological investigation showed that the inhibitory effects of a dopamine D_2 agonist on striatal neuronal activity was enhanced by antagonism of the A_{2A} receptor and reduced by an A_{2A} agonist (Stromberg et al. 2000). Moreover, the dose of the A_{2A} antagonist used in this study to achieve modulation of the D_2-mediated effects was ineffective when administered alone.

The cellular, pharmacological, microdialysis and electrophysiological findings are supported by a wealth of preclinical data looking primarily at the effect of A_{2A} antagonists on motor functions mediated by dopaminergic activity. The A_{2A} receptor is reported to have a behaviourally expressed antagonistic relationship with the D_2 receptor.

Systemic treatment with adenosine A_{2A} antagonists reverses the catalepsy and hypolocomotion induced by systemic administration of dopaminergic antagonists (Shiozaki et al. 1999), potentiates the effects of non-specific (Fenu et al. 1997; Koga et al. 2000; Lundblad et al. 2003) and D_2-receptor specific dopaminergic agonists (Stromberg et al. 2000) on turning behaviour in rat models of Parkinson's disease and reduces the motor impairments in D_2-receptor knockout mice (Aoyama et al. 2000). Direct injection of A_{2A} agonists into the rat striatum induces catalepsy (Hauber and Munkle 1995), suggesting that the effects of adenosine on motor performance are at least in part mediated via the A_{2A} receptor at the level of the striatum. Furthermore, catalepsy induced by direct infusion of D_1/D_2 receptor antagonists into the striatum in the rat is blocked when co-infused with an adenosine A_{2A} receptor antagonist (Hauber et al. 2001).

Complementary studies in MPTP-treated Parkinsonian marmosets suggest the use of the adenosine A_{2A} antagonist, KW-6002, alone or adjunctive with dopaminergic drugs. KW-6002 alone was less likely to result in dyskinesias (Grondin et al. 1999; Lundblad et al. 2003), whereas in conjunction with levodopa, the levodopa effects were potentiated without exacerbating dyskinesias

and thus KW-6002 may provide a means of reducing levodopa medication (Kanda et al. 2000). Clinically, KW-6002 has been shown to improve the motor symptoms in Parkinson's patients with low dose levodopa treatment, increasing "on" time and decreasing the incidence of dyskinesia by almost 50 % (Bara-Jimenez et al. 2003; Hauser et al. 2003).

1.5 The basal ganglia and behavioural control

The functional synergy of the structures that comprise the cortico-basal ganglia loops is greatly impaired in Parkinson's disease. Parkinson's disease is a neurodegenerative disorder that results in debilitating motor impairments including akinesia, bradykinesia and tremor. The cortico-basal ganglia loops loops are thought to be involved in contextually-dependent behavioural control. It has been hypothesised that information originating from cortical areas is channelled through the basal ganglia and back to the frontal cortex with an updated information signal that is then be incorporated into the production of a desired motor program which is transmitted to the motor system via the deep brain motor nuclei in the brainstem and the spinal cord. In other words, it has been suggested that the basal ganglia mediate motor output that is dependent on the relevance of sensory information in the context of previous experience.

The afferent glutamatergic input to striatal spiny neurons, mainly from cortex but also thalamus, is modulated by dopamine (Smith and Bolam, 1990), which is thought to provide a reinforcement signal that strengthens corticostriatal synaptic connections (Centonze et al. 2001). It has been suggested that the dopaminergic input acts as a "learning signal", priming striatal neurons to recognise relevant patterns of cortical input (Houk 1995). Behavioural support for this posit arises from neurophysiological studies showing that activity of dopamine neurons is largely context-dependent and increased activity is observed in response to unpredicted environmental events (Schultz 1998). The strengthening of corticostriatal synapses following dopamine release might serve to increase the probability of activation of the striatal neuron following subsequent cortical activity. Latter cortical activity would require a similar constellation of environmental events for the sensory input to activate the cortical neurons, and by this means the striatal neuron(s) would recognise the previous constellation of cortical activity. These mechanisms provide the system with a

means of recognising salient environmental events, with the signal propagated from the striatum contributing to appropriate motor output.

A recent study described the function of D_2 presynaptic autoreceptors as highly complex, selectively inhibiting a subset of corticostriatal neurons that have a low baseline probability of firing (Bamford et al. 2004), implying that dopamine permits the transmission of cortical signals that are most probable over less likely signals, in effect increasing the signal-noise ratio of information transmission (see also Flores-Hernandez et al. 1997). This is a very interesting finding that coincides with the idea of dopamine providing a "learning signal". The idea that the basal ganglia play a role in context-dependent behaviours can also be considered phylogenetically.

Cortical development and the corresponding development of the cortico-basal ganglia loops in man may be a consequence of evolutionary selection in response to the increase in behavioural demands provided by an ever-increasing complex environment. However, the basal ganglia are an evolutionary-preserved group of nuclei also involved in more rapid, instinctive behaviours that are not context dependent and do not require prefrontal cortical processing.

Components of the basal ganglia receive direct input from the sensory relay nuclei, such as the superior colliculus, and project directly to lower brainstem nuclei such as the pedunculopontine nucleus (PPN). Therefore, sensory information may be transmitted through the basal ganglia to the motor system without traversing the cortex. Also, sensory information may be transferred directly to the motor system via midbrain structures such as the superior colliculus. Thus, the cortico-basal ganglia loops are not the only possible routes of information flow through nuclei of the basal ganglia. The different levels of information transfer are thought to represent a functional hierarchy of action control (Hikosaka 1998).

By this premise, rapid reflexive actions, such as orienting responses, are mediated via the quickest possible route. More routine behaviours, such as walking, are mediated via an intermediate route whereby the pattern of motor output is generated (in the motor nuclei of the brainstem). Behaviours that are learned contingent on factors such as incentive motivation (see Robbins and Everitt 1996 for a discussion) and previous exposure to stimuli are mediated via the longest, most elaborate route which reflects the magnitude of demand on

these types of complex behaviours. It is this complex route that incorporates the basal ganglia and frontal cortex.

Within this system, Hikosaka (1998) argues that the basic function of the cerebral cortex is the combination of available actions in both spatial and temporal domains to create new action programs. Through development, many action programs will have accumulated in the motor/premotor cortices and the role of the basal ganglia is the selection of desired, learned motor programs (Hikosaka 1998).

1.5.1 Pathway-based model of contextual movement control

Mink (1996) provided a model as an explanation of how the basal ganglia control volitional movement based on the interplay between the "direct", "indirect" and "hyperdirect" pathways. This model suggests that the basal ganglia act in concert to block competing motor programs by inhibiting undesired motor programs and facilitating desired motor program output, which is schematically illustrated in Figure 1.6 (Mink 1996; Nambu et al. 2002). A motor program refers to a cumulative neuronal signal that encodes a subsequent motor output. The model assumes that the function of the hyperdirect pathway is to suppress previous network states, by increasing activity of the output nuclei, which ultimately inhibits the projection from the thalamus to the prefrontal cortex (thalamocortical projection) (Maurice et al. 1998; Nambu et al. 2002). The later signal from the direct pathway then inhibits the output nuclei, allowing selection of the desired motor program by propagation of the signal back to cortex through the active thalamocortical connection. Finally, the late signal from the indirect pathway (Maurice et al. 1998) serves to block competing motor programs. A key component of this model is the timing differences between the pathways. There is considerable support for inhibition and facilitation of motor programs by opposing pathways from the application of this model to pathological conditions such as Parkinson's disease and animal models of basal ganglia dysfunction.

The cardinal neuropathology of Parkinson's disease is a loss of striatal dopamine as a result of degeneration of the dopaminergic nigrostriatal projection, which results in over-activity of the STN and the output nuclei. It has been

suggested that the increased firing activity of these nuclei results in the typical motor impairments observed in Parkinson's disease.

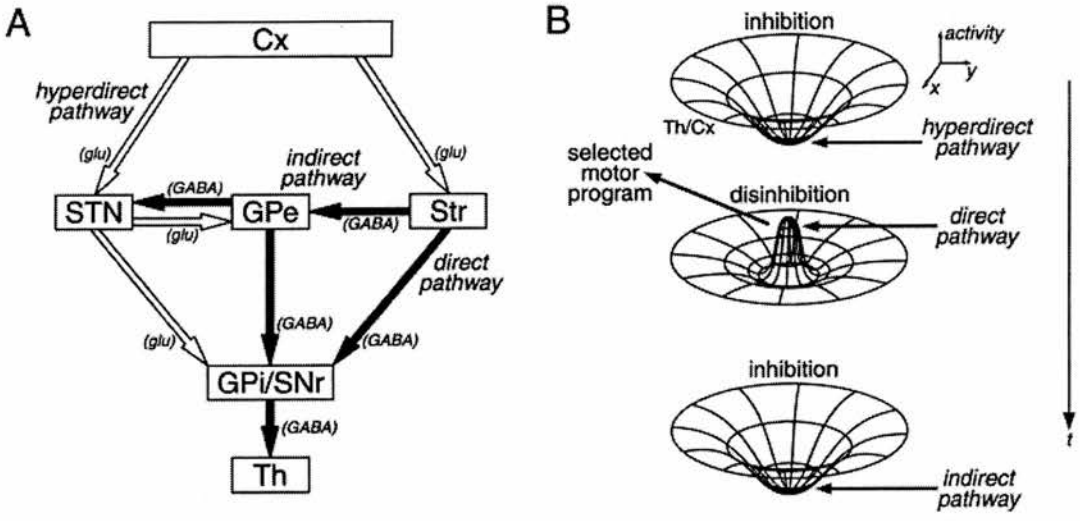

Figure 1.6 a) A simplified box-arrow diagram showing only the pathway projections from the cortex through the basal ganglia to the thalamus. Open and filled arrows represent excitatory glutamatergic (glu) and inhibitory GABAergic (GABA) projections, respectively. Cx, cerebral cortex; Th, thalamus. For all other abbreviations see Figure 1.4. b) Schematic illustration of the dynamic properties of the 'centre-surround model' of basal ganglia function, showing the change in activity in the thalamus and/or cortex caused by sequential inputs through the 'hyperdirect' (top), 'direct' (middle), and 'indirect' (bottom) pathways. The vertical downward arrow on the right (t) represents time. (Nambu et al. 2002, used with permission)

According to the Mink model, the STN is involved in the inhibition of responses. Over-activity of the STN is observed in animal models of Parkinson's disease (Bergman et al. 1994; Hassani and Feger 1999; Kreiss et al. 1997), and surgical ablation of this nucleus in humans has been considered as a possible treatment of Parkinson's disease, as well as a process known as deep brain stimulation of the STN, whereby (over-)stimulating the STN functionally inactivates the nucleus (Henderson and Dunnett 1998). Thus, if the STN is abnormally overactive, then an overriding inhibition may occur impairing the ability to initiate a desired motor program. In a rat model of Parkinson's disease, ablation of the STN reverses reaction time deficits, in accordance with an

improvement of Parkinsonian-type symptoms, but results in an increase in anticipatory responding (Baunez et al. 1995b; Phillips and Brown 1999), providing support for the role of the STN and the indirect pathway as an inhibitor of undesired motor output (Mink 1996).

1.6 Reaction time responding

1.6.1 Parkinson's disease and striatal dopamine

As well as the gross motor deficits observed as a result of Parkinson's disease, cognitive (premotor) processes are also impaired. These impairments span from lower, motor preparation type deficits (Briand et al. 1999; Brown and Marsden 1988; Flowers 1976) to higher, executive function deficits (Gotham et al. 1988; Owen et al. 1992; Swainson et al. 2000). The former refers to impairment in the initiation of voluntary movements that is thought to result from an impaired response selection system, whereby initiation of intentionally willed movement is impaired. Experimentally these effects are generally manifest as a slowing of reaction times, which are improved when patients are on levodopa medication, suggesting that striatal dopamine is critical for normal reaction time performance (see Robbins and Brown 1990).

Accordingly, depletion of striatal dopamine in rats results in slower reaction times (Amalric and Koob 1987; Baunez et al. 1995a; Brown and Robbins 1989; 1991; Smith et al. 2002; Ward and Brown 1996). Moreover, a study by Brown and Robbins (1991) showed that the delay-dependent speeding of reaction time is abolished after unilateral striatal dopamine depletion in rats. To recap on the discussion in Section 1.2.2, it has long been established that the preparation interval (foreperiod) before an imperative signal is an important factor in determining response speed, and that a random distribution of variable foreperiods results in a speeding of reaction time as foreperiod increases. The findings from the Brown and Robbins (1991) study suggest that striatal dopamine plays a critical role in this process. Moreover, a subsequent study by Brown et al. (1996) looked at the effects of amphetamine in a variable foreperiod task and found that the delay-dependent speeding of reaction time was dose-dependently enhanced by amphetamine. Although amphetamine affects the dopamine, noradrenaline and serotonin systems (Jones et al. 1998; Kuczenski et

al. 1995; Winstanley et al. 2003) and Brown et al. (1996) employed systemic administration, the opposing effects of striatal dopamine depletion and amphetamine administration are striking, suggesting a common mechanism of action mediated via dopamine perhaps within the striatum.

1.6.2 Possible role for the adenosine A_{2A} receptor in motor preparation

Surgical ablation of the STN has been used effectively in the treatment of Parkinson's disease as well as high frequency stimulation of the STN (see Henderson and Dunnett 1998). A recent pharmacological intervention under investigation in the treatment of Parkinson's disease may share commonalities with surgical ablation of the STN. The drug of interest is KW-6002 (discussed in Section 1.4.2.2), known clinically as istradefylline. KW-6002 blocks the effects of adenosine selectively at the adenosine A_{2A} receptor (Shimada et al. 1997).

There is compelling evidence demonstrating that A_{2A} receptor blockade can effectively alleviate motor impairment in Parkinson's disease without producing dyskinesia. Early clinical reports have indicated that the selective A_{2A} receptor antagonist KW-6002 reduces 'off' time in L-dopa treated patients (Hauser et al. 2003), and potentiates the anti- Parkinson's effect and duration of action of L-dopa without exacerbating dyskinesias (Bara-Jimenez et al. 2003). These clinical findings have been preceded and supported by a wealth of preclinical data. For example, adenosine A_{2A} receptor antagonists have been shown to improve striatal dopamine depletion-induced locomotor impairment in mice (Aoyama et al. 2000; Shiozaki et al. 1999) and rats (Fenu et al. 1997; Hauber et al. 2001; Koga et al. 2000; Pinna et al. 1996). In MPTP-treated monkeys (a model of Parkinson's disease), KW-6002 alone ameliorated the motor deficits and enhanced the beneficial effects of dopaminergic drugs without exacerbating dyskinesia (Grondin et al. 1999; Kanda et al. 2000). Thus, it appears that KW-6002 may be a useful tool in treating Parkinson's disease, either as a monotherapy or by reducing the required dose of dopaminergic drugs, namely L-DOPA, reducing the likelihood of dyskinesia; the main undesirable side-effect of such treatments (Bara-Jimenez et al. 2003; Hauser et al. 2003). The effects of adenosine via the A_{2A} receptor on motor performance are at least

in part mediated at the level of the striatum as direct injection of A_{2A} agonists into the rat striatum induces catalepsy (Hauber and Munkle 1995). Furthermore, catalepsy induced by direct infusion of D_1/D_2 receptor antagonists into the striatum in the rat is blocked when co-infused with an adenosine A_{2A} receptor antagonist (Hauber et al. 2001).

Thus, adenosinergic activity, possibly mediated at the level of the striatopallidal neuron, influences motor control. The possible site of action of adenosine antagonists (striatopallidal neuron) and the relationship of the A_{2A} receptor with the dopamine D_2 receptor suggest that the adenosinergic system (within the striatum) may be of considerable importance in motor preparatory activity. Thus, manipulations of the adenosine system may result in detectable changes in performance in reaction time tasks. The improvement in motor activity observed following KW-6002 treatment in cataleptic/Parkinsonian models and the improvement in anticipatory responses in Parkinsonian rats observed following lesions of the STN may be due to a similar mechanism of action mediated via the indirect pathway of the basal ganglia.

The effect of KW-6002 on motor preparation is directly tested in Chapter 2, which incorporates a cued variable foreperiod reaction time task (as used by Brown et al. 1996) and assesses the effects of different doses of KW-6002 on motor and motivational factors. Amphetamine has previously been tested in this task and resulted in a speeding of reaction time and an increase in anticipatory responses, both as a function of lengthening foreperiod (Brown et al. 1996). The authors concluded that this was due to an enhancement of 'motor readiness'. Expectation of reward also modulated reaction times, but this was not changed by amphetamine. This dissociation reveals that different psychological processes impact reaction times by different neuronal pathways and the role of striatal dopamine in the determination of reaction time is more complex than simply that of providing a signal indicating strength of final output. Rather, dopamine seems to be involved in fundamental processes determining response timing.

The hypothesis for the study in Chapter 2 was that KW-6002 would reflect the effects of amphetamine, with a more pronounced effect on anticipatory responses compared to reaction time. This is based on the proposed localisation of the A_{2A} receptor (and presumably the effects of KW-6002) on the 'indirect pathway'. Also, when the effects of STN lesions are considered in

comparison to the diffuse effect of amphetamine on dopamine function, which would presumably activate both D_1 and D_2 receptors indiscriminately, the 'indirect pathway' appears to be more crucial in response inhibition than on reaction time responding.

Tasks using variable foreperiods are typically unable to distinguish between a disturbance of underlying conditional probability (signal detection) processes or impaired time estimation processes (Brown et al. 1996; Risterucci et al. 2003).

1.6.3 Time estimation

Reaction time performance discussed so far as a function of increasing foreperiod - faster reaction times and increased anticipatory errors - may be under the control of an internal timing (clock) mechanism, the conceptual psychological function discussed in Section 1.3. There are striking similarities in the underlying neural structures and neurochemical systems that have been shown to mediate motor preparation and time estimation. For example, Parkinson's patients "off" medication show impairments in the ability to estimate time intervals, whereas "on" medication time estimation is reported as accurate (Gibbon et al. 1997; Meck and Benson 2002), implying striatal dopamine as a mediator of, or at least a contributor to, time estimation processes. However, caution should be exercised when considering the behavioural interpretation of the studies cited.

In these studies, the patient's report of estimated time is delivered by a motor response, a key press for example. As discussed above, part of the motor control deficit in Parkinson's disease may arise from an underlying deficit that results in an impairment of intentional motor control. If this is the case, then it is conceivable that a Parkinson's patient could estimate a time interval accurately but the behavioural expression of that estimate may be impaired, resulting in later key presses through an inability to select and initiate the adequate response.

As with reaction time investigations, experimental animal studies (utilising the concepts and tasks described in Section 1.3) have also implicated striatal dopamine in interval timing (MacDonald and Meck 2004; Meck 1996). Also, dopaminergic drugs have been championed as potentially influencing temporal processing. Dopamine agonists and antagonists are reported to increase

or decrease internal clock speed, respectively (Bizot 1997; Maricq and Church 1983; Meck 1986). Moreover, the binding affinity of D_2 receptor antagonists has been suggested to reflect the potency of these drugs to reduce clock speed (Meck 1986). An increase in internal clock speed is supposed to represent an enhanced perception of the passage of time and vice-versa for a decrease in clock speed. These effects are interpreted by analysis of the psychophysical functions described in Section 1.3. Thus dopamine transmission, particularly at D_2 receptors, appears to be critical for time estimation processes.

Also, amphetamine has been widely cited as having an effect on temporal processing. Most interestingly, Chiang et al. (2000) provided a rigorous assessment of the effects of amphetamine on timing. Their results suggest that amphetamine may be effect temporal regulation of ongoing behaviour rather than temporal discrimination. This is of particular relevance when considering the effect of amphetamine on performance in variable foreperiod tasks, as the former is the most likely to be incorporated in variable foreperiod behavioural performance (particularly anticipatory responses). Thus, the similarities between the effects of dopamine compounds on reaction time tasks and interval timing tasks provided the basis for the design of the task used in Chapter 3, which was used to test the effects of amphetamine and KW-6002 across three conditions where stimulus temporal probability was held constant and preparation time was varied.

Furthermore, recent investigations have implied that the effects of dopaminergic compounds may be more complex than previously suggested and may affect attention to temporal signals rather than selectively affecting the speed of an internal clock (Buhusi and Meck 2002; Chiang et al. 2000; Santi et al. 2001). These studies attempted to dissociate the possible involvement of dopaminergic mechanisms on clock and attentional effects by introducing non-temporal, salient stimulus attributes to stimuli that were to be monitored temporally (Buhusi and Meck 2002). The results from the Buhusi and Meck study lead them to describe the effects of amphetamine as reducing attentional resources to timing processes as rats were unable to maintain temporal processing under conditions with high attentional demands. On the other hand, haloperidol increased attentional resources, as the animals were able to maintain performance in the task that required temporal processing under conditions with

high attentional demands. Moreover, the effects of dopaminergic modulation of attentional factors required for temporal processing appear to be selectively modulated by the dopamine D_2 receptor (Buhusi and Meck 2002; Santi et al. 2001).

Thus, the experiment in Chapter 4 was designed to analyse the effects of selective blockade of the dopamine D_1 and D_2 receptors in a task which dissociated stimulus temporal conditional probability from spatial conditional probability and assessed attentional processing.

1.7 The void between reaction time and interval timing

To recap on the main points discussed; reaction time responding and time estimation processes appear to be mediated, at least in part, by the same underlying neural systems with the integrity of striatal dopamine transmission a critical component. However, interval timing and reaction time tasks are dissociable in many ways. Interval timing tasks in rats generally measure the rate of responding at levers with no consideration to the speed of responding. Note, however, that in a timing study by Maricq and Church (1983) they measured response latencies. Interestingly, latencies were slowest in conditions where reward probability was lowest, i.e. at the stimulus signal duration that was equally as likely to result in a response to the left or right lever (bisection point). Amphetamine speeded latencies more so in the conditions where a response was least certain, i.e., when reward probability was lowest, whereas haloperidol reduced latencies more so in the conditions where reward probability was highest, indeed in the only conditions which were rewarded. This profile of drug effect on latencies was very different from the psychometric functions of response rates but did not receive the same level of interpretation from the authors, even though the baseline latencies were reported as 'an independent measure of equality of psychological distance between the two extreme signal durations...the animals respond slowly following this signal duration either because of conflict or because of low probability of reinforcement' (Maricq and Church 1983, p14).

Furthermore, striatal neurons show differential activation during a delay while anticipating a stimulus and/or a movement (Hikosaka et al. 1989; Jaeger et al. 1993; Schultz et al. 1995), suggesting that these neurons are involved in both stimulus expectation and motor preparation. Moreover, in one study the length of

the delay period was the major determinant of the magnitude and timing of increased neuronal activity in the striatum, as the longer the delay between the cue and the imperative signal (i.e., foreperiod), the earlier was the onset and the greater the change in spike activity (Jaeger et al. 1993). Whether the activity of these neurons is related to temporal processing or is encoding the conditional probability of stimuli remains to be explored. An interval timing study recorded from striatal and prefrontal cortical neurons during a peak interval procedure reported temporally specific neurons in both regions (Matell et al. 2003). The areas recorded from are interconnected and constitute a subdivision (associative-related regions) of the corticostriatal circuits.

To date, there has yet to be a reaction time investigation of the neuropharmacology of interval timing processes, although a recent review by MacDonald and Meck (2004) highlights the similarities between the underlying neuronal substrates involved in reaction time and interval timing and emphasises the need for a reconciliation of these relatively separate fields of research. The possibility that time estimation processes contribute to reaction time responding in tasks that use preparation intervals in the millisecond range is particularly salient, especially in regards to responding in variable foreperiod tasks. For example, the increase in anticipatory responses as a function of increasing foreperiod duration by amphetamine observed by Brown et al. (1996) could be due to an enhancement in the perception of the passage of time, especially in the context of the aforementioned Chiang et al. (2000) study. Moreover, according to scalar expectancy theory (Gibbon et al. 1997), as aforementioned, this effect of amphetamine could be explained as heightened temporal expectation of the imperative stimulus occurring earlier than the actual stimulus and result in release of the pre-programmed motor program and an anticipatory response and this effect would be greatest at the longer foreperiods. The suggestion by Brown et al. (1996) that it is the summation of the temporal probability of the stimulus (signal detection) that reaches unity and results in an anticipatory response may still explain the incidence of anticipatory responses, as within the task used the effect of increasing temporal probability and foreperiod duration were confounded.

Therefore, the main part of this thesis examines the influence of preparation time *per se* and conditional probability on performance in the rat and

utilises adenosinergic and dopaminergic drugs that have been implicated in both processes to assess the relative effects of these compounds on these processes.

1.8 Experimental outline

In Chapter 2 the effects of the adenosine A_{2A} antagonist, KW-6002, were characterised in a cued variable foreperiod task that was previously used to define the effects of amphetamine on motor readiness (Brown et al. 1996). This experiment was intended to probe the effects of KW-6002 in a motor preparation task. The task does not enable dissociation of conditional probability and temporal processing.

Thus, in Chapter 3 a task was developed that dissociated the effects of elapsing time and the effects of increasing temporal probability. Due to the rationale discussed above and the previously reported effects of amphetamine on temporal probability, which are similar to the effects of KW-6002 reported in Chapter 2, the effects of both drugs were compared in this task.

Since both amphetamine and KW-6002 are considered to (indirectly) stimulate dopaminergic activity, in Chapter 4 the effects of antagonism of dopamine activity was investigated. The effect of manipulations of spatial and temporal characteristics of stimuli and selective blockade of dopaminergic transmission at the dopamine D_1 and D_2 receptors by SCH-23390 and raclopride, respectively, were investigated.

Finally, the anti-Parkinsonian potential of KW-6002 has been shown in many studies looking at motor effects but the ameliorative effects of KW-6002 on the cognitive deficits observed in Parkinson's disease are unknown. Deficits in reversal learning occur in rats with striatal dopamine depletion (J.M. Phillips, A.D. Blackwell and V.J. Brown, unpublished observations). Therefore, the effect of chronic KW-6002 was tested in rats with striatal dopamine depletion performing in a reversal learning task.

1.9 General materials and methods

1.9.1 Subjects

All rats used were male Lister-hooded and maintained on a restricted diet of 15-20 g lab chow. On days with behavioural testing, they received additional food given as a 'reinforcer' (either up to 5g of sucrose pellets – 45mg Noyes Formula A/I, Sandown Scientific, Middlesex, UK – or Honey Loops – Kellogg's, UK) per day. Water was freely available in the home cage. Prior to surgery, all rats were pair-housed on a 12 h light/dark cycle (lights on at 7am) with testing carried out in the light phase (post-operatively, rats were housed in single cages). Body weight was monitored weekly to ensure a healthy and steady gain, which did not fall below 85% of free-feeding weight. All practices were performed under UK Home Office License in accordance with the guidelines laid out in the *Handbook of Laboratory Animal Management and Welfare* (Wolfensohn and Lloyd 1998) and the requirements of the United Kingdom Animals (Scientific Procedures) Act 1986.

1.9.2 Apparatus

Figure 1.8 shows an example of the type of operant chambers used in Chapters 2, 3 and 4. These were 9-hole boxes, controlled by a Spider computer system (Paul Fray Ltd, Cambridge, UK). Each of the 8 chambers used were individually housed within wooden sound-attenuating cabinets ventilated by fans, which also served to mask any background noise. The rear wall of each chamber was concave and, at a height of 2.5 cm from the grid floor, was a horizontal array of nine holes. Illumination, when required, was provided by a standard 3-W bulb located at the rear of each hole and an infrared photocell beam was located at the front of each hole to monitor nose-poke entrance. Sucrose pellets were delivered to a food hopper, which was occluded by a hinged Perspex flap, located on the wall opposite the array of holes. The hopper was connected to a micro-switch that indicated its opening.

Auditory stimuli (duration of 0.1 s and ~80 dB) were produced from a tone generator, connected to a loudspeaker, located in the centre of the ceiling of the chamber. A 3-W houselight was illuminated throughout the session, other than when it was extinguished for 1.5 s (a "time-out") following an error.

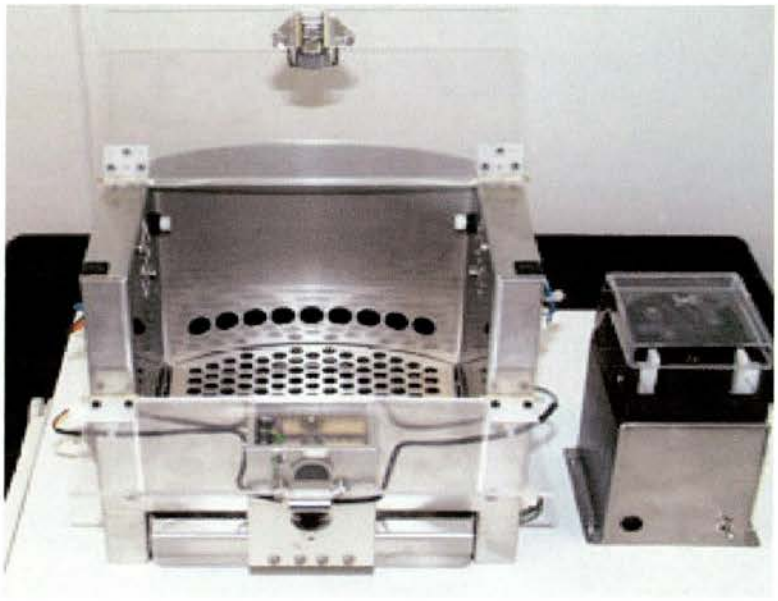

Figure 1.8 An example of the type of 9-Hole Box used. The centre hole in the array was always exposed; where the rat was required to maintain a nose-poke for a variable foreperiod. Transparent covers were secured over the four periphery holes on either side in Chapter 2; where the cue lights signalling trials to reward were presented. In Chapters 3 and 4 the holes to either side of the centre hole were both exposed and cue lights signalled the target location in which the rat was required to nose-poke following a maintained nose-poke in the centre hole. On the opposite wall from the array of holes is the food hopper; where reward was delivered for collection.
(http://www.lafayetteinstrument.com/animalcages.htm)

1.9.3 Training

For Chapters 2, 3 and 4 the initial behavioural training protocol involved the following stages:

1) Habituation
   - Rats were habituated to the testing chambers and food hoppers with reward available when the hinged flap of the food hopper was depressed. This continued until rats were reliably retrieving 100 reward pellets throughout a 30 min session, which generally required 5 sessions.

2) Associative conditioning
   - The light in the central hole illuminated brightly to signify trial commencement. Rats were required to sustain a nose-poke in the central hole for a delay (foreperiod; 0.1-0.5 s) after which the light was extinguished. Successful maintenance of the nose-poke resulted in the *imperative* signal, a brief (0.1 s) tone sound. Early withdrawals constituted *anticipatory responses* and resulted in a time-out (house-light extinguished for 1.5 s with no reward available).

Once rats were reliably retrieving pellets following successful nose-pokes in Stage 2 (after 16 sessions on average), the additional behavioural contingencies that were specific for each task, which are described in the Methods section of each experimental chapter, were introduced.

1.9.4 Measurements

The following measures were taken:

i) Reaction time
   - The time between tone onset and withdrawal of the snout from the hole. Due to the temporal requirements of signal detection and motor initiation any reaction time less than 10 ms is most likely to be a successful premature response, initiated before the imperative signal. Thus, minimum reaction time was set at 10 ms in all analyses. Timing resolution was 10ms.

ii) Movement time
   - The time between snout withdrawal and conclusion of the response (activation of a micro-switch at the food hopper panel or nose-poke in a response hole).

iii) Anticipatory responses
   - Withdrawal from the nose-poke hole before the imperative signal sounds.

iv) Omissions
   - Failure to complete the task requirement following successful nose-poke.

- In Chapter 2, this was simply reward retrieval. If the rat had not depressed the hinged flap at the food hopper within 10 s then a time-out ensued.
- In Chapters 3 and 4, a nose-poke in either hole adjacent to the centre hole was required. Failure to do so within 2 s resulted in a time-out.

v) Incorrect error (Chapters 3 and 4 only)

- A response in the non-contingent target (side) hole.

1.9.5 Drug administration

The following drugs were used:

- KW-6002 (Vernalis Research Ltd., Wokingham, UK)
  - An adenosine A_{2A} antagonist
  - Vehicle: 1% methyl cellulose
  - Administered orally, by gavage, using a dosing volume of 1 ml/kg.
  - Doses: vehicle, 0.3, 1.0, 3.0 mg/kg.
    - Orally administered KW-6002 has been shown to reach peak levels in rat brain at 30 min which is maintained for 60 min at the low dose employed here (Aoyama et al. 2002). Therefore the testing sessions (Chapters 2 and 3) were initiated at 60 min following drug administration and all sessions were completed or terminated after 30 min (to minimize the effect of the drug wearing off).
  - Dosing regime:
    - i) Chapters 2 and 3: Rats received one of each dose of KW-6002 and vehicle on consecutive testing days using a pseudo-counterbalanced dosing schedule based on a Latin square design.
    - ii) Chapter 5: chronic KW-administration. The dosing regime is described in the Methods section in Chapter 5.
- Amphetamine (Sigma-Aldrich, Dorset, UK)
  - Behavioural effects of amphetamine are ascribed to its potentiation of the action of dopamine, noradrenaline and

serotonin (for examples from behavioural studies see Kuczenski et al. 1995; Winstanley et al. 2003).

- Vehicle: sterile saline.
- Administered intraperitoneally using a dosing volume of 1 ml/kg.
- Doses: vehicle, 0.2, 0.4 or 0.8 mg/kg
  - These doses have previously been shown to be effective in the same task as used in Chapter 2 (Brown et al. 1996) and were the same doses used in the timing study of Chiang et al. (2000). Thus, these doses and dosing regime from the Brown et al. (1996) study was also used here: Rats began testing 10 min following drug administration and all sessions were completed or terminated after 30 min.
- Dosing regime:
  - Rats received one of each dose of amphetamine and vehicle on consecutive testing days using a pseudo-counterbalanced dosing schedule based on a Latin square design.

- Raclopride (Sigma-Aldrich, Dorset, UK)
  - Dopamine D_2-receptor antagonist.
  - Vehicle: sterile saline.
  - Administered subcutaneously 30 minutes prior to testing.
  - Doses: vehicle, 50, 100, 200 μg/kg.
    - Drug doses were selected based on previous studies in the lab, a pilot study (data not shown) and previous literature (Amalric et al. 1993; Courtiere et al. 2003).
  - Dosing regime:
    - Rats received one of each dose of raclopride and vehicle in a pseudo-counterbalanced dosing schedule over four days, with a drug free day in between each drug day.
- SCH-23390 (Sigma-Aldrich, Dorset, UK)
  - Dopamine D_1-receptor antagonist.
  - Doses: vehicle, 5, 10, 20 μg/kg.
  - All other factors were identical to those above for raclopride.

1.9.6 Data analysis

Mean reaction time, movement time, the percentages of anticipatory responses, omissions and incorrect responses were analysed. The reaction time distributions were analysed by considering the modal reaction time and the distribution height (the absolute probability of a modal reaction time) and normalised distribution height (the probability of a modal reaction time, correcting for errors).

The data were analysed together by repeated measures analyses of variance (ANOVA). The factors in each analysis, as well as any additional analysis, are mentioned in the Methods section at the beginning of each Chapter. If a within-subjects factor required further analysis, a restricted analysis was performed between the levels of interest. The F-ratio was corrected by dividing the mean square of the effect from the restricted analysis by the mean square error value from the original analysis. The new level of significance of the new F-ratio was defined using the degrees of freedom from the original analysis. For all analyses, where there was an indication that violation of homogeneity of variance might have occurred, the Huynh-Feldt correction was applied and a statistically significant effect was only accepted if it remained following the correction.

Graphs were plotted as means with two standard errors of the mean (SEM) as the error bars.

Chapter 2

The effect of the adenosine A_{2A} antagonist KW-6002 on motor and motivational processes in the rat

- *Amphetamine enhances motor preparatory processes that are impaired following striatal dopamine depletion. KW-6002 has been shown to reverse the motor impairments induced by a decrease in dopamine transmission. Does KW-6002 enhance motor preparatory processes in the same manner as amphetamine?*

- *The adenosine A_{2A} receptor, which is blocked by KW-6002, is predominantly expressed by the striatopallidal neuron, which belongs to the so-called 'indirect' pathway of the basal ganglia. Dysfunction of this pathway has been shown to influence response inhibitory processes. Are the effects of KW-6002 restricted to response inhibition?*

2.1 Introduction

As discussed in Section 1.6, striatal dopamine has been implicated in motor preparatory mechanisms in the rat. Striatal dopaminergic transmission plays a key role in the delay-dependant speeding of reaction time, thought to be due to the increasing temporal probability of a cue signal and reflected in increased motor readiness (Baunez et al. 1995a; Brown and Robbins 1991). Also, systemic amphetamine, which elevates dopamine levels and has a considerable effect on striatal neuronal activity (see Haracz et al. 1998), has been shown to enhance motor readiness (Brown et al. 1996). Based on these findings and the intimate antagonistic relationship of dopamine receptors with adenosine receptors (see Ferre et al. 1997) the effects of the adenosine A_{2A} antagonist KW-6002 on motor preparatory processes were examined. The methodology of Brown et al. (1996) was replicated to define the effects of the adenosine A_{2A} antagonist KW-6002 on motor preparation.

In the experimental procedure, each foreperiod is distributed equally within a testing session and the conditional probability of the imperative signal increases differentially, as shown schematically in Figure 2.1. This is due to the fact that 0.1 s from trial onset there is a 33% chance that the imperative signal will sound (instantaneous conditional probability). The reason for this level of likelihood is because there are two other possibilities; 1) the rat is required to maintain a nose-poke for a further 0.2 s before the imperative sounds or 2) the rat maintains nose-poke for a further 0.4 s before the imperative sounds. In the former, the instantaneous conditional probability of the imperative signal sounding is 50% (as the latter possibility remains) whereas in the latter the likelihood of the imperative is 100%. Additionally, the behavioural protocol included a motivational component, which allowed further assessment of any effects of KW-6002 on processing of cues that signal the amount of work required for reward.

2.2 Materials and methods

2.2.1 Subjects

Twenty male Lister hooded rats (Charles River, U.K), weighing between 319 and 456 grams at the start of testing, were used in this study.

2.2.2 Apparatus

In the operant box, only the centre hole was exposed for the purposes of this study, the four peripheral holes on either side were blocked with transparent covers so that light within the peripheral apertures could be seen.

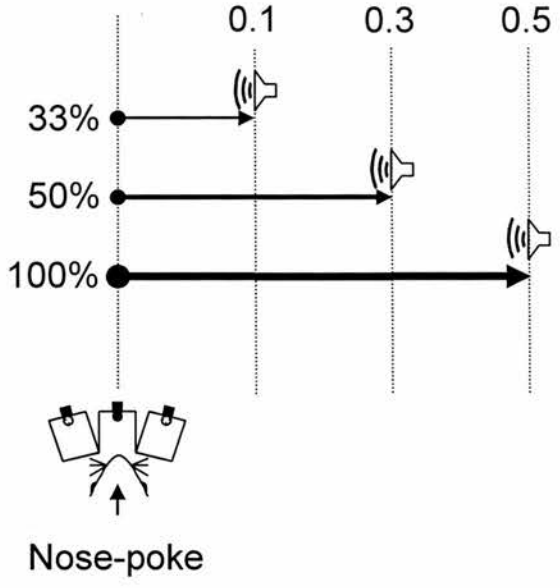

Figure 2.1 Schematic illustration of the three possible conditions following a nose-poke in the centre-hole. The arrows represent timelines from the initial nose-poke. There is a 33 % chance that following a nose-poke the tone will sound after 0.1 s. If time continues to elapse past 0.1 s then there is a 50 % chance that the tone will sound at 0.3 s, as two outcomes are possible. The other possible outcome is that time continues to elapse and the tone sounds at 0.5 s, which is 100% probable as no other condition is possible.

2.2.3 Behavioural task

Following Stage 2 of training (described in General Methods; Section 1.9.3), at the beginning of each trial the lights from the peripheral holes (4 to either side of the centre nose-poke hole) were illuminated either bright or dim. Bright lights signified that a reward would be available following a successfully sustained nose-poke. Dim lights indicated no reward available following the subsequent nose-poke. Unrewarded dim light trials were always followed by rewarded bright light trials. This final stage of training continued for 17 sessions. For the final behavioural protocol, unrewarded trials with no cue lights were also introduced (see below). Figure 2.2 provides a schematic illustration of a correctly performed trial.

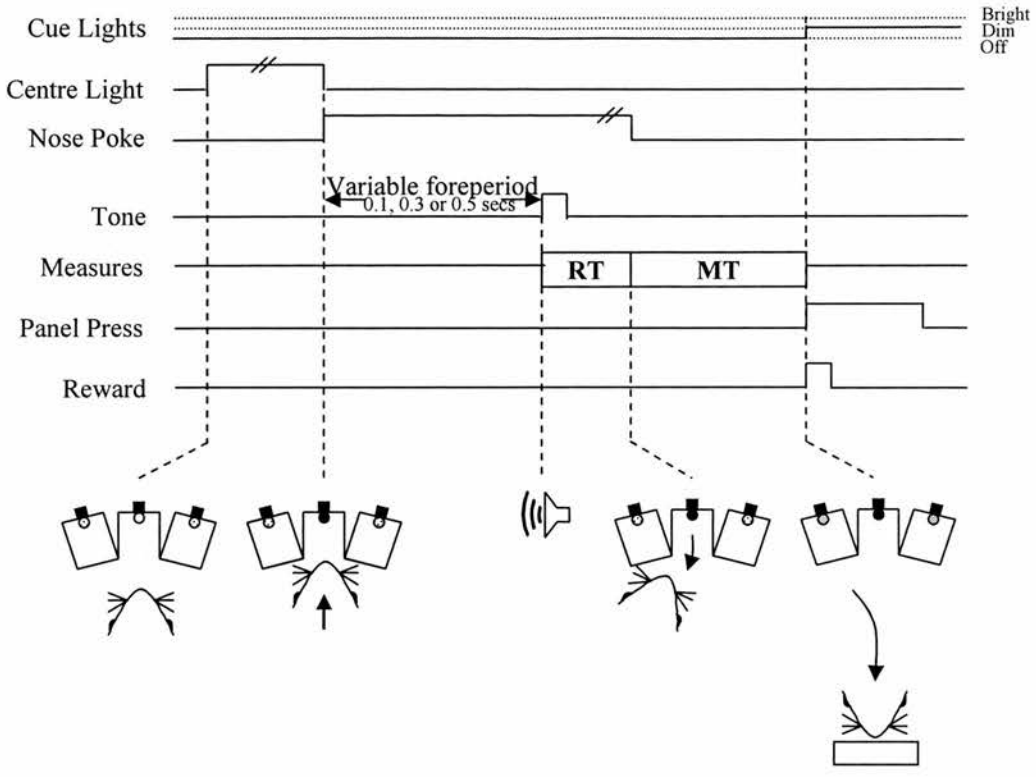

Figure 2.2 A schematic illustration of the trial events. The brightness of eight cue lights in an array, four to each side of the nose-poke hole, signaled the number of trials to complete (including the current trial) to earn a reward. A trial consisted of the rat making a sustained nose-poke, waiting for a variable foreperiod, before reacting to the onset of a tone. At the tone onset, the animal withdrew its snout from the hole and turned to press a panel. Pressing the panel resulted in the resetting of the cue lights and, on rewarded trials, the delivery of a food pellet. The schematic shows an unrewarded trial, with the cue lights off signaling three trials required for reward. At the end of the current trial the cue lights are reset (in this case to dim, indicating that two trials are required for reward). Reaction time (RT - tone onset to withdrawing the snout) and movement time (MT - snout withdrawal to panel response) recording epochs are shown.

The rat was not rewarded for every trial. To earn a food reward, the animal had to complete a series of correct responses, with a series consisting of one, two or three trials (see Figure 2.3 and Table 2.1). This is similar to a multiple ratio schedule, but with the progress through the current schedule signalled by cues (as used by Bowman et al. 1996). Cue lights in the occluded but transparent peripheral holes before the initial nose-poke indicated the number

of trials to reward: lights off indicated three completed trials required for a reward, dim lights indicated two trials to reward and bright lights indicated that reward would be available on completion of the current trial. The cue lights changed following a correct response and stayed at the same level of illumination throughout the trial. Error trials were repeated such that the cue lights did not change until the rat made a correct response at the food hopper.

The animals were trained in the final behavioural protocol to complete 120 correct trials within 30 min, which required 24 sessions. For drug testing, two blocks of testing (separated by ten weeks) were carried out to generate suitable data for a meaningful analysis.

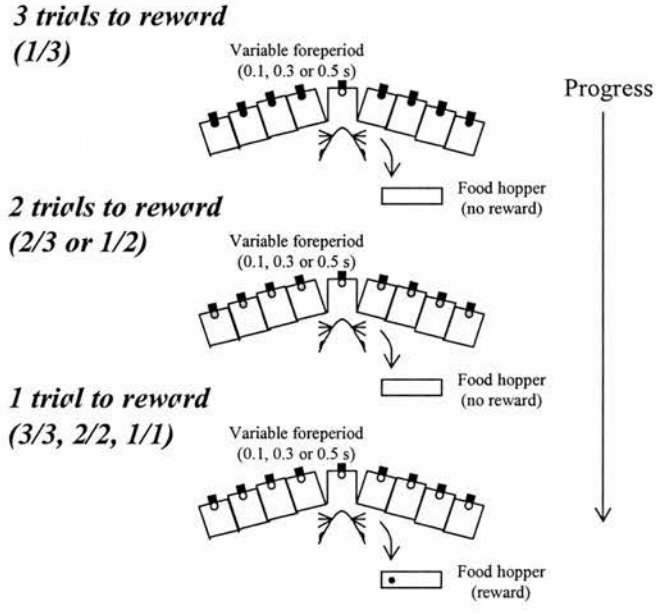

Figure 2.3 Schematic illustration of the progress through a schedule that required 3 trials to be completed before reward was available at the food hopper. In all trials, the rat was required to nose poke in the central hole for a variable foreperiod and then depress the panel covering the food hopper. In the example shown, a reward (food pellet) was only present on the final trial. The other schedules comprised either two trials for reward or one trial for reward (rewarded trial), as illustrated by the fractions in brackets. The progress through the current schedule was signalled to the rat from the cue lights in the apertures adjacent to the centre hole, with no lights, dim lights and bright lights signifying 3, 2 or 1 trial(s) required to be completed for reward.

2.2.5 Data analysis

The data from both test blocks were analysed together in a four-way, repeated measures analyses of variance (ANOVAs), with the factors: block, dose, trials to reward and foreperiod.

	Unrewarded			*Rewarded*		
	(1/3)	*(2/3)*	*(1/2)*	*(3/3)*	*(2/2)*	*(1/1)*
Trials to reward:	*3*		*2*		*1*	
Schedule of trials	*N trials*					
3	*20*		*20*		*20*	
2			*20*		*20*	
1					*20*	

Table 2.1 Distribution of number of trials in a complete session. There were three schedules of work, each requiring either one, two or three trials for a reward. There were 20 trials for each stage within each schedule (upper section represents the corresponding schedule fraction for each series of 20 trials in the lower section).

2.3 Results

There was no main effect of test block on any of the dependent variables (reaction time $F_{(1, 11)} = 2.68$; movement time $F_{(1, 11)} = 0.13$; anticipatory responses $F_{(1, 13)} = 2.40$; omissions $F_{(1, 13)} = 1.26$; all ns), so the data were collapsed across block for all analyses.

2.3.1 Foreperiod

2.3.1.1 Analysis of latencies

The upper panel in Figure 2.4 shows mean reaction times for the main effect of dose of KW-6002. KW-6002 dose-dependently decreased mean reaction times (main effect of dose: $F_{(3, 57)} = 17.26$, $p < 0.001$). Restricted analysis confirmed that reaction times were quicker as a function of dose, as each dose of drug speeded reaction times when compared to vehicle (data not shown), with the highest dose decreasing reaction times significantly more than the lowest dose (restricted main effect of dose: $F_{(3, 57)} = 8.15$, $p < 0.01$). Mean reaction time was faster as a function of increasing foreperiod (main effect of foreperiod: $F_{(2, 38)} = 537.34$, $p < 0.001$). KW-6002 resulted in faster mean reaction times at all foreperiods (dose x foreperiod interaction: $F_{(6, 114)} = 0.88$, ns).

Mean movement time was faster as a function of increasing foreperiod (main effect of foreperiod: $F_{(2, 38)} = 16.38$, $p < 0.001$). Figure 2.4 (upper panel) shows mean movement times for the main effect of dose of KW-6002. KW-6002 dose-dependently speeded mean movement times (main effect of dose: $F_{(3, 57)} = 21.04$, $p < 0.001$). There was no dose x foreperiod interaction ($F_{(6, 114)} = 0.52$, ns).

2.3.1.2 Anticipatory responses and omissions

As expected, the percentage of anticipatory responses increased with lengthening foreperiod (main effect of foreperiod: $F_{(2, 38)} = 283.71$, $p < 0.001$). In addition, KW-6002 dose-dependently increased the percentage of anticipatory responses (main effect of dose: $F_{(3, 57)} = 29.72$, $p < 0.001$; Figure 2.4, lower panel). Restricted analysis revealed that anticipatory responses were greater at the low dose compared to vehicle (vehicle versus 0.3 mg/kg; $F_{(3, 57)} = 4.15$, $p < 0.05$). Also, the medium and high dose of KW-6002 increased anticipatory responses more so than the low dose (0.3 mg/kg versus 1.0 mg/kg; $F_{(3, 57)} = 25.46$, $p < 0.01$, 0.3 mg/kg versus 3.0 mg/kg; $F_{(3, 57)} = 34.79$, $p < 0.01$). The effect of the medium and high dose was not significantly different (1.0 mg/kg versus 3.0 mg/kg; $F_{(3, 57)} = 0.73$, ns).

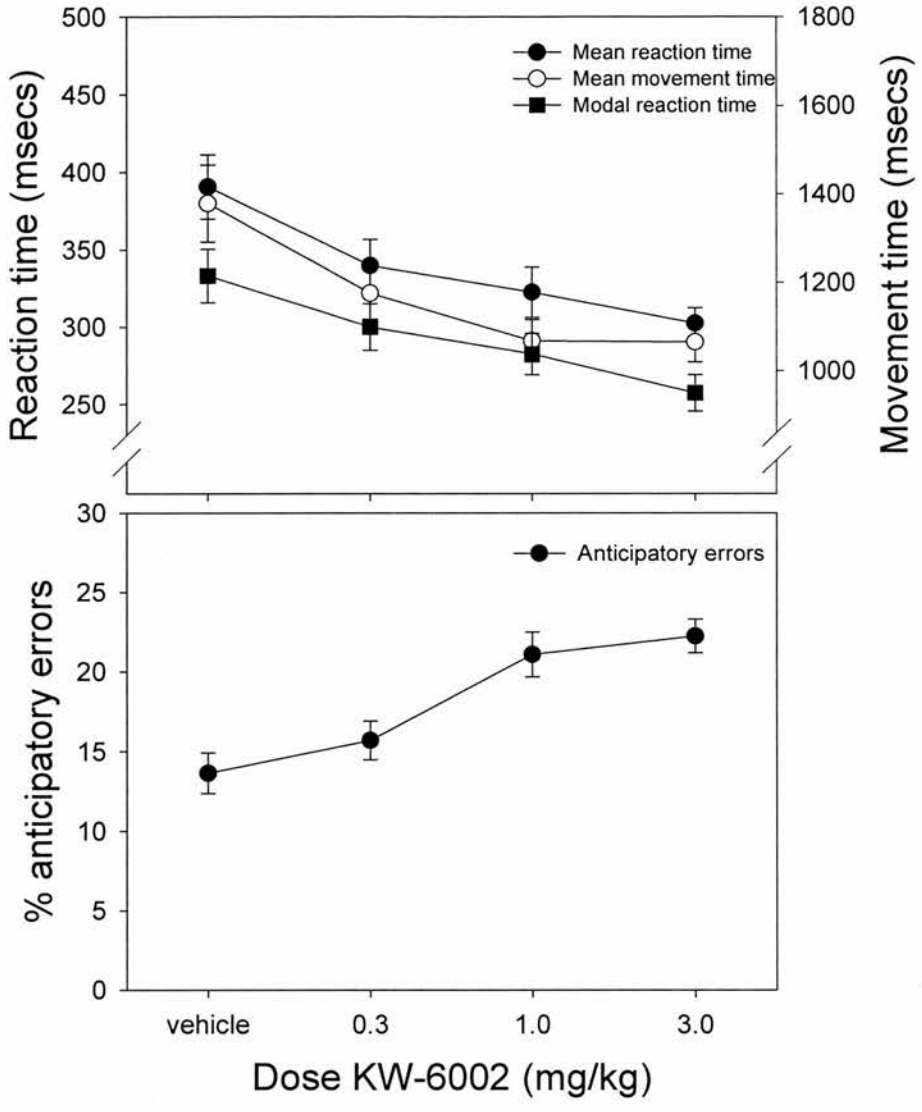

Figure 2.4 The upper panel shows the effect of KW-6002 on mean reaction times, mean movement times and modal reaction times. KW-6002 decreased reaction times and movement times as a function of increasing dose. The lower panel shows the effect of KW-6002 on anticipatory responses. KW-6002 dose-dependently increased the percentage of anticipatory responses.

Figure 2.5 shows that the increase in percentage of anticipatory responses by KW-6002 was most evident at the two longer foreperiods with the greatest effect at the highest dose and longest foreperiod (dose x foreperiod interaction: $F_{(6, 114)} = 12.26$, $p < 0.001$).

Omissions decreased with increasing foreperiod (main effect of foreperiod: $F_{(2, 38)} = 6.94$, $p = 0.003$). The effect of dose of KW-6002 on omissions was not significance (main effect of dose: $F_{(3, 57)} = 3.13$, ns).

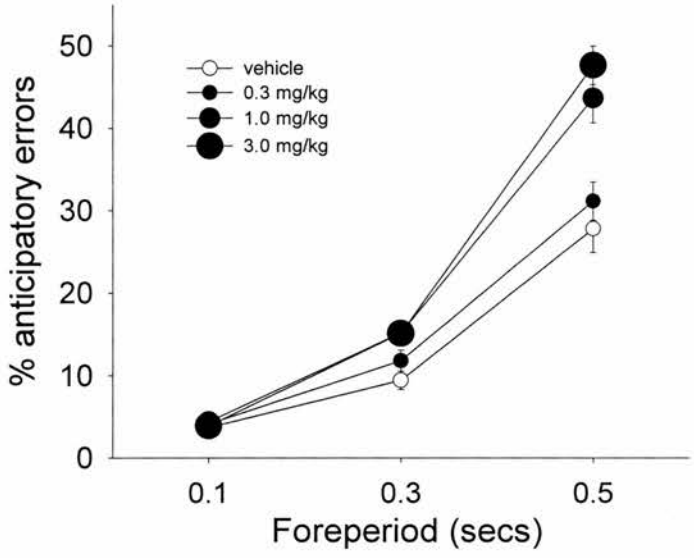

Figure 2.5 The effect of KW-6002 on the percentage of anticipatory responses. At the medium and high doses, KW-6002 increased the percentage of anticipatory responses at the longer foreperiods.

2.3.1.3 Analysis of reaction time distributions

If reaction time distributions are unimodal they may be characterized by the mean and the mode of the distribution (measures of central tendency) and the height of the distribution at the mode (i.e., the instantaneous probability of a modal response which also gives an indication of kurtosis). The modal reaction time is the reaction time (on the x-axis) which corresponds with the peak of the distribution; the distribution height, which is the value on the y-axis that corresponds with the peak of the distribution and provides a value representative of the probability of the modal reaction time compared to all other reaction times in the distribution. Since reaction time distributions are positively skewed, it is important to consider the modal reaction time as well as the mean reaction time, as the former may provide a more appropriate measure of central tendency than the latter. The reaction time distributions are displayed in Figure 2.7. As the reaction time distribution curves were unimodal, the mean of the modal reaction times for each rat and the mean of the distribution heights were examined in an attempt to further characterize the effects of KW-6002 on reaction times.

As a function of increasing foreperiod, modal reaction time was reduced (main effect of foreperiod: $F_{(2, 38)} = 252.29$, $p < 0.001$) and the likelihood of faster reaction times increased, reflected in an increase in the peak height of the reaction time distributions showing that the reaction times were becoming more tightly time-locked to the imperative stimulus. The increase in kurtosis occurred between the 0.1 and 0.3 s foreperiod, but the reaction time distribution peak height was lower at the 0.5 s foreperiod (main effect of foreperiod: $F_{(2, 22)} = 98.27$, $p < 0.001$). Like the effect of foreperiod, the effect of increasing dose of KW-6002 was to decrease modal reaction time (main effect of dose: $F_{(3, 57)} = 8.5$, $p < 0.001$; Figure 2.4, upper panel) and increase distribution peak height (main effect of dose: $F_{(3, 57)} = 16.8$, $p < 0.001$).

KW-6002 was found to interact with foreperiod in the analysis of errors, enhancing the increase in anticipatory responses as a function of foreperiod (see above). Distribution peak height is partly a function of error rate, as it is sensitive to changes in distribution area (changes in global response probability) as well as to changes in kurtosis. Therefore, to examine the effect of KW-6002 exclusively on the latter, the height of the distribution was analysed after normalizing the distribution, so correcting for the effect of errors.

Distribution peak height was divided by the proportion of correct responses in each condition to enable analysis of the relative probability of a response at the mode (responses per ms per correct trial). This provides a distribution curve that is representative of the pattern of reaction times that would be observed if all reaction times were correct, removing the contamination in the original distributions caused by fast anticipatory responses.

Because in some conditions there was a high incidence of anticipatory responses, fast correct reaction times are represented as less likely in the context of all reaction times observed, as these fast anticipatory responses are also taken into account. Removing this contamination provides the likelihood of a given correct reaction time relative to all other correct reaction times. After correcting for the effect of errors, there was still an effect of foreperiod (main effect of foreperiod: $F_{(2, 28)} = 23.33$, $p < 0.001$), except that distribution height now increased as a function of increasing foreperiod indicating greater kurtosis (a tighter time-locking of reaction time to the imperative signal onset). KW-6002 increased the normalized distribution height (main effect of dose: $F_{(3, 42)} = 5.36$,

$p = 0.017$) and this effect was strongest at the highest dose at the longest foreperiod (dose x foreperiod interaction; $F_{(6, 84)} = 4.57$, $p = 0.03$). Figure 2.8 shows the mean normalized probability density distributions of the highest dose of KW-6002 and vehicle at the shortest and longest foreperiods.

2.3.2 Trials to reward

2.3.2.1 Analysis of latencies

KW-6002 dose-dependently speeded mean reaction times (see above; Figure 2.4, upper panel). The effect of KW-6002 was analysed both separately on each schedule of work and collapsed across all three schedules. Both analyses provided the same results. Thus, for convenience, the collapsed data analysis is presented here (a detailed analysis of the effects of the schedules used here is provided by Bowman and Brown (1998)). Figure 2.6 shows reaction times and movement times collapsed across the three schedules as a function of trials to reward. Figure 2.6 (upper panel) shows mean reaction times for dose of KW-6002 as a function of trials to reward. Mean reaction times for rewarded trials were faster than unrewarded trials in a series of three trials (main effect of trials to reward: $F_{(2, 38)} = 30.78$, $p < 0.001$) although reaction times in the first of 3 trials were particularly fast (see vehicle data in Figure 2.6, upper panel). The effect of KW-6002 was greatest when reaction times for vehicle animals were slower, i.e., at two trials to reward (dose x trials to reward interaction: $F_{(6, 114)} = 4.45$, $p = 0.001$).

KW-6002 dose-dependently speeded mean movement times (see above; Figure 2.4, upper panel). Figure 2.6 (lower panel) shows mean movement times for dose of KW-6002 as a function of trials to reward. Mean movement times were faster as a function of decreasing trials to reward (main effect of trials to reward: $F_{(2, 38)} = 71.14$, $p < 0.001$). The effect of KW-6002 was greatest on unrewarded trials (dose x trials to reward interaction: $F_{(6, 114)} = 7.08$, $p < 0.001$).

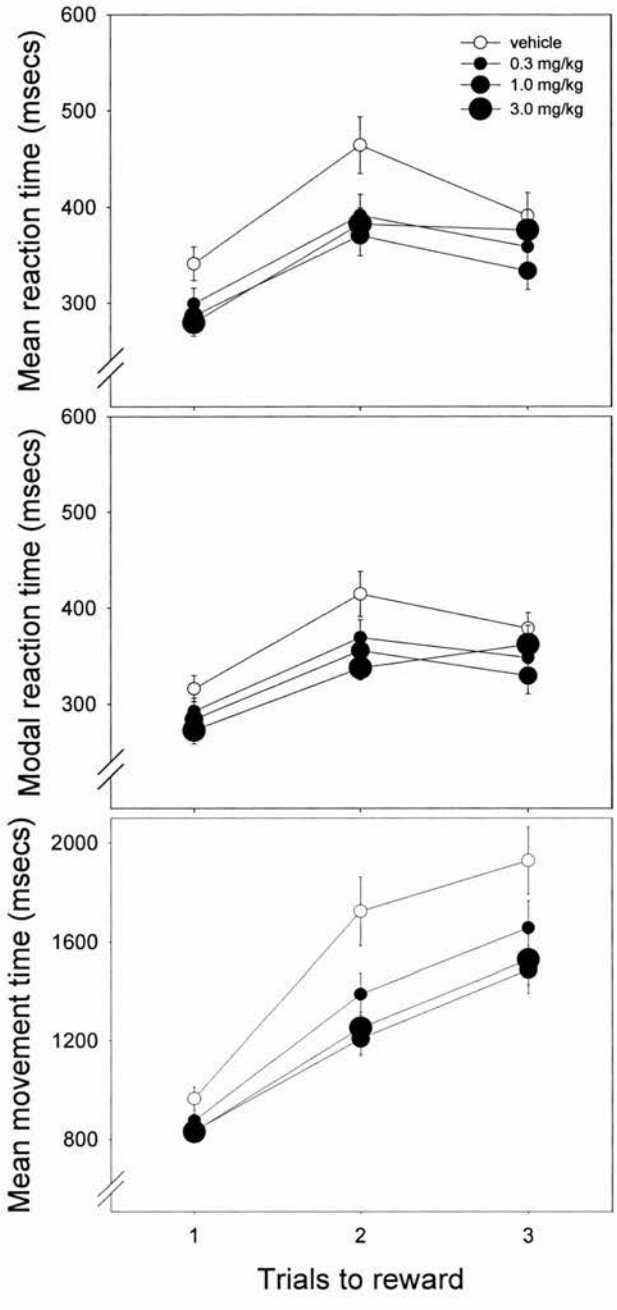

Figure 2.6 This shows reaction and movement times collapsed across all three schedules of work. The upper and middle panels show the effect of each dose of KW-6002 on mean and modal reaction times, respectively, as a function of trials to reward. KW-6002 decreased mean reaction times, particularly when the reaction times were slower, at two trials to reward. This effect was evident in mean and modal reaction times. The bottom panel shows the effects of KW-6002 on mean movement times as a function of trials to reward. KW-6002 speeded movement time and this effect was greatest on unrewarded trials.

2.3.2.2 Anticipatory responses and omissions

The percentage of anticipatory responses increased as a function of increasing reward cost (main effect of trials to reward: $F_{(2, 38)} = 33.63$, $p < 0.001$). KW-6002 dose-dependently increased the percentage of anticipatory responses (see above; Figure 2.4, lower panel). The effect of KW-6002 on anticipatory responses was the same across all trials to reward (dose x trials to reward interaction: $F_{(6, 78)} = 1.06$, ns).

As reward cost increased the percentage of omissions increases (main effect of trials to reward: $F_{(2, 38)} = 22.29$, $p < 0.001$). KW-6002 did not significantly affect omissions. The dose x trials to reward interaction was not significant ($F_{(6, 114)} = 2.18$, ns).

2.3.2.3 Analysis of reaction time distributions

When reward cost was high (i.e., unrewarded trials) the modal reaction time was slower than when reward cost was low (rewarded trials) (main effect of trials to reward: $F_{(2, 38)} = 27.42$, $p < 0.001$; see Figure 2.6, middle panel). KW-6002 dose-dependently decreased modal reaction times (see above) and this effect was greatest when vehicle modal reaction times were slowest, at two trials to reward (dose x trials to reward interaction: $F_{(6, 114)} = 3.45$, $p = 0.006$; Figure 2.6, middle panel).

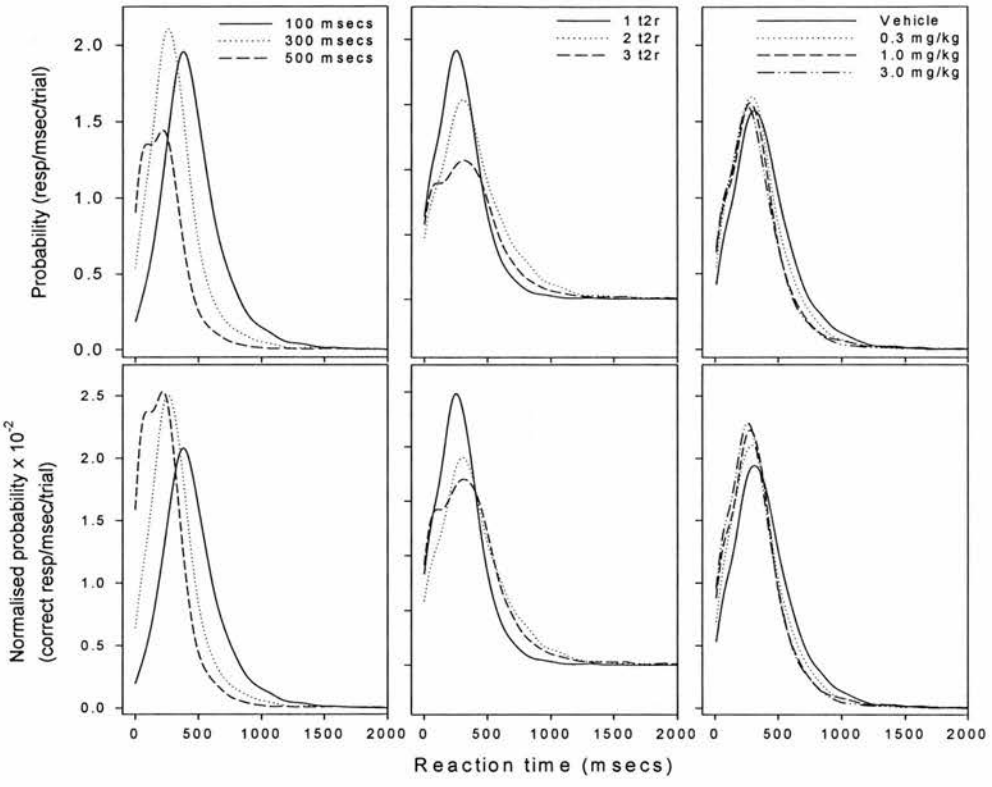

Figure 2.7 This shows the reaction time distributions averaged across all rats (responses with a given reaction time expressed as a proportion of all responses) for the main effects of foreperiod (left panels), trials to reward (centre panels) and dose of KW-6002 (right panels). The top panels display the overall distribution heights and the bottom panels show the distribution curves corrected for the effect of errors, such that they show the relative probability of a correct response. The effect of increasing foreperiod and dose of KW-6002 is more obvious after normalizing the data, suggesting that the anticipatory responses are responsible for the shape of the curves in the top panels. After normalizing the curves, an increase in kurtosis as a function of increasing foreperiod and increasing dose of KW-6002 is evident, whereas the shape of the curves across trials to reward remains similar.

Distribution height decreased on average as a function of increasing reward cost (main effect of trials to reward: $F_{(2, 38)} = 21.69$, $p < 0.001$). KW-6002 decreased distribution height without interacting with reward cost (dose x trials to reward interaction; $F_{(6, 114)} = 1.25$, ns). After correcting for the effect of errors, distribution height was only slightly greater on rewarded trials with very little apparent difference on unrewarded trials (main effect of trials to reward: $F_{(2, 28)} =$

7.65, p = 0.005). KW-6002 had no significant effect on distribution height as a function of trials to reward after correcting for the effect of errors (dose x trials to reward interaction: $F_{(6, 84)} = 1.02$, ns).

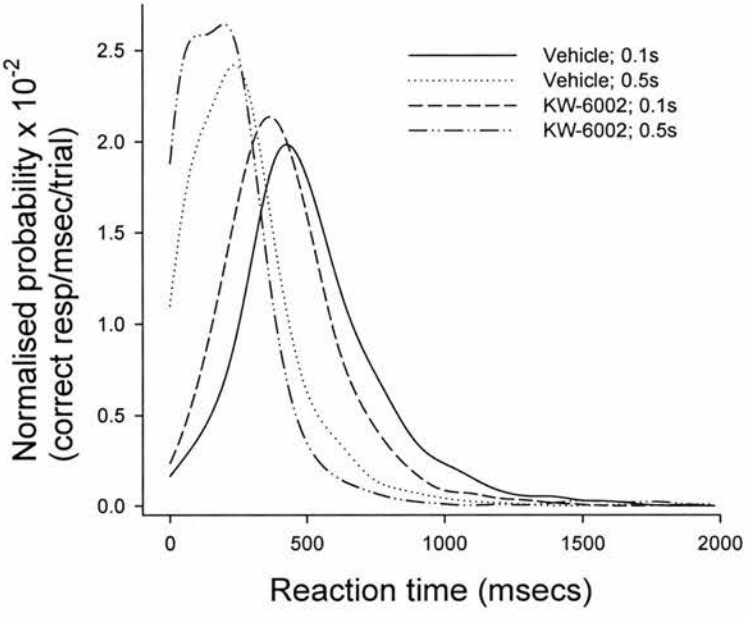

Figure 2.8 This shows the effects of vehicle and the highest dose of KW-6002 (3 mg/kg) on the normalized reaction time distributions at the shortest and longest foreperiods. The effect of 3 mg/kg dose of KW-6002 was greatest at the longest foreperiod.

2.4 Discussion

The effects of systemic KW-6002 were examined in a reaction time task in the rat. Performance within the task varied as a function of two parameters, motivation and motor preparation. Motivation was manipulated by varying the number of trials required for reward and was reflected in reaction time and percentage of correct trials. Motor readiness was defined as a speeding of reaction times as an inverse function of length of foreperiod preceding the imperative signal on each trial. Increasing doses of KW-6002 resulted in generally faster reaction times and increased the normalized distribution height at the longest foreperiod. In other words, KW-6002 increased the likelihood of a fast correct reaction time at the longest foreperiod, suggesting an enhancement of the effect of motor readiness. The effect of KW-6002 on motor preparatory processes is supported by the increase in anticipatory responses as a function of foreperiod, an interaction that is not evident with respect to trials to reward.

Brown et al. (1996) explain the occurrence of an anticipatory response as a consequence of enhanced temporal probability summation. Therefore, KW-6002, through its interaction with foreperiod, enhanced motor preparatory mechanisms possibly by an enhancement of temporal probability summation. Also, KW-6002 decreased reaction times (both mean and modal) at two trials to reward. Additionally, the speeding of mean movement times at rewarded trials compared to unrewarded trials was not as strong following KW-6002. However, this may have been due to a floor effect on unrewarded trials. Thus, it was further concluded that KW-6002 may act to lessen the impact of the cue lights on responding.

The dose x trials to reward interaction in the reaction time analysis was interpreted as a decrease in the gradient of the slope between unrewarded trials. This decrease occurs as a consequence of the increase in reaction time in vehicle performance (see Figure 2.6, upper panel) at two trials to reward, an effect previously observed (Bowman and Brown 1998; Brown et al. 1996). Therefore, consideration of this increase in reaction time at two trials to reward is necessary to evaluate the significant interaction observed.

Perhaps the rats were responding in the first trial in a manner that constitutes 'getting the series of trials started quickly', reflected in the relatively fast reaction times compared to two trials to reward. At two trials to reward, it is possible that the inevitability of forthcoming reward reduces the speed of responding. The fastest reaction times on the rewarded trials may result from the proximal association with the current trial and reward. Note that the data for each trial to reward is pooled from all schedule fractions, such that the two trials to reward data points include data not only from the schedule in which there were three trials required for reward (2/3) but also from the schedule in which there were only two trials required from reward (1/2). According to this explanation, the former trials (2/3) may be construed as having a negative/slowing influence on the overall mean reaction times. However, Bowman and Brown (1998) carried out an extensive analysis of the effects of the different schedules on baseline performance and they also observed a trend towards an increase in reaction time in trial two compared to trial one in a schedule of three trials (this was an analysis of the schedule of three trials in isolation, not including the first trial from the schedule of two trials), albeit a non-significant increase.

Another possible explanation is provided by the 'remaining-responses hypothesis' (Mazur 2002, p.160-161). This hypothesis attempts to explain why the strength of a response is classically weaker when it is closest to the previous reinforcer and furthest from the following reinforcer. If this were the case in the present task then the weakest response (i.e., slowest reaction time) would be expected at three trials to reward and not two. However, on further discussion of the 'remaining-responses hypothesis' in the context of real data, Mazur points out the required responses in a subsequent schedule influences the response in the preceding schedule. Although there are clear differences in Mazur's discussion (which compares post-reinforcement pause activity between schedules in a multiple schedule and the current considerations are of reaction times between discrete trials within a schedule) the rationale that a preceding response may be influenced by the following required response (which is cued and therefore known) remains. Further investigation of the type of multiple schedule ratio used here is required.

The general effect of KW-6002 was to lessen the steepness of the slopes in both the reaction times and movement times, indicating a decrease in the sensitivity to the information provided by the cues. The only conclusion that can be made from the current data is that KW-6002 decreased the impact of the cue lights. Alternatively, KW-6002 could simply act to speed up slower reaction times. Further investigation of the effects of KW-6002 on different schedules of reward is required to elucidate the role of KW-6002 and the adenosine A_{2A} receptor in motivational processes.

This study is the first to provide evidence of a role of adenosine A_{2A} receptors in motor-related processes underlying and predetermining locomotion. Brown et al. (1996) concluded that the effect of amphetamine, similar to the effect of KW-6002 reported here, was a result of an enhancement of the conditional probability of the imperative signal. It is the summation of this temporal probability that Brown et al. (1996) proposed is enhanced by amphetamine and could be the explanation for the effect of KW-6002 seen in our data but the alternative explanation regarding temporal information processing (described in Section 1.3) remains a possibility also.

In particular, scalar expectancy theory (Section 1.3.1) might provide an explanation for the increase in anticipatory responses following KW-6002. The

greater percentage of anticipatory responses at longer foreperiods could be due to the increasing time uncertainty, as hypothesised by scalar expectancy theory; the longer a time interval to be estimated then the greater the error distribution. If this is the case, the effect of KW-6002 could be to enhance this time uncertainty, reflected in the enhancement of the rate of anticipatory errors.

Motor preparatory processes in the rat have also been shown to be mediated by prefrontal cortical areas (Risterucci et al. 2003), striatal dopamine transmission (Baunez et al. 1995a; Brown and Robbins 1991) and enhanced by amphetamine (Brown et al. 1996). Thus, the neuroanatomy, neuropharmacolgy and neurophysiology of time estimation and motor preparation are intimately related to dopamine function and cortico-striatal circuitry. The present study has shown that KW-6002 enhances motor preparatory processes. This effect is characterised as an amplification of a response signal and does not require the interpolation of a mechanism involving an 'internal clock' (Brown et al. 1996). Nevertheless, it is not possible within the behavioural parameters imposed in the present task to detach timing processes from conditional probability processes, and thus although a timing function is not required to understand the data, such a process cannot be ruled out either.

In conclusion, KW-6002 enhanced motor preparatory processes. It is likely that motor preparation is subserved by the parallel cortico-striatal circuitry (Alexander and Crutcher 1990; Mink 1996) and that KW-6002, which is distributed to both cortical and striatal areas following oral administration (Aoyama et al. 2002), impinges upon this system. Chapter 3 assesses whether the effect of KW-6002 on anticipatory responding is due to an enhancement of temporal probability summation or an enhancement of time estimation processes. Furthermore, the effects of KW-6002 are compared to the effects of amphetamine, which has been shown to exert similar effects on anticipatory responding and is reported to enhance time estimation processes (see Meck 1996).

Chapter 3

Disentangling timing and probability mechanisms in a variable foreperiod task in the rat; effects of KW-6002 and amphetamine

- *KW-6002 and amphetamine enhance the foreperiod-dependent increase in anticipatory responding. Is this due to an enhancement of the perception of the passage of time or due an enhancement in the perceived likelihood of stimulus occurrence?*

- *KW-6002 and amphetamine have distinct pharmacological mechanisms of action. Are these reflected in the behavioural profile of these compounds?*

3.1 Introduction

The main focus of the previous experiment was to elucidate the effects of KW-6002 on motor readiness, in order to ascertain whether KW-6002 had a similar profile of effects as amphetamine (Brown et al., 1996). This hypothesis was confirmed; KW-6002, like amphetamine, enhanced the effect of foreperiod on anticipatory responding, with an enhancement of the effect of foreperiod on reaction time distributions. The specific underlying processes involved in this foreperiod effect have yet to be clarified.

Studies that have attempted to define the mechanisms underlying the computational processes involved in the summation of the conditional probability of an imperative signal, and the contribution of this process to motor preparatory mechanisms, draw conclusions from their data that infer the contribution of time perception processes (Baunez et al. 1995a; Risterucci et al. 2003). Such conclusions are not strictly conjectural, they are derived from the considerable overlap in the neuronal constituents involved in the two curiously separate fields of research; time perception and motor preparation, described in the General Introduction (see also MacDonald and Meck 2004).

Studies looking at timing mechanisms have largely focused on the effect of amphetamine, which has also been shown to enhance motor readiness (Brown et al. 1996) and partial blockade of dopamine transmission, by the D_2 receptor antagonist raclopride at non-cataleptic doses, has been shown to slow reaction times (Amalric et al. 1993; Baunez et al. 1995a; Courtiere et al. 2003). Also, the results from Chapter 2 suggest that the adenosine A_{2A} antagonist KW-6002 enhances motor preparation in a similar manner to amphetamine.

Based on these findings, the effects of amphetamine and the adenosine A_{2A} antagonist KW-6002 were compared between 3 groups of rats in a variable foreperiod task in which the effect of absolute time and the effect of increasing temporal probability were dissociated. Rats were trained to respond to an auditory stimulus (tone) presented (unpredictably, from trial to trial) at one of three times (foreperiods). Conditional probability was the same for each group: the probability of a stimulus at the 1st foreperiod was 33%, at the 2nd, it was 50% and at the 3rd, it was 100% (see Figure 2.1). Preparation time, however, was varied between the three groups of rats (Table 3.1). This enabled isolation of

the dominant determinate of responding as either the probability of the stimulus or amount of time elapsed before the stimulus and further characterization of the effects of amphetamine and KW-6002 on motor preparatory mechanisms.

3.2 Materials and methods

3.2.1 Subjects

Twenty four male Lister hooded rats (Harlan, U.K), weighing between 308 and 440 g at the start of testing, were used in this study.

3.2.2 Apparatus

In the operant chamber, in addition to the centre hole the two adjacent holes were exposed and cue (target) lights were provided from these apertures.

3.2.3 Behavioural task

Following successful associative conditioning (Stage 2 as described in General Methods; Section 1.9.3), when the imperative signal sounded following a sustained nose-poke in the central hole, a target light was simultaneously illuminated in one of the side holes with equal probability (50% to each side throughout a complete session). The rat was required to nose-poke in the target hole for reward to be available from the food hopper. The foreperiod was reduced to 0.2 s for this stage, and gradually increased over subsequent training sessions on an individual performance basis. This final stage of training required 21-31 sessions.

Two additional contingencies were simultaneously introduced for the final behavioural protocol. Rats were required to respond in the target hole within 2 s, failure resulted in a time-out and was recorded as an *omission*. This insured tighter time locking of the rats' reaction times to the imperative signal. Also, the imperative signal now occurred after a variable, unpredictable, foreperiod of varying length. Rats were divided into 3 groups (see Table 3.1). For group 1 the foreperiods were 0.2 s, 0.3 s and 0.4 s; group 2, 0.4 s, 0.5 s, and 0.6 s; group 3, 0.2 s, 0.4 s, and 0.6 s. Within each session, each of the three foreperiods was randomly allocated in equal proportion such that each foreperiod was presented a total of 40 times per complete session. Thus, the instantaneous

conditional probability of the imperative signal for each of the foreperiods in ascending order for each group equals 33%, 50% and 100%. This final stage continued for 41-48 sessions before rats were regularly performing at criteria, which was on average 100+ (with a maximum of 120) correct trials within 30 m.

	Group 1			Group 2			Group 3		
Foreperiod (secs)	**0.2**	**0.3**	**0.4**	**0.4**	**0.5**	**0.6**	**0.2**	**0.4**	**0.6**
Conditional probability (%)	**33**	**50**	**100**	**33**	**50**	**100**	**33**	**50**	**100**

Table 3.1 The randomly distributed temporal foreperiods from nose-poke in the central hole till the imperative signal for each group and the respective conditional probabilities.

3.2.4 Drug administration

Rats received one of each dose of KW-6002 or amphetamine and vehicle on consecutive testing days using a pseudo-counterbalanced dosing schedule based on a Latin square design. To test for any drug sensitization effects of previous treatment with KW-6002 or amphetamine, half of the rats from each foreperiod group received KW-6002 in the first block of testing, followed by amphetamine in the second block and vice-versa for the other half. Both test blocks were 4 weeks apart to allow for confirmation of return to baseline performance and an adequate drug wash-out period.

3.2.5 Data analysis

3.2.5.1 Repeated measures ANOVA

The data were analysed with repeated measures ANOVA with drug order, drug, dose and conditional probability (foreperiod is used to refer to the absolute time of the wait, which varied between groups, whereas conditional probability was the same for all groups) as within-subjects factors. Dose was analysed with four levels, dose 0 (vehicle), dose 1, dose 2 and dose 3. The dose range for both amphetamine and KW-6002 were selected based on the dose-responses observed previously: Chapter 2 showed the dose-dependent effects of KW-6002 on motor preparation and Brown et al. (1996), using the same behavioural procedure, observed comparable dose-dependent effects of amphetamine. Therefore, these

doses were used here and analysed based on the rationale that for both amphetamine and KW-6002 dose 0 (vehicle) had no effect, with a relative increase in effect through dose 1 to dose 3. Rather than analysing the effects of each drug separately, they were analysed together to enable a comparison of the dose effects of both drugs. Nevertheless, as the pharmacokinetics of amphetamine and KW-6002 are obviously different, absolute comparison of 'dose' needs to be made with caution.

Group was a between-groups factor. Tukey's honestly significant difference (HSD) post-hoc test was employed for multiple comparisons of between-subject factors.

An effect of group on a dependent variable or a group x conditional probability interaction would signify that different foreperiod durations between the groups had a significant impact, whereas a main effect of conditional probability in absence of the a main effect of group or a group x conditional probability interaction would imply that the conditional probability of the imperative signal within each group, irrespective of absolute time (foreperiod length), was having an impact. Figure 3.1 shows examples of the expected patterns the slopes would display if conditional probability or foreperiod duration were the defining factors of performance.

If the conditional probability dominated performance, then horizontally juxtaposed slopes between the groups would be expected if the data were plotted across foreperiod duration (Figure 3.1; upper-left panel). If plotted over conditional probability, then the slopes should be superimposed (Figure 3.1; upper-right panel). On the other hand, if foreperiod duration dominated performance then the slopes would be parallel when plotted across foreperiod duration (Figure 3.1; lower-left panel). If plotted across conditional probability, then the difference in foreperiod duration would be reflected in a steeper slope in the group with the largest difference between foreperiods (i.e., Group 3; Figure 3.1; lower-right panel).

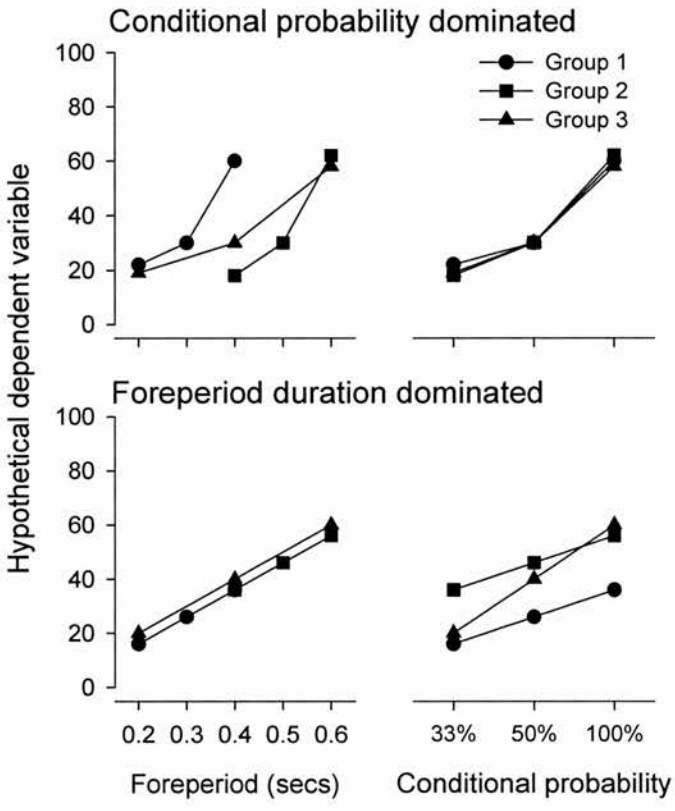

Figure 3.1 This shows the predicted outcome for a hypothetical dependent variable determined by the conditional probability of the stimuli in each group (upper panels) or the foreperiod duration (lower panels). If the dependent variable is determined by conditional probability, when plotted across foreperiod duration then foreperiods with equal probability will align along the horizontal axis with the slopes juxtaposed (upper left). When plotted across conditional probability, the slopes would be superimposed (upper right). If, however, the dependent variable was determined by foreperiod duration, then when plotted across foreperiod duration the slopes would display a continuum (lower left). When plotted across conditional probability, the slope from Group 3 would be the steepest, representing the time differences between the foreperiods (lower right).

3.2.5.2 Principal components analysis

The data were further analysed to define the relative contribution of conditional probability and foreperiod duration on performance. The vehicle data from the amphetamine and KW-6002 days were not significantly different and were therefore pooled and analysed. This was required to provide a baseline level

of performance for comparison with drug-dose effects. Principal components analysis (SPSS v10.0) was performed on the dependent variables of reaction time, movement time and anticipatory responses. The purpose of this analysis was to determine which constellations of dependent variables, if any, co-varied across the combinations of rats and vehicle conditions. The steps used in performing the PCA are listed below.

First, the raw data from each variable was converted to standardised *z*-scores, taking into account the mean and standard deviation of all scores for the given variable across rats and vehicle conditions. Second, PCA was used to extract three factors that represented orthogonal, linear combinations[2] of the standardised dependent variables. The factors were calculated sequentially to account for the greatest residual variance in the standardised data at each step in the analysis.

Third, eigenvalues were then assessed to determine which factors accounted for a meaningful proportion of the data. The eigenvalues provided a standardised measure of the variability accounted for by each factor. In general an eigenvalue of > 1 is considered to account for a meaningful amount of the variability in the data (Hardy and Bryman 2003). Fourth, the pattern of factor coefficients across the standardised dependent variables was examined to interpret each factor defined in the PCA.

Using the PCA analysis, factor scores were calculated for each combination of rat and condition. Multiple regression was then used to describe the relationships between the predictors (independent variables); foreperiod and conditional probability and the factor scores (predicted variables). The two regression analyses performed were defined by the following equations:

Estimated score for PC1 = a + (b)(foreperiod) + (c)(conditional probability)
Estimated score for PC2 = a + (b)(foreperiod) + (c)(conditional probability)

Where a is the estimated value of the factor score if foreperiod and conditional probability are equal to zero, and b & c are coefficients.

[2] The linear combinations took the following form: PCA score = (a)(z-score of reaction time) + (b)(z-score of movement time)+(c)(z-score of % anticipatory responses, where a, b and c are coefficients calculated according to the algorithm described in the text.

The regression analyses were performed in SPSS (v10.0), using the general linear model with foreperiod and conditional probability as predictors and the factor score as the predicted variable. Parameter estimates from these regression analyses were examined for the intercept (coefficient *a* above), foreperiod (coefficient *b)* and conditional probability (coefficient *c*). The value for each coefficient was then extracted, its level of statistical significance (where H_0: coefficient=0) and the observed power.

In order to interpret the effects of amphetamine and KW-6002 on the relative contribution of conditional probability and foreperiod duration, it was necessary to devise a means to assess the effect of each dose of drug on the variance accounted for in PC1 and PC2 in the vehicle condition.

Standardised z-scores were calculated for reaction time, movement time and anticipatory responses. Factor scores were then calculated (using the algorithm described above) for each dose of amphetamine and KW-6002 using the factor coefficient values from PC1 and PC2 from the vehicle data. This provided factor scores at each dose representative of a shift from the pattern observed in the vehicle data. Multiple regression analysis was carried out on the recalculated factor scores (recalculated PC1 and PC2) for each dose of amphetamine and KW-6002. This enabled assessment of the impact of conditional probability and foreperiod at each dose, expressed as the Eta squared value, relative to the vehicle condition. In other words, a measure of the extent to which each dose of amphetamine and KW-6002 affected the proportion of variance accounted for by conditional probability and foreperiod in PC1 and PC2.

3.3 Results

3.3.1 Baseline performance

After 16 weeks of training, when correct responding on the final behavioural protocol had stabilised (average cumulative error percentage = 30 %; 19 % anticipatory responses, 7 % incorrect responses and 4 % omissions), two blocks of data, six and five days respectively, separated by one day in which the rats were not run, were compared. The reaction time data and the percentage of correct responses from each block were analysed using a repeated measures

ANOVA with block, side and conditional probability as within-subjects factors and group as a between-groups factor.

There was no main effect of block on reaction times ($F_{(1, 21)} = 0.06$, ns). However, it should be noted that there was a significant block x conditional probability interaction ($F_{(2, 42)} = 6.00$, $p = 0.006$). Analysis of each block independently showed that conditional probability accounted for a large amount of variance in blocks 1 (Eta squared = 90%) and 2 (Eta squared = 91%). Neither side of target location nor group significantly affected reaction time in either block (main effects; $F_{(1, 21)} = 0.67$, ns and $F_{(2, 21)} = 1.22$, ns, respectively).

There was a main effect of block on the percentage of correct responses ($F_{(1, 21)} = 27.93$, $p < 0.001$), which was most likely due to a small improvement in performance (i.e., the rats made fewer errors) in the second block. Moreover, there was no significant difference in the effect of conditional probability or group on accuracy of performance between both blocks (block x conditional probability x group interaction; $F_{(4, 42)} = 0.08$, ns).

Thus, performance was generally stable across both blocks and drug testing proceeded thereafter.

3.3.2 Effects of amphetamine and KW-6002

Four rats in total were not included in the final analysis due to poor performance during testing, resulting in missing data in some conditions. These included two rats from group 1 and two rats from group 3. As the drug order was counterbalanced, with one group receiving amphetamine in the first block of testing and KW-6002 in the second block and vice versa for the other group, an initial analysis was carried out on each drug individually to test for cross sensitisation effects. Repeated measures ANOVA revealed no significant difference in performance for any measures following amphetamine, regardless of previous treatment with KW-6002 (main effect of drug order on mean reaction time; $F_{(1, 14)} = 2.43$, ns: anticipatory responses; $F_{(1, 14)} = 1.91$, ns: incorrect responses; $F_{(1, 14)} = 0.56$, ns: omissions; $F_{(1, 14)} = 1.61$, ns). Moreover, there was no interaction of drug order and dose with group or conditional probability (data not shown).

Overall, there was no significant effect of drug order on performance following KW-6002 injections (main effect of drug order on mean reaction time;

$F_{(1, 14)} = 3.97$, ns: anticipatory responses; $F_{(1, 14)} = 1.77$, ns: omissions; $F_{(1, 14)} = 0.89$, ns). One exception was incorrect responses, which were higher in block 1 (5.09(±0.45)) than block 2 (3.11(±0.65)) (main effect of block; $F_{(1, 14)} = 6.15$, $p = 0.027$). A dose x drug order interaction would indicate that drug order had an effect on performance (for example, by sensitisation or tolerance). However, for no measure of performance was this interaction significant, including incorrect responses following KW-6002 (dose x drug order interaction; $F_{(3, 42)} = 0.18$, ns), and therefore drug order was not considered further.

Therefore, within the behavioural parameters of the task, cross sensitisation of amphetamine and KW-6002 did not occur. As this is the case, the drug data from each test block were pooled and analysed further.

3.3.2.1 Anticipatory responses

Figure 3.2 and 3.3 show the effect of amphetamine and KW-6002, respectively, on the percentage of anticipatory responses. With rising conditional probability there was an increase in anticipatory responses, an effect that was different between the groups and therefore dependent on foreperiod length (conditional probability x group interaction; $F_{(4, 34)} = 5.37$, $p=0.002$). Restricted analysis revealed that the different foreperiod lengths between the groups did not have an effect on anticipatory responses in the vehicle condition (restricted conditional probability x group interaction in vehicle condition; $F_{(2, 17)} = 0.55$, ns). This corresponded with an analysis of the baseline data (data not shown). The conditional probability x group interaction is most likely explained by the main effect of drug, as discussed below.

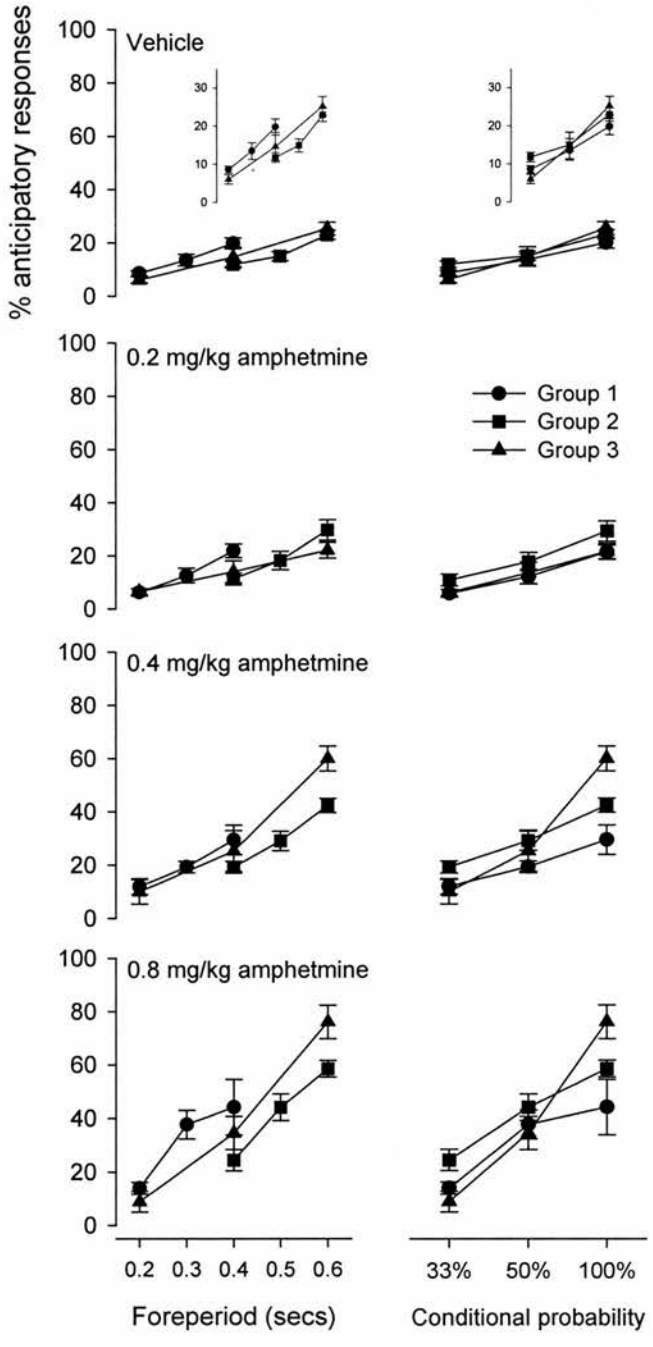

Figure 3.2 Percentage of anticipatory responses for each dose of amphetamine plotted across foreperiod (left panels) and conditional probability (right panels).The insets show the vehicle data with expanded y-axes. Amphetamine dose-dependently enhanced the effect of foreperiod. The graphs suggest an enhancement of the effect of conditional probability as the slopes in all groups are progressively steeper with increasing dose of amphetamine. Also, at the 0.4 s foreperiod the slopes are increasingly diverging. However, there is also the suggestion that the slope enhancement by amphetamine was greatest in group 3.

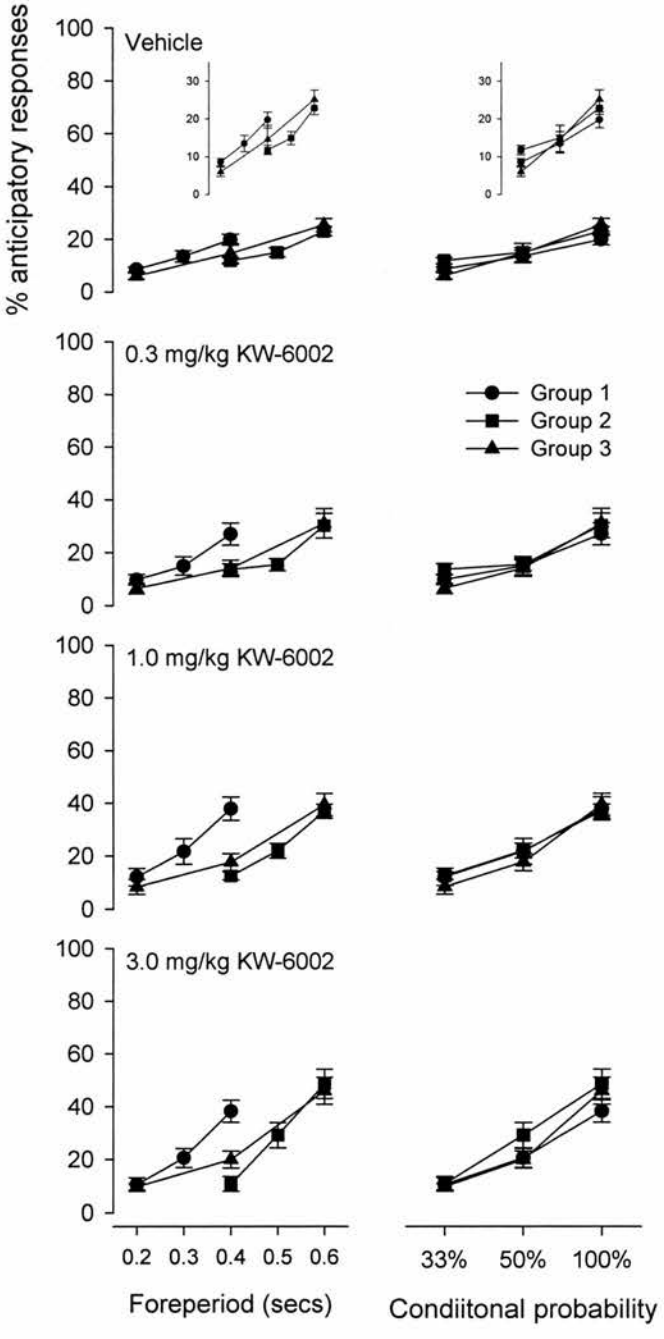

Figure 3.3 Percentage of anticipatory responses for each dose of KW-6002 plotted across foreperiod (left panels) and conditional probability (right panels). The insets show the vehicle data with expanded y-axes. KW-6002 dose-dependently increased anticipatory responses as a function of increasing foreperiod. The graphs suggest an enhancement of the effect of conditional probability as the slopes in all groups are progressively steeper with increasing dose of KW-6002. Also, at the 0.4 s foreperiod the slopes are increasingly diverging. The enhancement of the effect of conditional probability by KW-6002 between the groups appears similar as the slopes remain parallel at all doses.

Amphetamine and KW-6002 increased anticipatory responses dose-dependently (main effect of dose; $F_{(3, 51)} = 83.48$, $p<0.001$) with the greatest increase occurring at the longer foreperiods (dose x conditional probability interaction; $F_{(6, 102)} = 19.71$, $p<0.001$). Figure 3.4 clearly shows the dose-dependent enhancement of the conditional probability effect by amphetamine and KW-6002 when foreperiod duration is constant at 0.4 s.

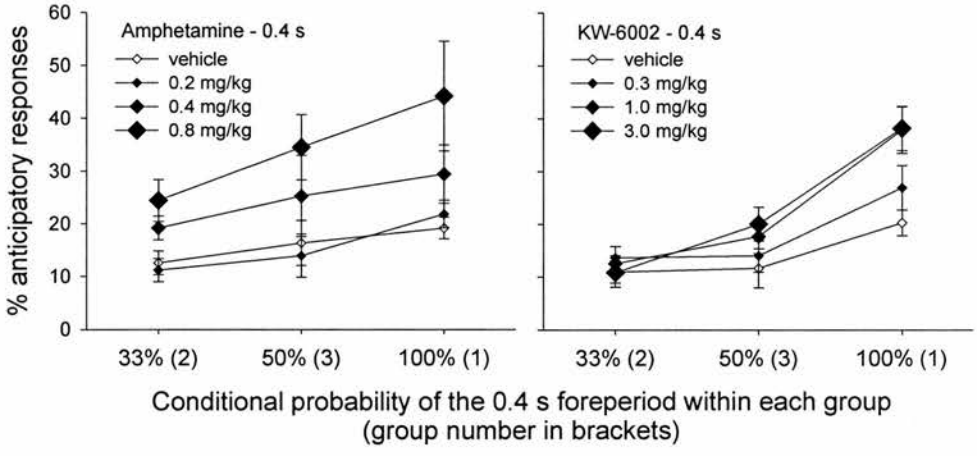

Figure 3.4 Effect of amphetamine (left panel) and KW-6002 (right panel) on anticipatory responses as an absolute function of conditional probability. These graphs show that when the stimulus was presented at the same temporal location, anticipatory responding was dictated by the prior likelihood of that stimulus and this effect was dose-dependently enhanced by amphetamine and KW-6002.

Amphetamine increased anticipatory responses more so than KW-6002 (main effect of drug; $F_{(1, 17)} = 6.07$, $p = 0.025$) particularly at the highest dose (drug x dose interaction; $F_{(3, 51)} = 12.27$, $p < 0.001$). Although amphetamine and KW-6002 did not generally affect the level of anticipatory responding between the groups (dose x group interaction; $F_{(6, 51)} = 1.09$, ns), amphetamine, at the medium and high dose, increased anticipatory responses as a function of increasing foreperiod most in group 3, as can be seen from the non-parallel slopes in the bottom panels of Figure 3.2 (drug x dose x conditional probability x group interaction; $F_{(12, 102)} = 2.84$, $p = 0.002$). To further test this finding, linear regression was carried out for each group at each dose and the line of best fit for the slopes in Figures 3.2 and 3.3 was calculated. Figure 3.5 shows the

corresponding gradient of the slopes for each group at each dose of both amphetamine and KW-6002. The increase in the steepness of the slopes by increasing dose of KW-6002 was similar among the groups. Amphetamine, on the other hand, increased the steepness of the slopes in all groups with a much greater effect on the slope in group 3 at the medium and high dose.

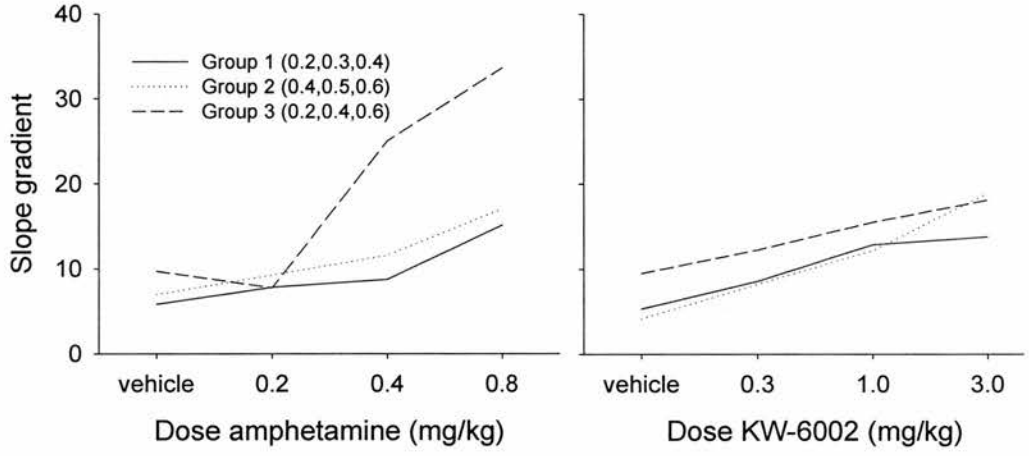

Figure 3.5 The effect of increasing dose of amphetamine (left panel) and KW-6002 (right panel) on the gradient of the slopes plotted in Figures 3.2 and 3.3. KW-6002 had a similar effect on all groups whereas amphetamine had a greater effect on group 3.

Thus, these results indicate that KW-6002 did not differentially effect anticipatory responding between the groups whereas amphetamine had a greater effect on group 3, particularly at the higher doses. This indicates that the effect of KW-6002 was a greater enhancement of the effect of stimulus conditional probability compared to foreperiod length. Amphetamine, on the other hand, as well as enhancing conditional probability also enhanced the effect of foreperiod duration, particularly when the difference between variable foreperiods was greater (i.e., a 0.2 s difference in foreperiods in group 3 compared to 0.1 s in groups 1 and 2). However, to reiterate, an absolute comparison of 'dose' needs to be interpreted with caution, as a higher dose of KW-6002 may result in a similar pattern seen here by the highest dose of amphetamine.

3.3.2.2 Mean reaction time

Mean reaction time decreased as a function of increasing conditional probability (main effect of conditional probability; $F_{(2, 34)} = 187.45$, $p<0.001$).

Although mean reaction times did not generally differ between groups (main effect of group; $F_{(2, 17)} = 1.15$, ns), Figure 3.6 shows that the effect of conditional probability was greater in the group with the longest foreperiods (group 3; conditional probability x group interaction; $F_{(4, 34)} = 6.57$, $p=0.002$). Amphetamine and KW-6002 dose-dependently decreased mean reaction time (main effect of dose; $F_{(3, 51)} = 31.03$, $p < 0.001$). There was no significant difference between the effect of amphetamine and KW-6002 on mean reaction time (main effect of drug; $F_{(1, 17)} = 0.19$, ns). Also, amphetamine and KW-6002 did not enhance mean reaction time as a function of conditional probability (dose x conditional probability effect; $F_{(6, 102)} = 0.72$, ns) and there was an interaction between dose and group that approached significance (dose x group interaction; $F_{(6, 51)} = 2.20$, $p = 0.058$). Thus, amphetamine and KW-6002 speeded mean reaction times and this effect was additive with the effect of foreperiod duration and conditional probability.

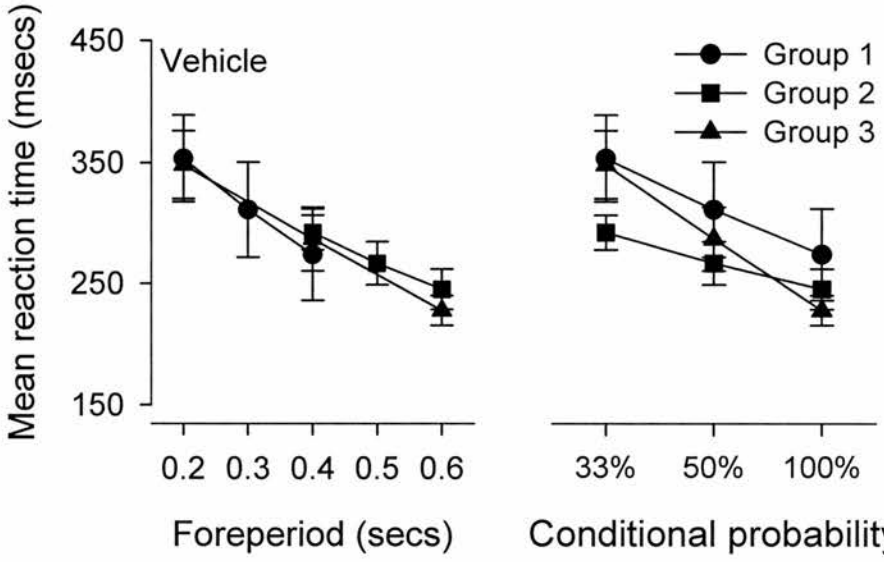

Figure 3.6 Mean reaction times from the vehicle condition plotted across foreperiod (left panel) and conditional probability (right panel) for each group. Mean reaction times decreased as a function of increasing foreperiod and this effect was greatest in group 3.

3.3.2.3 Mean movement time

The effect of amphetamine and KW-6002 on mean movement times are displayed in Figure 3.7 and 3.8. Mean movement time increased as a function of conditional probability (main effect of conditional probability; $F_{(2, 34)} = 120.34$, p

< 0.001). There was no significant difference in mean movement times between groups (main effect of group; $F_{(2, 17)} = 0.09$, ns). The effect of conditional probability was not significantly different between groups (conditional probability x group interaction: $F_{(4, 34)} = 1.26$, ns). Both amphetamine and KW-6002 dose-dependently increased mean movement times (main effect of dose; $F_{(3, 51)} = 40.72$, $p < 0.001$), enhancing the effect of conditional probability (dose x conditional probability interaction; $F_{(6, 102)} = 3.05$, $p = 0.009$). Neither drug had significantly different effects on movement time between the three groups (drug x dose x group interaction; $F_{(6, 51)} = 0.93$, ns).

3.3.2.4 Incorrect responses

The percentage of incorrect responses increased with increasing conditional probability (main effect of conditional probability; $F_{(2, 34)} = 13.20$, $p = 0.001$). Amphetamine and KW-6002 did not affect the overall level of incorrect responses (main effect of dose; $F_{(3, 51)} = 1.71$, ns) nor did they interact with any other factor in the analysis on incorrect responses.

3.3.2.5 Omissions

The percentage of omissions was not significantly affected by conditional probability (main effect of conditional probability; $F_{(2, 34)} = 1.57$, ns) or group (main effect of group; $F_{(2, 17)} = 0.16$, ns). Figure 3.9 shows that amphetamine and KW-6002 had different effects on omissions. Amphetamine generally increased omissions and KW-6002 generally had no effect, with a greater effect of amphetamine at the medium and high doses (drug x dose interaction; $F_{(3, 51)} = 3.68$, $p = 0.018$).

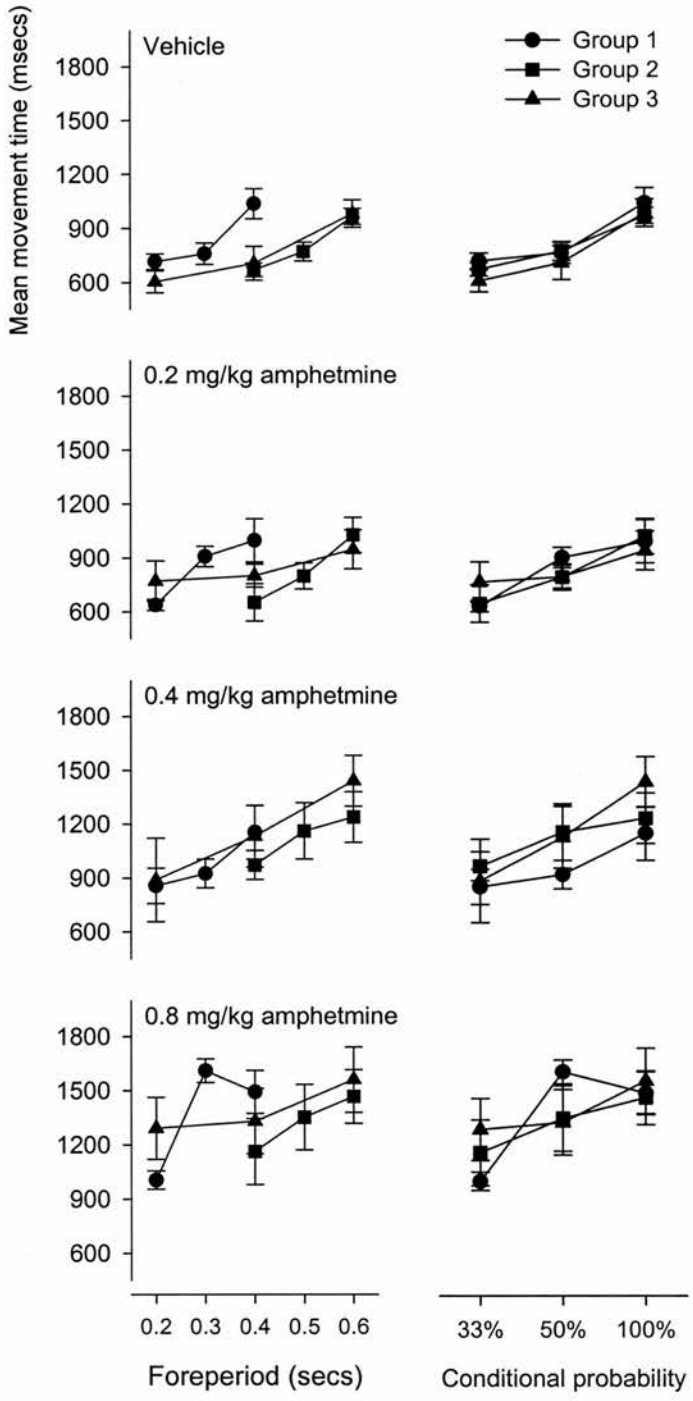

Figure 3.7 Effect of increasing dose of amphetamine (top to bottom panels, respectively) on mean movement time plotted across foreperiod (left panels) and across conditional probability (right panels) for each group. Amphetamine increased mean movement times as a function of foreperiod in all groups and this effect is most apparent at the 0.4 mg/kg dose.

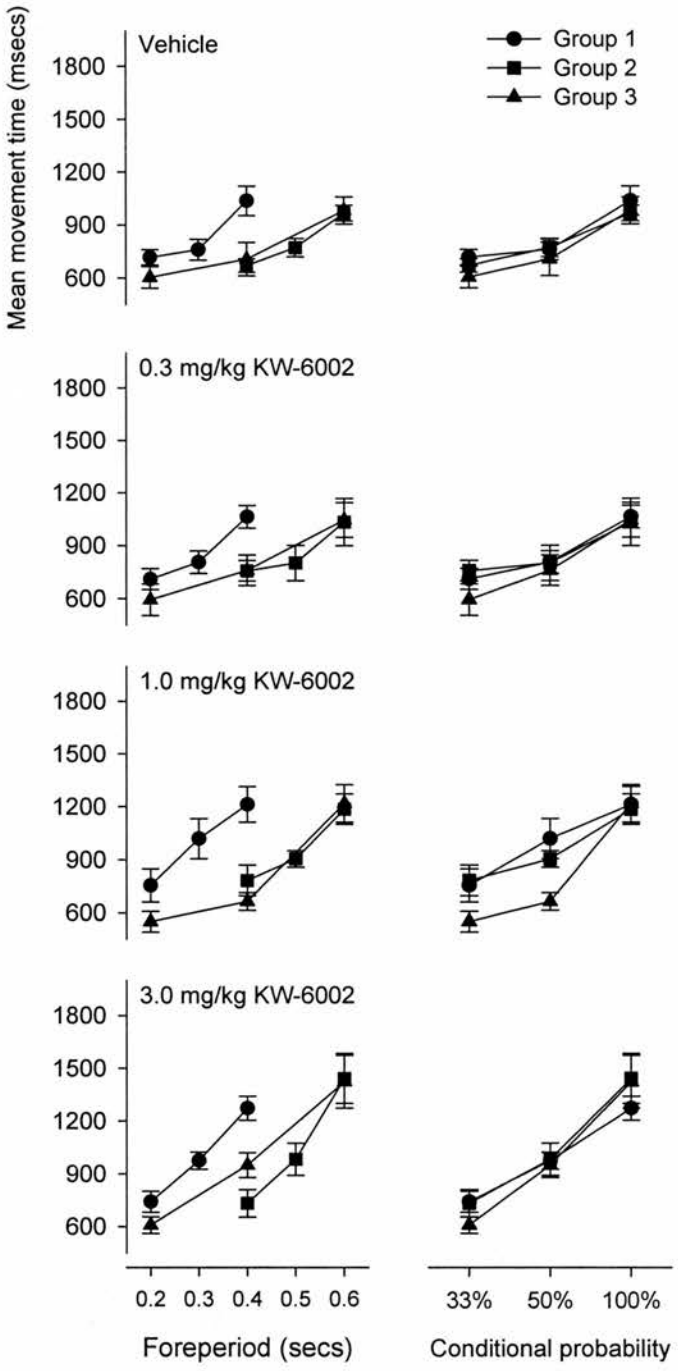

Figure 3.8 Effect of increasing dose of KW-6002 (top to bottom panels, respectively) on mean movement time plotted across foreperiod (left panels) and across conditional probability (right panels) for each group. KW-6002 dose-dependently increased mean movement times as a function of foreperiod. This effect was equal in all three groups.

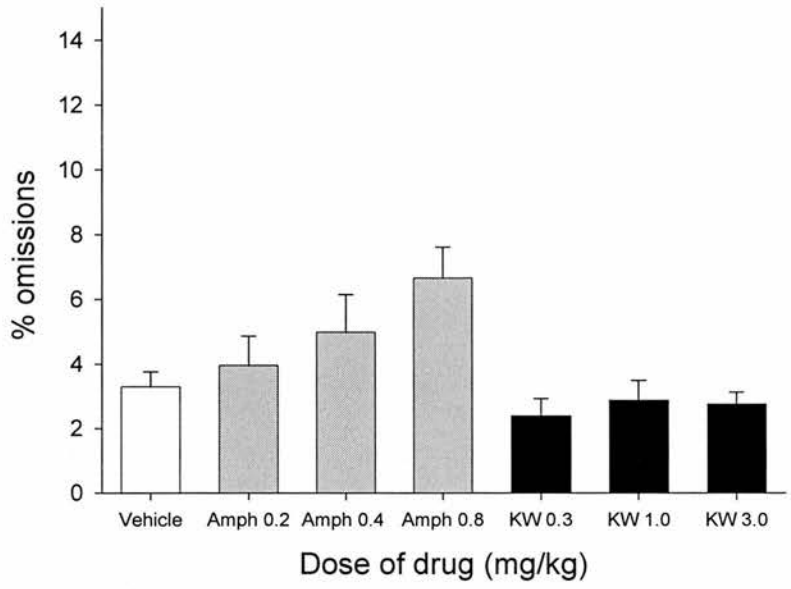

Figure 3.9 The effect of amphetamine and KW-6002 on the percentage of omissions. KW-6002 did not affect the percentage of omissions whereas amphetamine increased the percentage of omissions at the medium and high doses.

3.3.3 Summary 1

In the mean reaction time analysis, there was no significant interaction between drug dose and conditional probability or group. This indicates that the effects of both amphetamine and KW-6002, when given separately, were additive with conditional probability and foreperiod duration. Modal reaction times were also considered (data not shown) and there was a similar (close to significance) interaction of dose with conditional probability; amphetamine and KW-6002 had a tendency to reduce modal reaction times at the shorter foreperiods but this was likely due to a ceiling effect on reaction time performance.

Both drugs enhanced the effect of conditional probability and not foreperiod duration on movement times. In the analysis of anticipatory responding, there was a 4-way drug x dose x conditional probability x group interaction. This appears to be explained by an isolated effect of KW-6002 on conditional probability whereas, although amphetamine also appeared to enhance conditional probability, this effect was greatest in group 3. Thus, amphetamine

appeared to enhance both the effect of conditional probability and the effect of foreperiod duration on anticipatory responding.

3.3.4 Principal components analysis

3.3.4.1 Vehicle data

In order to further assess and clarify the effects of amphetamine and KW-6002 on conditional probability and foreperiod duration, principal components analysis was employed to reduce the dataset to *components* or *factors* (estimated dependent variables) representative of the variability in all experimental conditions. This allowed assessment of which factor, conditional probability or foreperiod duration, was a greater predictor of the variability in the data and how amphetamine and KW-6002 affected the predictive value of each factor.

There was a strong positive relationship between mean movement time and the proportion of anticipatory responses (r=0.74, p<0.001). The proportion of anticipatory responses and mean reaction time had a weak negative correlation (r=-0.36, p<0.05). Mean reaction time and mean movement time also had a weak negative correlation (r=-0.28, p>0.05).

The eigenvalues obtained from the PCA provide a measure of the amount of variance in the data that is explained by each component. The eigenvalues indicated that the two first components each accounted for a high percentage of the data. The eigenvalue for PC1 was 1.95, whereas PC2 was 0.79. Although the latter was not greater than 1 (generally considered a cut-off level for an eigenvalue that accounts for significantly more or less variance than one of the dependent variables, but see Hardy and Bryman (2003, p.29) for an explanation of the arbitrary nature of this value), it was considerably higher then the eigenvalue for PC3 (0.26). Together, PC1 and PC2 captured 91% of the variability in the standardised data (Figure 3.14). Therefore, only these components shall be considered further.

The factor loading values for the dependent variables in the PCA provide a measure of the amount of variance accounted for within each component (i.e., PC1 and PC2) by the dependent variables. Figure 3.14 shows the factor loading values for each dependent variable for PC1 and PC2. The factor loading values for mean movement time and anticipatory responses for the PC1 were very high

(r=0.88 and 0.91, respectively). Mean reaction time had a moderately negative factor loading value for PC1 (r=-0.6). In contrast, mean reaction time had a very high factor loading value for PC2 (r=0.8), with very low factor loading values for mean movement time and percentage of anticipatory responses (r=0.33 and 0.21, respectively). Thus, the results of the PCA were interpreted as indicating that there were two meaningful factors: PC1, which is dominated by mean movement time and proportion of anticipatory responses, and PC2, which is dominated by mean reaction time.

Contribution of foreperiod and conditional probability to variations in PC1 and PC2:

For PC1, in which movement time and anticipatory responding accounts for most of the variability, both conditional probability and foreperiod were significant predictors of the variability ($p<0.001$ and $p< 0.05$, respectively). The Eta squared values indicated that 30% of the variability in scores on PC1 was accounted for by conditional probability, with 10% of the variance accounted for by foreperiod.

In the regression analysis of PC2, which is predominated by mean reaction time, only foreperiod was significant (p=0.034), accounting for 8% of the variance. Although this relationship is not particularly strong, the lack of a significant effect of conditional probability is in sharp contrast to the results from the first regression analysis. These findings suggest that conditional probability and foreperiod had different influences on the variance in the two factors described by the PCA. This might be due to different underlying psychological processes detected by the PCA analysis.

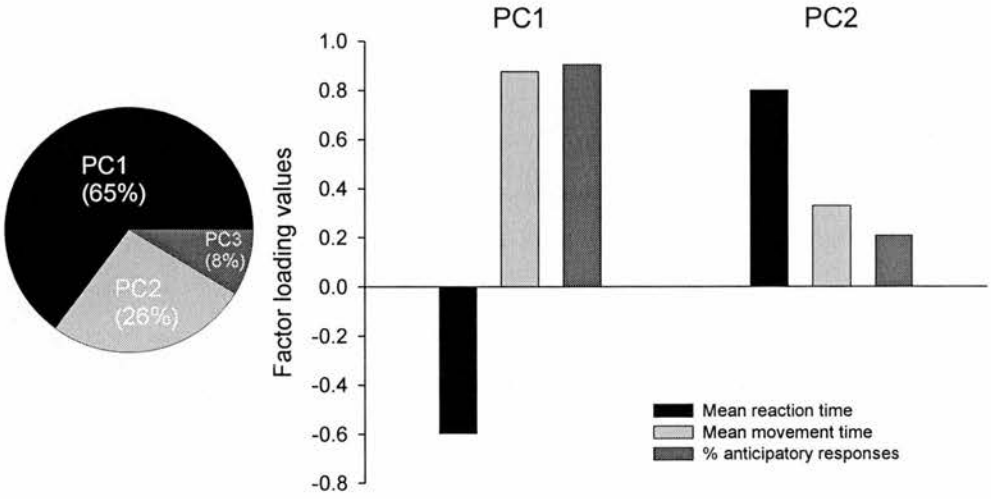

Figure 3.14 The pie chart on the left shows the variance in the data accounted for by each of the three components extracted by principal components analysis. The bar graph on the right shows the factor loading values for each of the dependent variables for both PC1 and PC2. Mean movement time and anticipatory responses contributed most strongly to the variability in the data captured by PC1, whereas mean reaction time was predominant in PC2.

Validation of conditional probability and foreperiod effects:

Table 3.4 shows the results of the multiple regression analyses of the subsets. For PC1, the significant effect of foreperiod was confirmed in only 7/20 subsets, with no significant effect in 13/5 while conditional probability was significant in 19/20. For PC2, the significant effect of foreperiod was confirmed in 7/20 subsets while conditional probability was not significant either in the overall dataset or in 17/20 subsets. Thus, the variability in scores in PC1 was consistently accounted for by conditional probability, whereas the variability in PC2 was more consistently accounted for by foreperiod.

	PC1				PC2			
	Foreperiod		Probability		Foreperiod		Probability	
	p	Eta^2	p	Eta^2	p	Eta^2	p	Eta^2
Vehicle :	√	**10%**	√	**30%**	√	**8%**	**X**	**6%**
Subset1 :	X	-	√	30%	√	16%	X	-
Subset2 :	√	26%	√	23%	X	-	X	-
Subset3 :	X	-	√	33%	√	22%	X	-
Subset4 :	√	12%	√	21%	√	15%	X	-
Subset5 :	√	13%	√	24%	√	20%	√	15%
Subset6 :	X	-	√	35%	X	-	X	-
Subset7 :	X	-	√	16%	√	27%	√	14%
Subset8 :	X	-	√	32%	X	-	X	-
Subset9 :	√	46%	X	-	√	32%	√	31%
Subset10 :	X	-	√	27%	X	-	X	-
Subset11 :	X	-	√	36%	X	-	X	-
Subset12 :	X	-	√	15%	X	-	X	-
Subset13 :	X	-	√	31%	√	21%	X	-
Subset14 :	X	-	√	27%	X	-	X	-
Subset15 :	√	24%	√	39%	X	-	X	-
Subset16 :	X	-	√	35%	X	-	X	-
Subset17 :	X	-	√	29%	X	-	X	-
Subset18 :	X	-	√	54%	X	-	X	-
Subset19 :	X	-	√	30%	X	-	X	-
Subset20 :	X	-	√	19%	X	-	X	-
Summary:	√	5/20	√	19/20	√	7/20	**X**	17/20

Table 3.4 Summary of the variance accounted for by foreperiod and conditional probability in PC1 and PC2 in the overall vehicle analysis and for each of the subset analyses. For PC1, conditional probability reliably accounted for a high percentage of the variance in all but 1 of the subset analyses (19/20). Foreperiod accounted for a significant percentage of the variance in 5/20 subset analyses for PC1. For PC2, foreperiod accounted for a low but significant percentage of the variance in the overall analysis and in 7/20 subsets. Conditional probability did not account for a significant percentage of the variance in PC2 in the overall analysis nor did it account for a significant percentage of the variance in 17/20 subsets.

3.3.4.2 Drug effects

PC1 and PC2, by definition independent of each other, were predominated by mean movement time and anticipatory responding (PC1) and mean reaction time (PC2) in the vehicle data. However, Figure 3.15 shows that with increasing dose of amphetamine there is a change in the relationship between the dependent variables and the two predominating principal

components. The strength of the correlation between a dependent variable and a principal component is expressed as a factor loading value. Factor loading values can range from -1 to 1, with the absolute value representing the strength of the correlation and the sign (negative or positive) indicative of the direction of the correlation.

Before considering the effect of amphetamine and KW-6002 on the variance accounted for by conditional probability and foreperiod in PC1 and PC2, it is important to consider the relationship between the dependent variables and the estimated factors (i.e., PC1 or PC2). This has considerable implications for any conclusions drawn from the regression analysis. If a drug affects the predictive value of an independent variable (conditional probability or foreperiod) on an estimated factor (PC1 or PC2), the effect upon the estimated factor can be confidently inferred upon the dependent variables only if the relationship with the dependent variables is unchanged. Otherwise, if the effect of drug is to reduce the strength in the relationship between a dependent variable and an estimated factor (expressed as the factor loading value), then the estimated factor is less likely to reflect the pattern in the dependent variable(s).

3.3.4.2.1 Amphetamine

The effect of amphetamine on the factor loading values for reaction time, movement time and anticipatory responding are shown in Figure 3.15 (upper panels) for both PC1 and PC2.

As a function of increasing dose of amphetamine, there was a weak increase in the factor loading value for mean reaction time in PC1 (Figure 3.15; upper left panel). In contrast, there was a strong decrease in the factor loading value for mean movement time (Figure 3.15; upper right panel). The factor loading value for anticipatory responding in PC1 was not affected by increasing dose of amphetamine (Figure 3.15; upper right panel).

For PC2, there was a moderate decrease in the factor loading value for both mean reaction time and anticipatory responses with increasing dose of amphetamine (Figure 3.15; upper left and upper right panels, respectively), whereas there was a strong dose-dependent increase in the factor loading value for mean movement time (Figure 3.15; upper right panel).

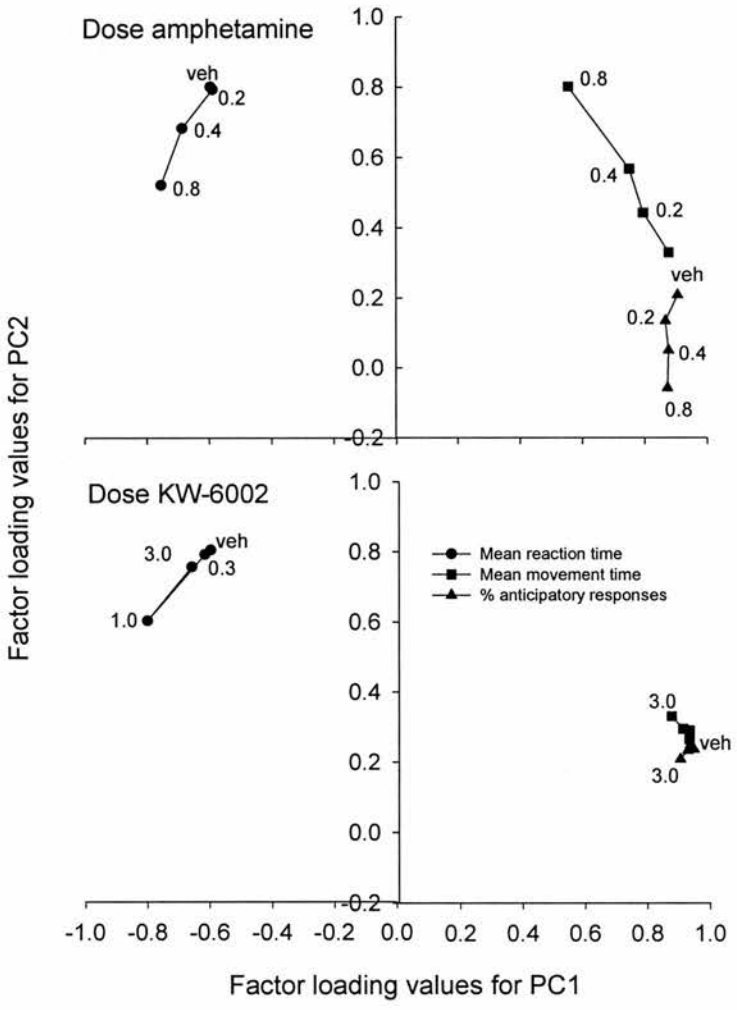

Figure 3.15 The factor loading values for the dependent variables mean reaction time, mean movement time and percentage of anticipatory responses at each dose of amphetamine (upper panels) and KW-6002 (lower panels) for PC1 and PC2. Only at the medium dose did KW-6002 have a moderate effect on the factor loading value for mean reaction time, strengthening the value in PC2 and weakening the value in PC1. Amphetamine dose-dependently affected the factor loading values for all the dependent variables. There was a low and moderate effect of increasing dose of amphetamine on the factor loading value for mean reaction time and mean movement time in PC1, respectively; the reaction time value increased and the movement time value decreased. In PC2, amphetamine had a moderate weakening effect on the loading value for reaction time and anticipatory responses, whereas amphetamine strongly strengthened the loading value for mean movement time.

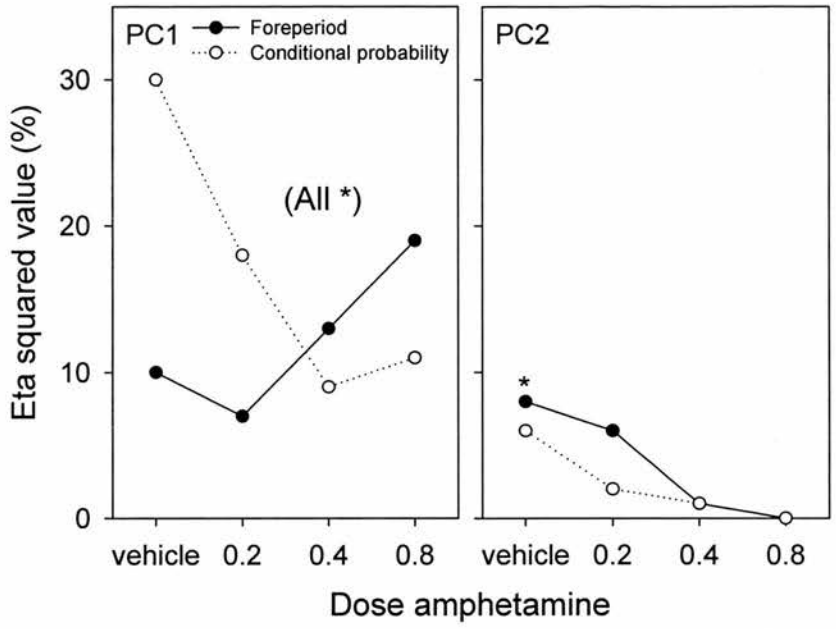

Figure 3.16 The effect of dose of amphetamine on the proportion of variance accounted for by conditional probability and foreperiod for PC1 (left panel) and PC2 (right panel) – the estimated dependent variables from the principal components analysis. At each dose, the relative impact of conditional probability and foreperiod on variance is expressed as the eta squared value (a standardised measure of the magnitude of effect). For PC1 the impact of conditional probability decreased as a function of increasing dose of amphetamine, whereas the impact of foreperiod increased. Both foreperiod and conditional probability accounted for a significant amount of the variance in all conditions in PC1. For PC2, at all doses foreperiod did not account for a significant level of variance. Conditional probability did not significantly account for the variance in PC2 at any dose, including vehicle. Asterisks indicate conditions where a significant amount of variance was accounted for.

The left panel of Figure 3.16 shows that for PC1, the variance accounted for by conditional probability decreased as a function of increasing dose of amphetamine. Note however that although there was a decrease in the amount of variance accounted for by amphetamine, conditional probability accounted for a significant proportion of the variance at all doses. Figure 3.16 (right panel) shows that for PC2, the percentage of variance accounted for by foreperiod decreased and was no longer significant following amphetamine at all doses.

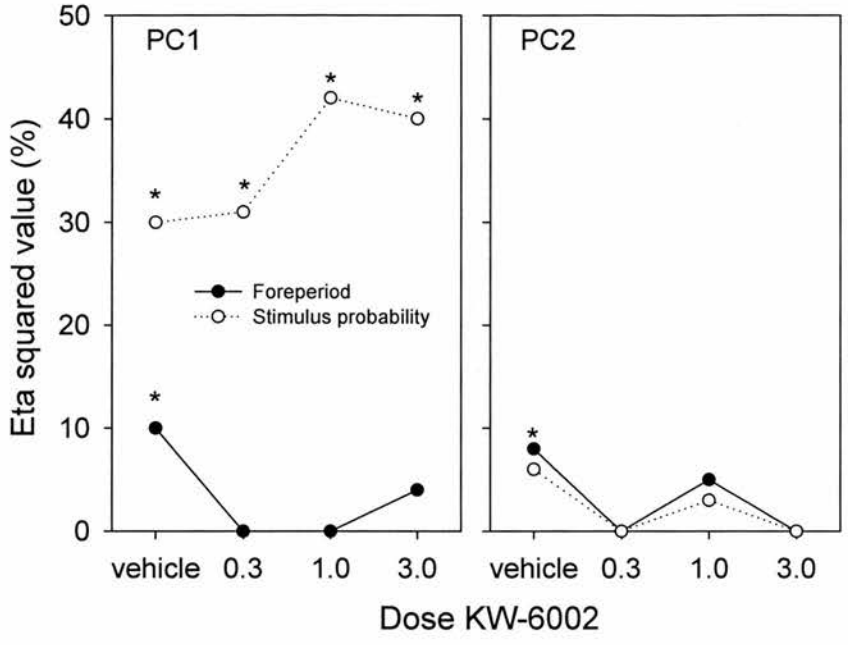

Figure 3.17 The effect of dose of KW-6002 on the proportion of variance accounted for by conditional probability and foreperiod for PC1 (left panel) and PC2 (right panel). For PC1 the impact of conditional probability increased as a function of increasing dose of KW-6002, whereas the impact of foreperiod decreased. Foreperiod did not account for a significant amount of variance in the data at any dose of KW-6002. For PC2, at all doses foreperiod did not account for a significant level of variance. Conditional probability did not significantly account for the variance in PC2 at any dose, including vehicle. Asterisks indicate conditions where a significant amount of variance was accounted for.

3.3.4.2.2 KW-6002

In contrast to the effects of amphetamine on the factor loading values, increasing dose of KW-6002 did not generally affect the factor loading values (Figure 3.15; lower panels). In further contrast to the effect of amphetamine, Figure 3.17 (left panel) shows that for PC1, KW-6002 dose-dependently increased the percentage of variance accounted for by conditional probability. Also, there was a decrease in the percentage of variance accounted for by foreperiod with KW-6002, such that foreperiod no longer significantly accounted for the variance at any dose of KW-6002.

On the other hand, for PC2 the effect of KW-6002, shown in Figure 3.17 (right panel), reflected amphetamine, significantly reducing the variance accounted for by foreperiod at all doses.

3.3.5 Summary 2

KW-6002 did not generally affect the relationship of reaction time, movement time and anticipatory responding within both principal components. For PC1, in which movement time and anticipatory responding accounted for most of the variance, KW-6002 increased the impact of conditional probability whilst decreasing the impact of foreperiod. For PC2, in which the variance was mostly accounted for by reaction time, KW-6002 reduced the impact of foreperiod. This implies that KW-6002 generally enhanced the effect of conditional probability and reduced the effect of foreperiod.

Similar to KW-6002, amphetamine also reduced the impact of foreperiod in PC2. However, in contrast to KW-6002, for PC1 amphetamine decreased the impact of conditional probability and increased the impact of foreperiod. Also, in further contrast to KW-6002, amphetamine disrupted the relationship of reaction time, movement time and anticipatory responding within both principal components.

3.4 Discussion

The effects of systemic amphetamine and KW-6002 were examined in a reaction time task in which elapsing time and increasing temporal probability were dissociated. It was found that conditional temporal probability of a stimulus and elapsed time before a stimulus exerted differential effects on reaction times in comparison to anticipatory responses and movement times. Reaction times were faster as a function of time elapsed, with probability having less influence. Both amphetamine and KW-6002 resulted in a speeding of mean reaction time, which was additive with the effects of preparation time and conditional probability. Principal components analysis revealed that the effect of conditional probability on mean reaction time was no longer significant following amphetamine and KW-6002.

Mean movement times and anticipatory responses, however, were more strongly influenced by conditional probability than preparation time. This is

supported by the fact that the principals of scalar expectancy theory (Section 1.3.1) are unable to explain the pattern of anticipatory responses, which were generally influenced by the conditional probability of stimuli independent of the foreperiod duration preceding the stimuli. More specifically, the percentage of anticipatory responses was greatest in the group where the 0.4 s foreperiod duration had a conditional probability of 100% compared to 50% or 33% (see Figure 3.7, vehicle data). This is a violation of the assumptions of scalar expectancy theory. Amphetamine decreased the impact of conditional probability on movement times and anticipatory responses and increased the impact of foreperiod, particularly on anticipatory responses. In contrast, KW-6002 increased the effect of conditional probability on mean movement times and anticipatory responses whilst decreasing the effect of preparation time.

Thus, KW-6002 influenced motor preparation activity that is determined by the decreasing uncertainty of the occurrence of the imperative stimulus (increasing instantaneous conditional probability), independent of the amount of preparation time preceding the foreperiod. Moreover, once the response had been initiated, KW-6002 enhanced the effect of the stimulus instantaneous conditional probability on the vigour with which the response was executed (movement time). It is a curious finding that the rate of anticipatory responses and movement times were strongly influenced by conditional probability and enhanced by increasing dose of KW-6002, whereas the drug did not interact with the initiation phase of movement (reaction time). Also, movement time increased as a function of lengthening foreperiod whereas reaction time decreased.

The increase in movement time as a function of lengthening foreperiod may be explained by consideration of principals of associative learning. As foreperiod increases, the value of the reward increases as a function of the amount of time a nose-poke had to be maintained to receive reward. Initially, at the imperative signal withdrawal of the snout was an absolute requirement for reward to be available, in other words the rat simply had to respond (as measured by reaction time). Thereafter, even though the target location was cued a decision as to which side to respond was required in order to receive reward. With greater demands on the inhibition of an anticipatory response at the longer foreperiods, a similar mechanism could be incorporated in validating (as the side was cued) which is the correct side to respond. Thus, the increase in movement time as a

function of lengthening foreperiod might be a consequence of the rat ensuring a response to the correct side after a protracted nose-poke rather than a function of increasing foreperiod duration per se. By extending this notion, perhaps at shorter foreperiods the stimulus (cue light) drives the faster movement times, whereas at longer foreperiods an internally driven signal interrupts the stimulus control over movement time, which arises as a consequence of increasing cost of making an error.

Both drugs enhanced this effect of foreperiod on movement time, thus suggestive of an enhancement of the control of movement time by an internally driven process. This is not surprising in the context of the motor deficits observed in Parkinson's patients, which appears to be an impairment of internally derived (willed) actions. Thus, it seems reasonable that a drug (KW-6002) which counteracts Parkinson's symptoms and a drug that increases dopamine in the striatum (amphetamine) may act to enhance an internal signal involved in the control of action. A further consideration is the lack of effect of amphetamine on reaction time, which was previously shown to enhance the foreperiod-dependent effect on reaction time (Brown et al. 1996).

It might be that the rats' had not formed a suitable representation of the conditional probability of the stimuli in the task. Indeed, a critical period or minimum amount of exposure to a constellation of stimuli may be required for the differential conditional probabilities of the stimuli to be internally represented and expressed in reaction times. If this was not attained then reaction times might have been less sensitive to drug effects. This line of argument suggests that timing properties of stimuli are more readily available than conditional probabilities of stimuli, as in the vehicle days there was a reliable and strong foreperiod-dependent effect on reaction times. A longitudinal study of the effects of elapsed time and conditional probability on responding is required to address this issue and would provide interesting insight into the time course of conditional probability coding.

However, when one considers the effect of amphetamine on modal reaction times this argument becomes less convincing. There was a dose x conditional probability interaction, however these data were not presented here as this appeared to be due to a ceiling effect. The slopes across all groups were flattened at the highest doses, suggesting that rats were performing as fast as they

were likely to at the longer foreperiods and the effect of drug was simply to speed up modal reaction times at the shorter foreperiods to the same optimal speed. If this was the case, then one would expect that a degree of optimum performance was attained and this may suggest that with further training the mean reaction times would also have displayed this pattern thereby not revealing a pattern of responding contingent on the stimulus conditional probability. However, it is outwith the scope of the current data to further evaluate these possibilities.

Nonetheless, KW-6002 enhanced the effect of conditional probability on anticipatory responses and movement times more so than the effect of preparation time *per se*. The effects of systemic KW-6002 (A_{2A} antagonism) on motor activity are largely attributed to the striatopallidal neurons, constituents of the so-called 'indirect pathway'. Dopamine D_2 receptors are also expressed by striatopallidal neurons, with dopamine D_1 receptors expressed predominantly by the striatonigral neurons of the so-called 'direct' pathway. Anatomically, the extent of segregation of these receptors to the respective pathways is contentious. Therefore, the following experiment sought to investigate the functional implications of D_1 and D_2 receptors in motor preparation processes.

Chapter 4

An investigation of spatiotemporal processing in the rat: effects of the dopamine D_1 receptor antagonist SCH-23390 and the dopamine D_2 receptor antagonist raclopride.

- *KW-6002 and amphetamine, drugs that indirectly stimulate dopamine activity, have both been shown to enhance the effect of foreperiod on responding in the rat whereas striatal dopamine depletion has been shown to eradicate the foreperiod-dependent speeding of reaction time. Do drugs that inhibit dopamine activity have any effect on responding as a function of foreperiod?*

- *Do drugs that differentially affect the dopamine D_1 and D_2 receptor subtypes have different effects on motor preparatory processes?*

4.1 Introduction

A modified version of the task used in Chapter 3 was developed that allowed assessment of the effects of conditional temporal probability and conditional spatial probability. The location (spatial probability) of the directional target light changed as a function of the position in the foreperiod (temporal probability) such that early in the foreperiod left targets were more probable than right targets and later in the foreperiod, right targets were more likely. Thus, the spatiotemporal probability was symmetrical through the foreperiod and determined *a priori*.

This manipulation of spatiotemporal stimulus probability could be expected, based on previous literature in humans (Carpenter and Williams 1995; Frith and Done 1986), to affect performance in the task, with responses to more probable stimuli being both faster and more likely to be correct than responses to less likely locations. Similarly, if responses were prepared based on response probability, rightward responses (being more likely later in the foreperiod) would have been more accurate with elapsed time. If attentional allocation occurred, faster reaction times would have been expected to the left target early in the foreperiod and to the right target later in the foreperiod.

Chapters 2 and 3 looked at the effects of drugs that indirectly stimulate dopamine activity on motor preparation. In this experiment, the effects of the D_2- and D_1-receptor antagonists, raclopride and SCH-23390, respectively, were examined in an attempt to dissociate the contributions of the two receptor subtypes (believed to differentially modulate the direct and indirect pathways). Although there would have been interest in comparing the effects of KW-6002 and amphetamine in this task (indeed, this is a study which will require to be done), initially (and in the absence of any other data) I chose to take a wider view. Since KW-6002 and amphetamine stimulate neuronal systems, I decided to focus on the effects of antagonism of the dopamine system in this study to provide a broader overview of the underlying processes involved in motor preparation.

4.2 Materials and methods

4.2.1 Subjects

Twenty four male Lister hooded rats (Harlan U.K), weighing between 333 and 479 grams at the start of testing, were used in this study.

4.2.2 Behavioural task

Rats were trained on the same regime described in Section 3.2.3. The final behavioural protocol differed in the range of foreperiods used and the probability of the target location cues. After 57 sessions, the majority of rats were consistently completing 120 correct trials within 30 m. However, on average 25% of rats had reached an asymptote of 100 correct trials on average. Since there were many possible trial contingencies and some trials were low in number (to achieve the desired probability weighting; see below), the time limit was removed for 5 sessions in attempt to increase the number of correct trials. A further 30 sessions was required for performance to asymptote with an average 100+ correct trials (with a maximum of 120) within 35 m and an average of 25% incorrect trials.

Rats were required to sustain a nose-poke in the centre hole for randomly allocated variable foreperiods of 0.2, 0.3, 0.4, 0.5 or 0.6 s. The probability of the target stimulus occurring to either side was weighted, such that as foreperiod length increased the probability of the target stimulus to the right adjacent hole increased whilst the probability of the target stimulus to the left adjacent hole decreased.

An equal number of foreperiods of different lengths (20 of each foreperiod, equalling 120 trials in total) were randomly distributed, so that temporal conditional probability of the tone increased logarithmically with increasing foreperiod. The spatial conditional probability of the cue lights was also determined by the foreperiod length. Table 4.1 shows the temporal probability of the tone at all foreperiods, with the corresponding spatial probabilities of the cue lights to the left and right target locations. These probabilities were absolute in that they were determined *a priori*. In addition, the combined spatiotemporal (absolute) conditional probability of each event type (i.e., the likelihood of a left target cue occurring at the 0.5 s foreperiod for

example) was also determined *a priori*. However, the actual instantaneous conditional spatial probability was dynamic, with a subsequent target stimulus equally likely to either side at the beginning of the foreperiod becoming more likely to the right side as foreperiod elapsed. Therefore, the conditional spatial probability of the cue signal to the left or right side was recalculated to provide the instantaneous conditional spatial probability of a cue signal at each foreperiod (Table 4.1).

Foreperiod (secs):	0.2	0.3	0.4	0.5	0.6
Temporal probability (tone):	20%	25%	33%	50%	100%
Trial n per session (cue light)					
Left	22	16	12	8	2
Right	2	8	12	16	22
Spatial probability (cue light)					
Left	92%	67%	50%	33%	8%
Right	8%	33%	50%	67%	92%
Spatiotemporal (absolute) conditional probability					
Left	18%	17%	17%	17%	8%
Right	2%	8%	17%	33%	92%
Instantaneous conditional spatial probability					
Left	50%	40%	31%	21%	8%
Right	50%	60%	69%	79%	92%

Table 4.1 The relative probabilities of the tone and the target lights. The probability of the tone follows a temporal pattern which rises logarithmically, shown in the table as the 'temporal probability (tone)'. The probability of the cue light occurring to either side at any given foreperiod ('spatial probability') was weighted a priori such that as foreperiod increased, the probability of the cue light appearing to the left decreased, whereas the probability of the cue light to the right increased. The 'spatiotemporal (absolute) conditional probability' of the imperative stimulus was also determined a priori and represents the combined spatial and temporal probabilities of the imperative stimulus. The 'instantaneous conditional spatial probability' of the target lights is the likelihood of which side the target light will occur as time advances through the foreperiod (at the first foreperiod (0.2 s), the target light is equally likely to occur to either side, with the probabilities diverging dynamically through the foreperiod).

Thus, the target cue probabilities can be calculated and interpreted in different ways and it was the focus of this experiment to investigate which

constellation of probabilities were best reflected in the rats performance and whether these were affected by raclopride or SCH-23390. In order to best understand the rat's performance, models of predicted behaviour were developed based on the above probability calculations.

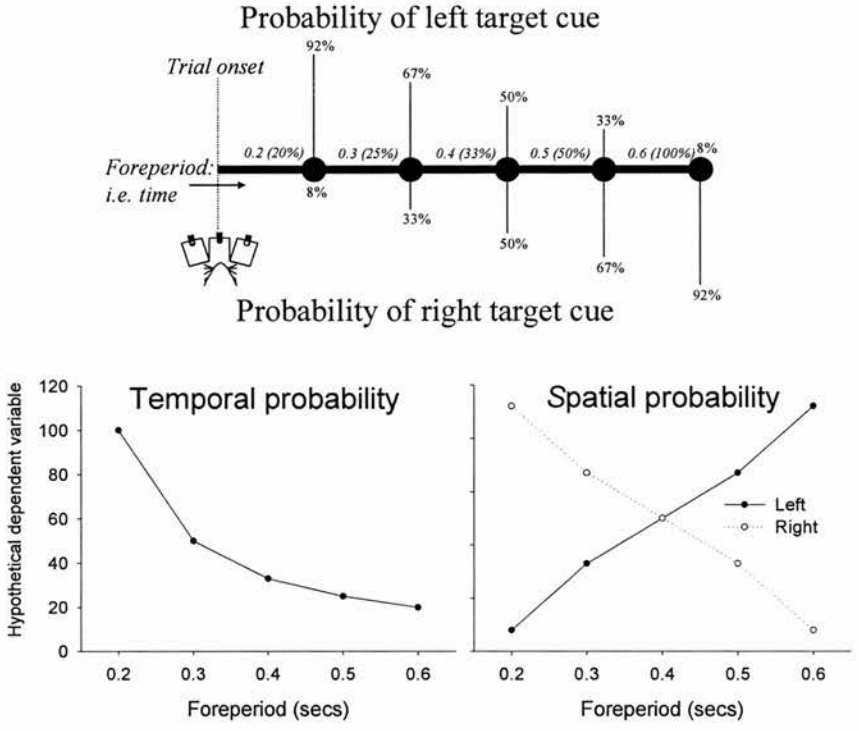

Figure 4.1 The upper diagram is a schematic illustration showing the temporal and spatial probabilities of the target cues to the left and right target locations, which were determined a priori. The lower graphs are examples of predicted behaviour contingent on the probabilities shown in the upper diagram. The data are plotted as a function of lengthening foreperiod. In this example, the hypothetical dependent variable could represent reaction time, which would be expected to decrease with increasing stimulus probability. The left graph shows the expected pattern of effect if foreperiod length (temporal probability) affected the hypothetical variable/reaction time, independent of an effect of the spatial probabilities. The graph on the right shows the expected effect if the spatial probabilities determined performance.

The upper part of Figure 4.1 is a schematic illustration of the temporal and spatial probabilities of the target cues to the left or right at each given foreperiod, as determined *a priori* ('temporal probability' and 'spatial

probability' in Table 4.1, respectively). The lower part of Figure 4.1 shows corresponding graphs of predicted behaviour determined by these probabilities. The hypothetical variable in these graphs may be reaction time for instance, which would be predicted to decrease with increasing stimulus probability. Figure 4.2 shows the probabilities and predicted behaviour if the spatiotemporal (absolute) conditional probabilities determined behaviour.

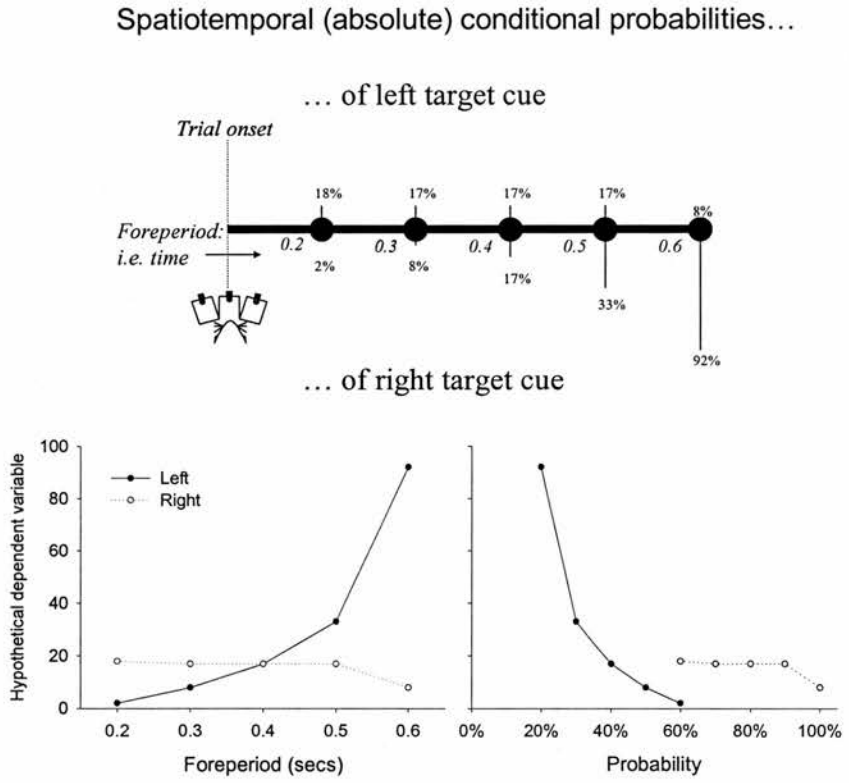

Figure 4.2 *The upper diagram is a schematic illustration showing the spatiotemporal (absolute) conditional probabilities of the target cues to the left and right target locations, which were determined a priori. The lower graphs are examples of predicted behaviour (i.e., reaction time, which is predicted to decrease with increasing spatiotemporal probability) contingent on the spatiotemporal probabilities. The data are plotted for each target location as a function of foreperiod length (left graph) and spatiotemporal probability (right graph).*

Thus, both Figure 4.1 and 4.2 show models of behaviour that reflect the *a priori* probability constellations. As these probabilities are determined *a priori*, such patterns of behaviour would indicate that a representation of these probabilities had been hard-wired (through training) and guided the rat's

behaviour through an awareness of future events. Furthermore, if performance reflected the spatial probabilities, as shown in the graph on the right in Figure 4.1, this would suggest that no information contributing to performance is unfolding through time but rather that time is being used as a tag or cue to indicate the more probable spatial location, thus focussing attention. On the other hand, Figure 4.3 shows a predicted model of performance that would be expected if the rat was capable of dynamically coding the probabilities of the target cue lights unfolding throughout the foreperiod. This pattern of performance would suggest a dynamic attentional allocation that incorporates temporal and spatial information into motor preparation.

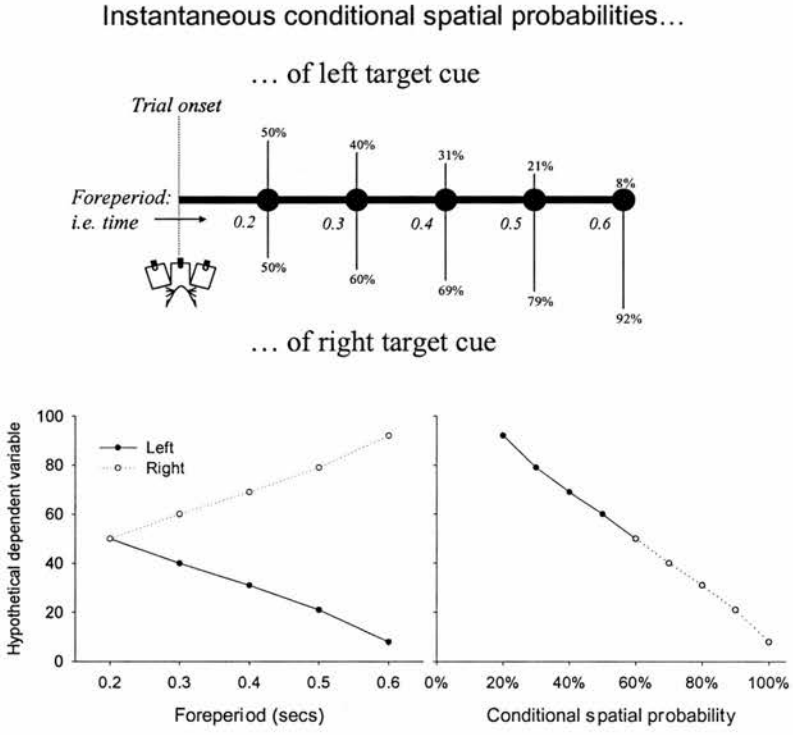

Figure 4.3 The upper diagram is a schematic illustration showing the instantaneous conditional spatial probabilities of the target cues to the left and right target locations, which changed dynamically throughout the aging foreperiod. The lower graphs are examples of predicted behaviour (i.e., reaction time, which is predicted to decrease with increasing probability). The data are plotted for each target location as a function of foreperiod length (left graph) and probability (right graph).

4.2.4 Drug administration

Rats were split into two groups, one group received one of each dose of raclopride (and vehicle) and the other received one of each dose of SCH-23390 (and vehicle) in a pseudo-counterbalanced dosing schedule over four days, with a drug free day in between each drug day. After the drug-free day following the complete regime on one drug, each group received the alternate drug by the same protocol.

4.2.5 Data analysis

There were 11 rats with complete datasets from raclopride testing days and 8 from SCH-23390 testing days. The data for each drug were analysed separately with repeated measures ANOVA with dose, side and foreperiod as within-subject factors.

4.3 Results

4.3.1 Effects of raclopride

When the incorrect and anticipatory responses comprised 25% of responses on average, baseline data was collected for five weeks and assessed. There was no significant difference in any dependent variable across the five weeks so drug testing commenced thereafter.

To assess if performance returned to baseline levels two days following a drug dose (i.e., on the day of subsequent drug testing), vehicle data from the drug testing days was compared with the pre-testing baseline data. Although mean reaction times were generally faster in the vehicle data compared to the baseline data (main effect of condition; $F_{(1, 10)} = 6.9$, $p = 0.025$), there was no significant difference in the effects of foreperiod (condition x foreperiod interaction; $F_{(4, 40)} = 2.51$, ns) or side (condition x side interaction; $F_{(1, 10)} = 0.04$, ns) on the baseline or vehicle data. Also, from the vehicle data on drug testing days there were generally more correct responses compared to the pre-testing baseline data (main effect of condition; $F_{(1, 10)} = 12.12$, $p = 0.006$), with no difference in the effects of foreperiod (condition x foreperiod interaction; $F_{(4, 40)} = 0.74$, ns) or side (condition x side interaction; $F_{(1, 10)} = 2.39$, ns) between the baseline and vehicle data. Therefore, the effect of side and foreperiod on the vehicle data was not

significantly different from the baseline data, indicating that 48 hrs was an adequate washout period for raclopride, at least in terms of the behavioural parameters of interest in this task.

4.3.1.1 Anticipatory responses

Anticipatory responses increased as a function of lengthening foreperiod (main effect of foreperiod; $F_{(4, 40)} = 13.77$, $p < 0.001$). Raclopride decreased the percentage of anticipatory responses (main effect of dose; $F_{(3, 30)} = 7.47$, $p = 0.001$). This effect was greatest at the longest foreperiods and Figure 4.4 shows that with increasing dose of raclopride anticipatory responding no longer increased as a function of increasing foreperiod (dose x foreperiod interaction; $F_{(12, 120)} = 3.30$, $p = 0.002$).

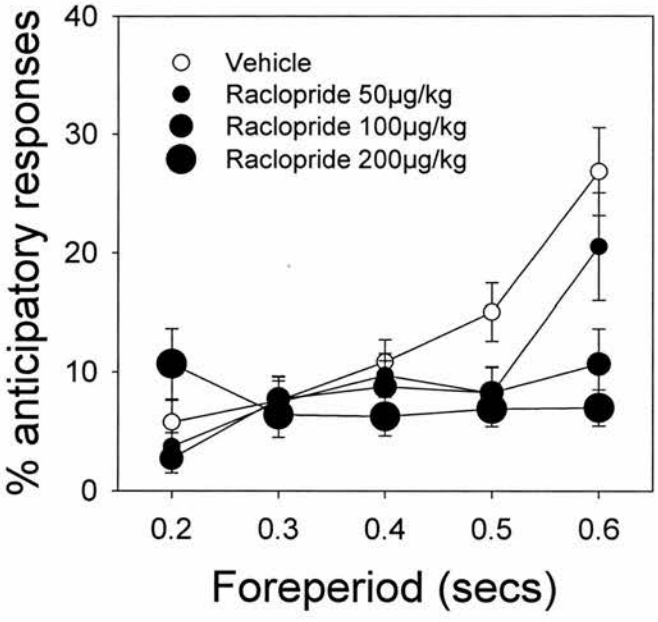

Figure 4.4 Effect of raclopride on the percentage of anticipatory responses at each foreperiod. Raclopride decreased anticipatory responding at the longest foreperiods.

4.3.1.2 Incorrect errors and omissions

The percentage of incorrect errors was not significantly affected by foreperiod or raclopride. Foreperiod did not have a significant effect on the percentage of omissions, which were increased by raclopride (main effect of dose; $F_{(3, 30)} = 10.25$, $p < 0.001$) at all foreperiods.

4.3.1.3 Mean reaction time

Figure 4.5 shows that mean reaction times increased with increasing dose of raclopride (main effect of dose; $F_{(3, 30)} = 37.63$, $p < 0.001$). Mean reaction times decreased as a function of increasing foreperiod (main effect of foreperiod; $F_{(4, 40)} = 27.84$, $p < 0.001$). Figure 4.6 shows that raclopride increased mean reaction times more so at the shortest foreperiods (dose x foreperiod interaction; $F_{(12, 120)} = 2.32$, $p = 0.003$). Thus, although raclopride slowed mean reaction times, the foreperiod dependent speeding of reaction time remained intact and was more pronounced following raclopride. This effect was not significantly different between both target locations (dose x side x foreperiod interaction; $F_{(12, 120)} = 1.23$, ns).

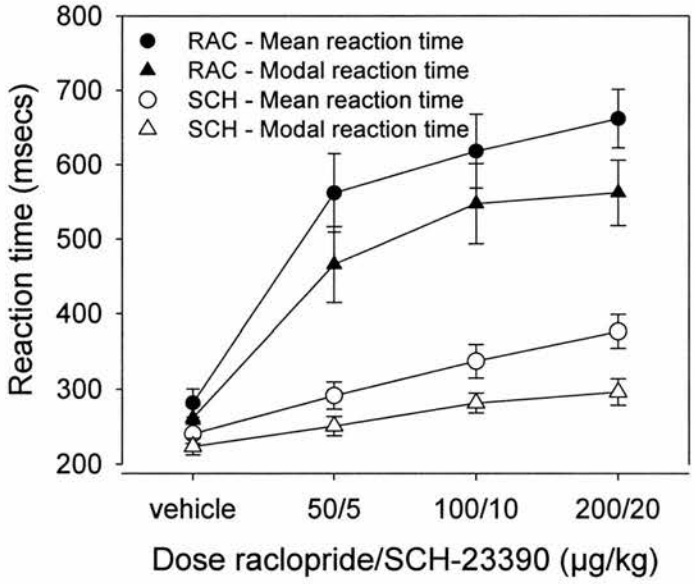

Figure 4.5 This shows the effect of increasing dose of raclopride (black circles) and SCH-23390 (open circles) on mean and modal reaction times. Raclopride and SCH-23390 dose-dependently increased mean and modal reaction times.

4.3.1.4 Modal reaction time

As foreperiod increased, modal reaction time decreased (main effect of foreperiod; $F_{(4, 40)} = 30.15$, $p < 0.001$). The inset in the top graph in Figure 4.7 showing the vehicle data shows that the effect of foreperiod was greatest when the target location was to the right (side x foreperiod interaction; $F_{(4, 40)} = 3.09$, $p = 0.032$), indicating that increasing spatial probability had a greater effect on modal reaction times than decreasing spatial probability (as was the case to the

left side). Figure 4.7 also shows that raclopride increased modal reaction times (main effect of dose; $F_{(3, 30)} = 18.05$, $p < 0.001$) with an increase in the effect of foreperiod (dose x foreperiod interaction; $F_{(12, 120)} = 2.53$, $p = 0.034$) for both sides (dose x side x foreperiod interaction; $F_{(12, 120)} = 1.25$, ns).

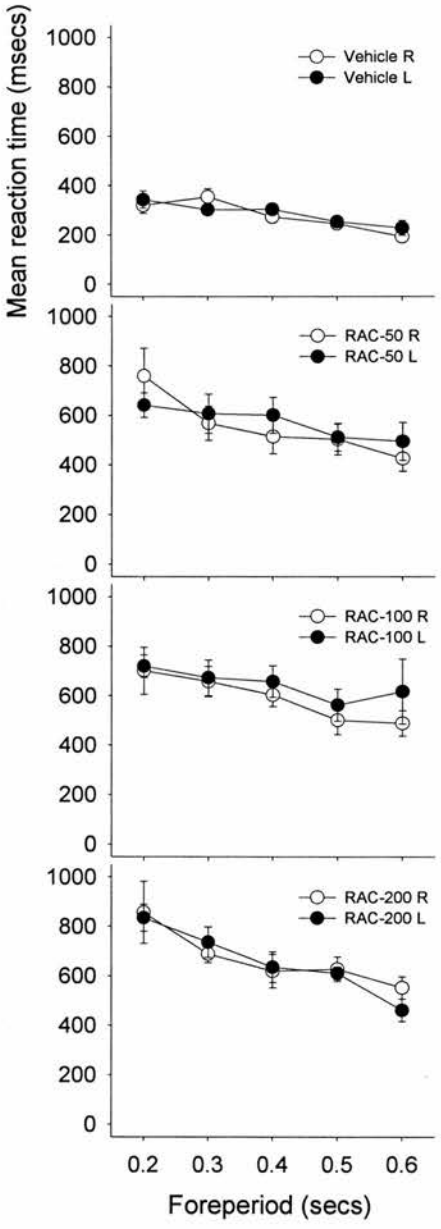

Figure 4.6 Effect of increasing dose of raclopride on mean reaction time at each foreperiod to the left (L) and right (R) target locations. Increasing dose of raclopride decreased mean reaction times and enhanced the foreperiod-dependent speeding of reaction time. (Legend shows the dose of raclopride (RAC) in μg/kg).

4.3.1.5 Mean movement time

There was no significant effect of foreperiod or raclopride on mean movement times.

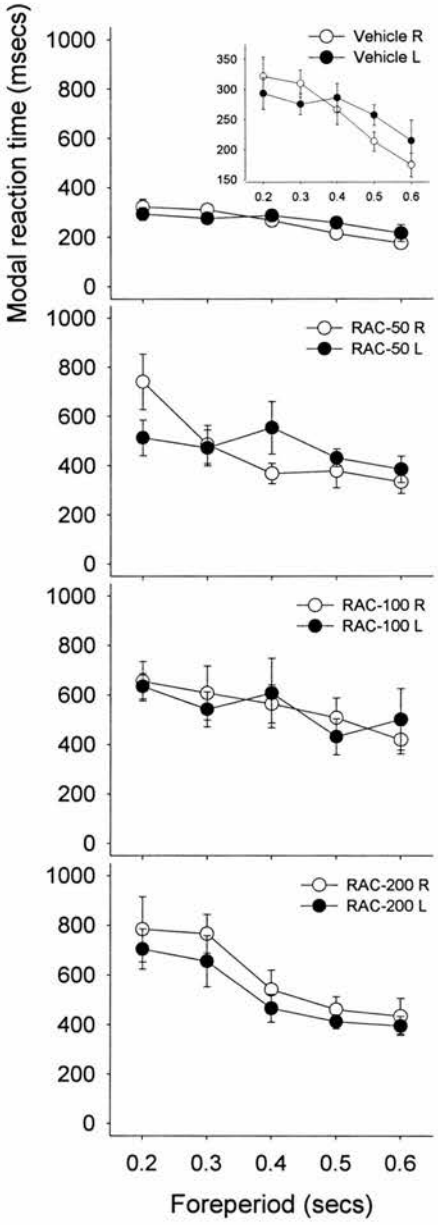

Figure 4.7 *Effect of raclopride on modal reaction times at each foreperiod to the left (L) and right (R) target locations. Inset shows an expanded y-axis scale for the vehicle data. In the vehicle condition, modal reaction times decreased with increasing foreperiod. Foreperiod had a greater effect on modal reaction times to the right target location. Raclopride slowed modal reaction times and enhanced the effect of foreperiod. This enhancement was not significantly different to either side of target location.*

4.3.2 Effects of SCH-23390

The vehicle data from the SCH-23390 testing days was compared to the pre-drug testing baseline data. There was no significant difference in the effect of foreperiod or side on mean reaction time or the percentage of correct responses (data not shown).

4.3.2.1 Anticipatory responses

In contrast to raclopride, SCH-23390 did not generally decrease anticipatory responses (main effect of dose; $F_{(3, 24)} = 0.51$, ns). However, as Figure 4.8 shows, SCH-23390 did decrease anticipatory responses at the longest foreperiod (dose x foreperiod interaction; $F_{(12, 96)} = 3.1$, $p = 0.002$).

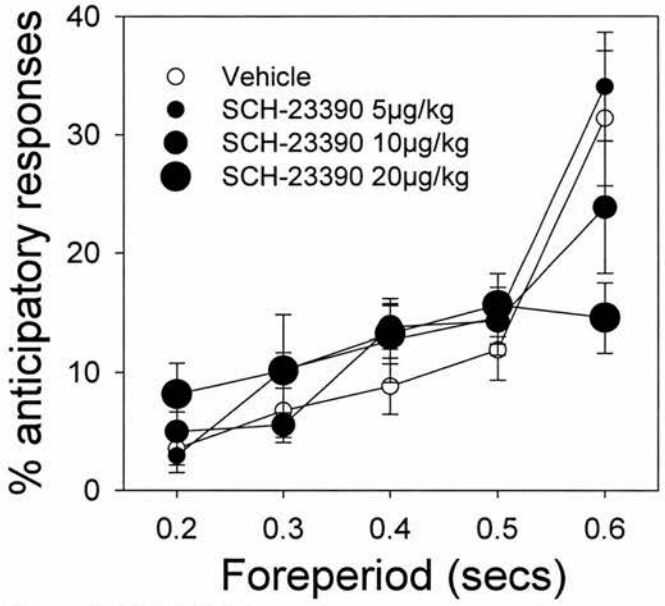

Figure 4.8 Effect of SCH-23390 on the percentage of anticipatory responses at each foreperiod. SCH-23390 decreased anticipatory responding at the longest foreperiod.

4.3.2.2 Incorrect errors and omissions

Figure 4.9 shows that the percentage of incorrect errors were greatest to the right side at the longest foreperiods (side x foreperiod interaction; $F_{(4, 32)} = 7.54$, $p = 0.001$), an effect that was no longer present after SCH-23390 administration (dose x side x foreperiod interaction; $F_{(12, 96)} = 1.92$, $p = 0.048$). There was a tendency towards an increase in omissions by SCH-23390, an effect which approached significance (main effect of dose; $F_{(3, 24)} = 2.85$, $p = 0.059$).

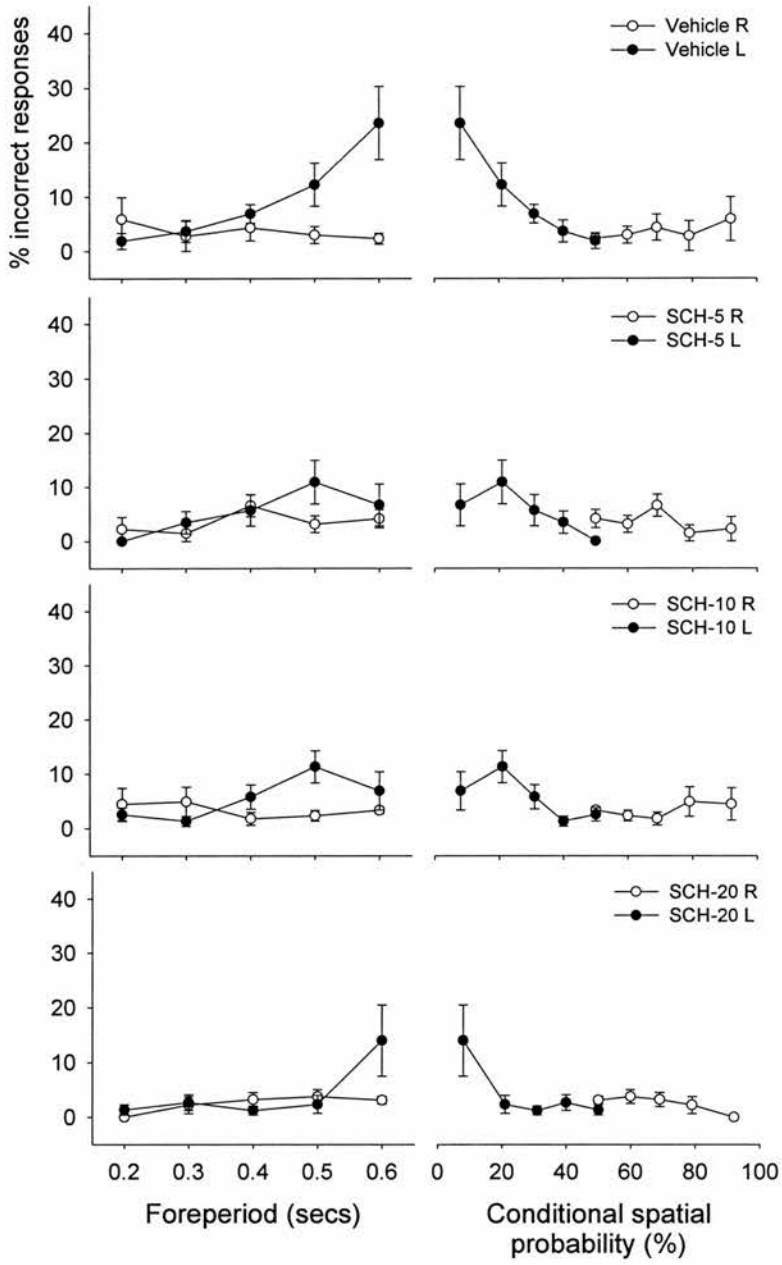

Figure 4.9 Effect of SCH-23390 on the percentage of incorrect responses at each foreperiod (left panels) and plotted as a function of the instantaneous conditional spatial probability (right panels). Incorrect responses were greatest at the longer foreperiods when rats were required to respond to the left target location. The instantaneous conditional spatial probability of a cue to the left was low later in the foreperiod. SCH-23390 decreased the percentage of incorrect responses at the longest foreperiods to the side (left) with the lowest probability.

4.3.2.3 Mean and modal reaction times

Figure 4.5 shows that SCH-23390 increased mean and modal reaction times (main effect of dose; $F_{(3, 24)} = 13.51$, $p < 0.001$; $F_{(3, 24)} = 7.48$, $p = 0.001$), an effect which was the same across all foreperiods for each side (dose x side x foreperiod interaction; $F_{(12, 96)} = 0.89$, ns; $F_{(12, 96)} = 1.12$, ns).

4.3.2.4 Mean movement time

There was no significant effect of SCH-23390 on mean movement times.

4.4 Discussion

In concordance with Chapters 2 and 3, reaction times were faster as a function of lengthening foreperiod. In addition, modal reaction times were faster to the left target early in the foreperiod and to the right target later in the foreperiod. The instantaneous probability of a target cue to the right increased as foreperiod lengthened and decreased to the left. The pattern observed in the modal reaction times suggests that this information was incorporated when the rats' prepared a response, suggestive of an attentional allocation to the most probable target location as determined by the temporal position in the foreperiod. Both raclopride and SCH-23390 decreased reaction times (mean and mode) to both target locations.

However, only raclopride interacted with the effect of foreperiod on reaction times in a rather unexpected manner. Although reaction times were slower, raclopride facilitated the effects of foreperiod, with the greatest foreperiod-dependent speeding of reaction times most apparent at the highest dose of raclopride. Both drugs also decreased anticipatory responding at the longer foreperiods.

Thus, raclopride decreased the foreperiod-dependent effect on anticipatory responses yet facilitated the foreperiod-dependent effect on reaction times. This suggests that after raclopride rats were more likely to withhold a response until the imperative signal and when the imperative signal sounded rats were still able to utilise the information from the foreperiod to produce a response, albeit generally slower. Moreover, although there was no significant interaction with the effect of raclopride and target location on reaction times, in the modal reaction times at the highest dose of raclopride it appeared as though

rats were responding equally to both sides regardless of the divergent spatial probability of the target locations. However, this effect was not significant so it was not possible to conclude that processing of the spatial probability of the target cues was affected by raclopride. In the analysis of the effects of raclopride there was no significant effect of any factor on incorrect responses.

On the other hand, in the SCH-23390 analysis more incorrect responses were made when a response to the left side was required. The instantaneous conditional probability of a target cue decreased to the left as foreperiod lengthened which resulted in more incorrect responses (i.e., responses to the right side when the cue appeared to the left). This suggests that a mnemonic representation of the spatiotemporal (absolute) probability was affecting response preparation and (incorrect) response inhibition. SCH-23390 removed the increase in incorrect responses that occurred later in the foreperiod, suggesting that there was a reduction in response preparation bias to the more probable side. A lack of effect of raclopride on incorrect errors must be interpreted with caution as the increase in incorrect errors to the right target was not observed in the raclopride data and may have confounded any significant effect of raclopride on this measure.

In conclusion, during a temporal delay when rats are preparing a motor response, additional information, such as the probability of the spatial location of a target cue, may also be incorporated into the response preparation process. Moreover, in the current task this information could only be processed within the context of the temporal information provided dynamically by the delay. This is supported by the rats behaviour (modal reaction times and incorrect responses), which reflected the divergent probabilistic properties of the target cues. These findings suggest that rats are capable of incorporating complex temporal and spatial information into response preparation. It is likely that the dopamine system plays a role in mediating these processes; blockade of D_2 receptors enhanced the effect of foreperiod on reaction times and blockade of D_1 receptors reduced the percentage of incorrect responses to the least probable target location.

Chapter 5

The effect of striatal dopamine depletion and KW-6002 on reversal learning in rats

- *Striatal dopamine depletion has been shown to impair reversal learning in the rat. KW-6002 reverses motor impairments that result from depletion of striatal dopamine. Does chronic KW-6002 ameliorate the cognitive flexibility deficits, as well as the motor deficits, that result from striatal dopamine loss?*

5.1 Introduction

KW-6002 is a likely candidate as an adjunctive drug therapy to dopamine replacement in the treatment of Parkinson's disease (Bara-Jimenez et al. 2003; Hauser et al. 2003). KW-6002 ameliorates the motor symptoms in animals with striatal dopamine depletion (Grondin et al. 1999; Kanda et al. 2000; Lundblad et al. 2003; Shiozaki et al. 1999), yet the influence of the compound on 'cognitive' functions, both in isolation and in conjunction with striatal dopamine depletion, remains untested, with studies to date of the effects of KW-6002 restricted to motor functions, with the interest focused on the potential to reduce the required dosage of L-DOPA and consequently reducing the incidence of dyskinesia (Bara-Jimenez et al. 2003). This is surprising considering that, in addition to dyskinesia, cognitive impairments are both a consequence of Parkinson's disease and a complication of dopamine replacement therapy (Cools et al. 2001; Gotham et al. 1988; Swainson et al. 2000).

Furthermore, the medial region of the striatum in the rat is implicated in cognitive functions (see Voorn et al. 2004). Previous work has shown that medial striatal dopamine depletion results in impaired reversal learning in the rat (J.M. Phillips, A.D. Blackwell and V.J. Brown, unpublished observations). Thus, the present study was designed to examine the role of KW-6002 and its interaction with dopamine in the medial striatum in cognitive flexibility. In the present study, the task was modified to permit examination of the effects of chronic KW-6002 on reversal learning (so-called 'affective shifts'; Dias et al. (1996)) as well as attentional set-shifting (so-called 'attentional shifts'; Dias et al. (1996)). Rats were trained to dig in texture-covered bowls of scented digging media. Rats are able to learn to retrieve food bait from digging bowls according to the specific texture on the bowls outside surface, the digging medium within the bowl or the odour of the bowl.

The test comprised four stages, conducted on different days. Initially, rats were exposed to baited bowls overnight ('familiarization'), and were then given a series of three simple discriminations – one for each of 3 perceptual discriminations: odour, digging medium and the texture covering the bowl – followed by reversals of

these ('training stage'). The next stage (reversal learning: 'SD/REV') consistent of expose to three novel simple discriminations and each discrimination was followed by a reversal. The final stage ('set-shifting') followed the protocol described in Birrell and Brown (2000). Novel stimuli were used in each block so as the rats were never exposed to the same stimulus twice.

The advantage of using this task is that it is self-paced and does not involve a high degree of motor coordination. Analogous to the Wisconsin Card Sorting Test, it is formally the same as the ID/ED task used extensively in monkeys to explore the neural basis of attentional flexibility (see Dias et al. 1996).

The contribution of striatal dopamine and of KW-6002 to performance of affective and attentional shifts and the possibility that chronic KW-6002 would ameliorate reversal deficits resulting from striatal dopamine depletion, was the focus of interest.

5.2 Materials and Methods

5.2.1 Subjects

Thirty six male Lister hooded rats (Harlan, U.K), weighing between 310 and 590 g at the start of testing, were initially pair-housed on a 12 h light/dark cycle (lights on at 7am). Post-surgery the rats were single-housed. Best efforts were made for testing to be carried out in the light phase; however due to the unconstrained nature of the timing of the behavioural task, testing of the final rat did, on occasion enter the dark phase. This generally occurred in the first block when rats were being habituated to the task. Rats were maintained on a restricted diet of 15-20 g of lab chow per day and additional honey loops (Kellogg's, U.K), which served as reinforcement, consumed during testing with water freely available in the home cage.

5.2.2 Surgery

Rats were pretreated with the monoamine oxidase inhibitor, pargyline (50mg/kg; Sigma-Aldrich, Dorset, UK) intraperitoneally 30 m prior to surgery and carprofen (Rimadyl™, 0.1ml/kg; 5% w/v; Pfizer Ltd., Kent, UK) subcutaneously.

Anaesthesia was induced with Sagatal (60mg/kg pentobarbitone sodium BP; Rhône Mérieux Ltd, Essex, UK) intraperitoneally. Bilateral lesions of medial portion of the striatum were made by injection of 6-hydroxydopamine (6-OHDA; Sigma-Aldrich, Dorset, UK) 2.5 mm anterior to Bregma, 1.8 mm medial and lateral to the midline and 4.0 mm below skull surface, with the nosebar set at +5.0 mm, using a cone-tipped 5.0 µl Hamilton (SGE) syringe. 6-OHDA was administered in a vehicle of sterile phosphate buffered saline at a volume of 2 µl/site. Injections were made at a rate of 0.1 µl/10sec and left *in situ* for a further 3 min. Control rats received equivalent injections of vehicle only, at the same co-ordinates. Skin incisions were cleaned and closed using sterile metal clips. Three days were allowed for post-operative recovery before proceeding with drug administration.

Of the original 36 rats, 2 were excluded as a result of post-surgical complications; following histology, 7 were excluded as having either unilateral or asymmetric lesions. Thus, the 25 rats in the final analysis comprised 11 sham-operated and 14 rats with lesions. With these groups, 5 controls and 8 lesion rats received vehicle and 6 controls and 6 lesion rats received KW-6002. Note that a lesion rat that received KW-6002 did not complete the task in the SD/REV testing, rendering this group one rat fewer for the SD/REV analysis.

5.2.3 Drug administration

KW-6002 (1 mg/kg) was delivered bi-daily (morning and late afternoon) for a total of 15 days plus one dose on the sixteenth day prior to the final set-shifting task. On habituation and testing days, the first dose was always given at least 2 hours before testing with the second dose administered within 5-8 hours. The dosing regime was staggered accordingly on testing days, as it was impossible to determine the amount of time any given rat would take to complete the task. Vehicle (1% methyl cellulose at 1 ml/kg) dosing followed the same regime.

	DAY	
	1	**Lesion surgery**
Sham surgery	2	
	3	
	4	Begin chronic drug administration
Begin chronic drug administration	5	
	6	
	7	
	8	
	9	SD/REV (i.e. after 5 days drug admin)
SD/REV (i.e. after 5 days drug admin)	10	
	11	
	12	
	13	
	14	SD/REV (i.e. after 10 days drug admin)
SD/REV (i.e. after 10 days drug admin)	15	
	16	
	17	
	18	
	19	ID/ED (i.e. after 15 days drug admin)
ID/ED (ie after 15 days drug admin)	20	

Table 5.1 Timeline of the experimental procedures, which was repeated until a suitable amount of successfully lesioned animals were available for analysis. Chronic drug administration (either vehicle or KW-6002) commenced 3 days after surgery. Simple discrimination (SD/REV) testing was carried out after 5 and 10 days of chronic drug administration. Set-shifting (ID/ED) testing was carried out after 15 days of chronic drug administration.

5.2.4 Behavioural procedure

Simple discrimination and reversal (SD/REV) testing

A protocol was designed to test the ability of lesioned rats on reversal learning in particular, with chronic drug administration (see Table 5.1 for an outline of the overall experimental regime). Note that due to incomplete or insignificant lesions identified post-histology (hence, after testing) and also the nature of the task (a maximum of 3 rats could be tested per day), the full protocol of testing was repeated 5 times over a period of 32 weeks to obtain reasonable group sizes for analysis.

At the familiarization stage, which occurred after 5 days of drug administration and the day before the training stage, the rat was provided with a bowl (the same type used in the task) filled with homecage-type sawdust and 6 honey loops with 4 buried in the sawdust and 2 exposed on the surface. The bowl was left in the rat's homecage overnight for the rat to investigate – all loops were always consumed by the following morning.

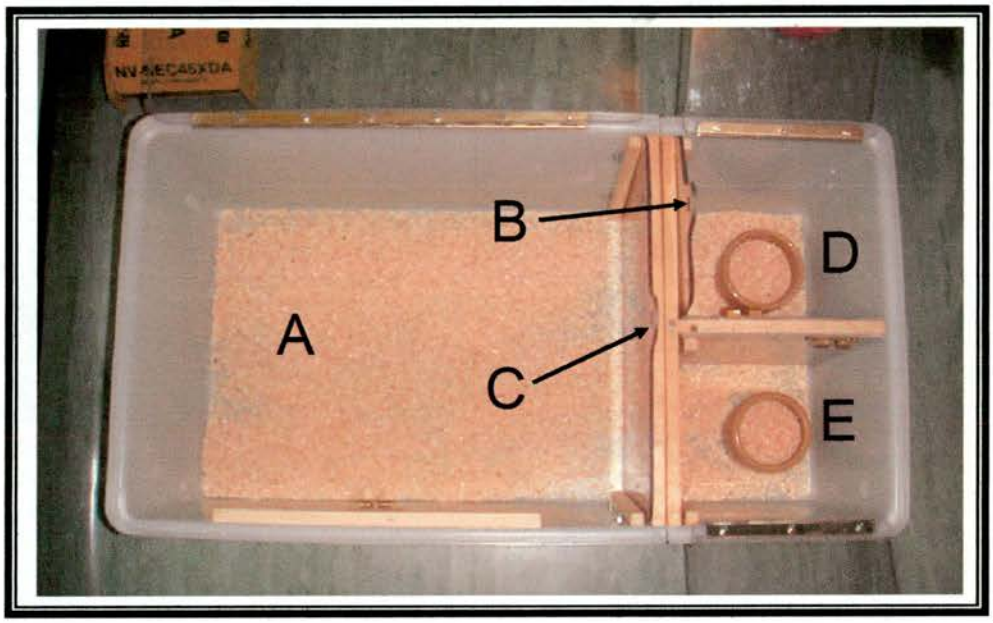

Figure 5.1 The set shifting testing box. The rat was placed in the open chamber (A) at the beginning of the session with the divider (C) in place. Digging bowls were then placed in the closed chambers (D and E). The divider was raised allowing access to both digging bowls. During testing, once the rat began to dig in one of the bowls the single divider (B) was placed over the chamber to the other digging bowl, blocking access. The rat was freely permitted to dig and the full divider was only put in place once the rat had left the chamber, signifying the end of the trial. Digging bowls were then removed and re-baited accordingly.

On the following day, the rat was placed in the set shifting testing box. Two bowls were filled with homecage-style sawdust and a single honey loop buried in the sawdust of each bowl (Figure 5.1). The bowls were placed in the closed off sections of the set shifting testing box and the rat was given access to them by removal of the partition. The rat was left unattended for 10 min to retrieve the two honey loops

from the bowls. If neither or only one of the honey loops was retrieved then the rat was left for a further 10 min, this was repeated until both the honey loops had been retrieved and continued until the rat had retrieved 12 honey loops. If after a prolonged period, about 40 – 60 min, it appeared as though the rat was unlikely to attempt a dig, the reward was semi-exposed. This was never done during testing (to avoid manipulation of the experimental conditions and undesirable auto-shaping of the rats' behaviour). Once completed the rat was exposed to 3 simple discriminations with exemplars from a medium, odour and texture dimension (see Table 5.2 for exemplars used). Each simple discrimination was followed by a reversal of the relevant (positively reinforced) exemplar from the preceding simple discrimination, i.e., a reversal of the reward contingency. SD/REV testing followed the same discrimination protocol without the initial habituation stage and was carried out following 10 days of drug administration.

Dimension	Exemplars			
	Digging training		SD/REV	
1) Odour	Thyme	Paprika	Mint	Oregano
2) Medium	Pebbles	Wooden beads	Sawdust	Wood shavings
3) Texture	Smooth sandpaper	Coarse sandpaper	Smooth velvet	Coarse velvet

Table 5.2 Exemplars used during digging training and SD/REV.

A simple discrimination is as follows. The rat was in the testing box and, for example, odour was the dimension for the first simple discrimination. The order of the dimensions and positively reinforced exemplars was randomly allocated across rats within the 4 experimental groups (control and vehicle, control and KW-6002, lesion and vehicle, lesion and KW-6002) and replicated between the groups (see Table 5.3). Thus, thyme- and paprika-scented sawdust may be the two exemplars. Half a honey loop was buried in the bowl containing the positive (reinforced) exemplar, for example thyme as the positive exemplar. The two bowls were placed

in the closed off section of the box. The rat was given access to the bowls by removal of the partition and the stopwatch was started.

Dimension Order

Block1 Habituation (after 5 days drug admin)	Block 2 SD/REV (after 10 days drug admin)	Block 3 Set shifting (after 15 days drug admin)
O-M-T	T-M-O	O-M
T-O-M	M-O-T	M-O
M-T-O	O-T-M	T-O
T-M-O	O-M-T	O-T
M-O-T	T-O-M	M-T
O-T-M	M-T-O	T-M

Table 5.3 The order of the dimensions presented. Each row represents the order of presentation for each individual rat within each group across each phase. O – odour; M – medium; T – texture.

During the first 4 trials of each new discrimination, if the rat dug in the incorrect bowl it was allowed to dig subsequently in the correct bowl. Thus the first 4 trials constitute a discovery period and the rat always retrieved the bait in these trials. After the first 4 trials, as soon as the rat started to dig in either bowl, the partition to the other bowl was lowered. Thus if the rat had selected the incorrect bowl, he was not permitted to gain access to the correct bowl subsequently. He was allowed to dig in the selected bowl until he moved away from the bowl. The rat had up to 10 min in total to dig per trial. If after this time the rat had not dug in either bowl, the trial was marked as a fail. If the rat failed 3 times in one stage, then the task was aborted. The rat was considered to have attained criterion performance when it dug correctly 6 times consecutively (including the initial 4 such that it was possible for the rat to achieve criterion in only 6 trials).

Set-shifting protocol

Within the SD/REV, rats were required to learn the correct exemplar and then switch responding from that to a previously incorrect exemplar (rule reversal).

The final set-shifting protocol corresponded to that of Birrell and Brown (2000). Testing, conducted in a single session, consisted of a series of two-choice discriminations of bowls that differed on multiple dimensions. Only one of the dimensions was relevant for making the discrimination of the correct bowl at each stage within the test. After a series of discriminations (simple, compound, a reversal, acquisition of new exemplars and another reversal) based on responding to one perceptual dimension, the relevant dimension was changed and the rat had to learn a new discrimination (with novel exemplars) based on the previously irrelevant dimension. For example, if the initial discriminations were based on the odour of the bowl, the new discrimination was based on digging medium or vice versa. Subjects were randomly assigned to a particular shift type prior to testing (see Table 5.3). Each discrimination required subjects to learn which of two exemplars from any given dimension was positively correlated with reinforcement. Table 5.4 shows the stimuli used during testing. Exemplars were always presented in pairs. For example basil and rosemary were always presented together in either sand or gravel digging medium, or with vinyl paper or matt paper covering the digging bowl in a neutral digging medium (homecage-style sawdust).

	Exemplars					
	Pair 1		Pair 2		Pair 3	
Dimensions:	1	2	3	4	5	6
Odour	Basil	Rosemary	Cinnamon	Cumin	Nutmeg	Cloves
Medium	Sand	Gravel	Shredded paper	Polystyrene	Cork	Smooth pebbles
Texture	Vinyl paper	Matt Paper	Bubble wrap	Plastic	Rubber	Masking tape

Table 5.4 Exemplars used for set-shifting testing in block 3. Exemplars labelled 1 and 2 are always paired together, as are 3 and 4, and 5 and 6.

The order in which pairs of exemplars were presented was pseudo-randomized *a-priori* (based on a Latin square design), such that no two rats in the

same group received the same combination/order of exemplars. Furthermore, the relevance of perceptual dimension has been extensively studied previously (Birrell and Brown, 2000) and the authors showed that performance at each stage in the task was not differentially affected by the nature of the dimension used (odour, texture or medium) in the initial acquisition and reversal stages and the extradimensional shift (for example, odour-medium or texture-medium). Therefore, in this task the order and type of dimension used were also pseudorandomized *a-priori*, such that no two rats in the same group received the same dimension type/switch (i.e., they may have received the same dimensions but the order would have been reversed).

Discrimination	Relevant dimension	Irrelevant dimension	Illustrative exemplar combinations
SD	Odour	-	**O1** + O2
CD	Odour	Medium	**O1**/M1 + O2/M2 OR O2/M1 + **O1**/M2
Rev1	Odour	Medium	O1/M1 + **O2**/M2 OR **O2**/M1 + O1/M2
ID	Odour	Medium	**O3**/M4 + O4/M3 OR O4/M4 + **O3**/M3
Rev2	Odour	Medium	O3/M4 + **O4**/M3 OR **O4**/M4 + O3/M3
ED	Medium	Odour	**M5**/O6 + M6/O5 OR M6/O6 + **M5**/O5
Rev3	Medium	Odour	M5/O6 + **M6**/O5 OR **M6**/O6 + M5/O5

Table 5.5 The order of the discriminations and examples of the positive (shown in bold) and negative exemplars (Based on Birrell and Brown, 2000).

To summarize the procedure: The rat was required to perform 7 discriminations/shifts. First, a simple discrimination (SD) that, as before, required the rat to discriminate between two exemplars from a single perceptual dimension.

This was followed by a compound discrimination (CD), where the rewarded exemplar remained the same, but a second (irrelevant) perceptual dimension was added. This was followed by a reversal (Rev 1) where the attended dimension remained the same but the correct and incorrect exemplars were reversed. During reversals, the rat not only had to maintain an attentional set to a particular dimension, but also had to learn and unlearn specific stimulus-reward associations. There was then a total change of exemplars, but the correct attended dimension remained the same (an intradimensional shift, ID), followed by another reversal (Rev 2) where correct and incorrect exemplars are reversed. Following another total change of exemplars, the previously incorrect dimension now became correct, an extradimensional (ED) shift. This was followed by a final reversal (Rev 3). The discriminations were always presented in this order. The relevant and irrelevant dimensions (shift type) and order of exemplar presentation was pseudo-randomized across rats (Table 5.3).

5.2.5 Immunocytochemistry

Following experimentation, rats were deeply anaesthetised with Dolethal (0.7ml; 200mg/l pentobarbitone sodium BP; Univet Ltd., Oxford, UK) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1M phosphate buffered saline (PBS). Brains were removed and stored in a fridge overnight in 20% sucrose solution in PBS. Brains were cut coronally in 50μm sections using a freezing microtome. The sections were processed as follows: 45 minute wash in blocking solution (39ml PBS, 10ml goat serum, 0.5ml Triton X); two PBS rinses; 24 hours incubation in primary tyrosine hydroxylase antibody (1:100); five PBS rinses; 90 minute incubation period on a shaker in Vector IgG solution (5μl per ml; Vectastain mouse ABC Kit, Vector Laboratories, Peterborough, England); five PBS rinses; 60 minute incubation in Avidin-Biotin complex solution on a shaker; five PBS rinses; incubated for 2 – 10 min in diaminobenzidine peroxidase substrate (Sigma Fast 3-3'- diaminobenzidine tetrahydrochloride tablet sets; Sigma-Aldrich, Dorset, England). Sections were mounted and covered with the xylene-based mountant DPX and cover slips.

5.2.6 Data Analysis

Trials to criterion were recorded for each rat for all discriminations. For the SD/REV data a repeated measures ANOVA was carried out with two within-subjects factors (stage: discrimination and reversal; shift: SD1/Rev1, SD2/Rev2, SD3/Rev3) and two between-subjects factors (surgery: lesion and sham; drug: KW-6002 and vehicle). Repeated measures ANOVA was also employed for the set shifting analysis, with three factors, one within-subjects (shift: SD, CD, Rev1, ID, Rev2, ED, Rev3) and two-between subjects (surgery: lesion and sham; drug: KW-6002 and vehicle). Separate analyses were performed on the reversal discriminations (to compare initial acquisition and the three reversals) with two within-subjects factors (stage: discrimination and reversal; shift: SD/Rev1, CD/Rev2, ID/Rev3) and two-between subjects factors (surgery: lesion and sham; drug: KW-6002 and vehicle).

5.3 Results

5.3.1 Histology

In total, fourteen rats sustained bilateral depletion dopamine as assessed by loss of tyrosine hydroxylase staining of the medial striatum. Figure 5.2 shows the areas of tyrosine hydroxylase staining common to all rats in the KW-6002 and vehicle groups. Depletion of tyrosine hydroxylase was generally discrete medially, occasionally extended mediodorsal and never extended ventrally towards the nucleus accumbens.

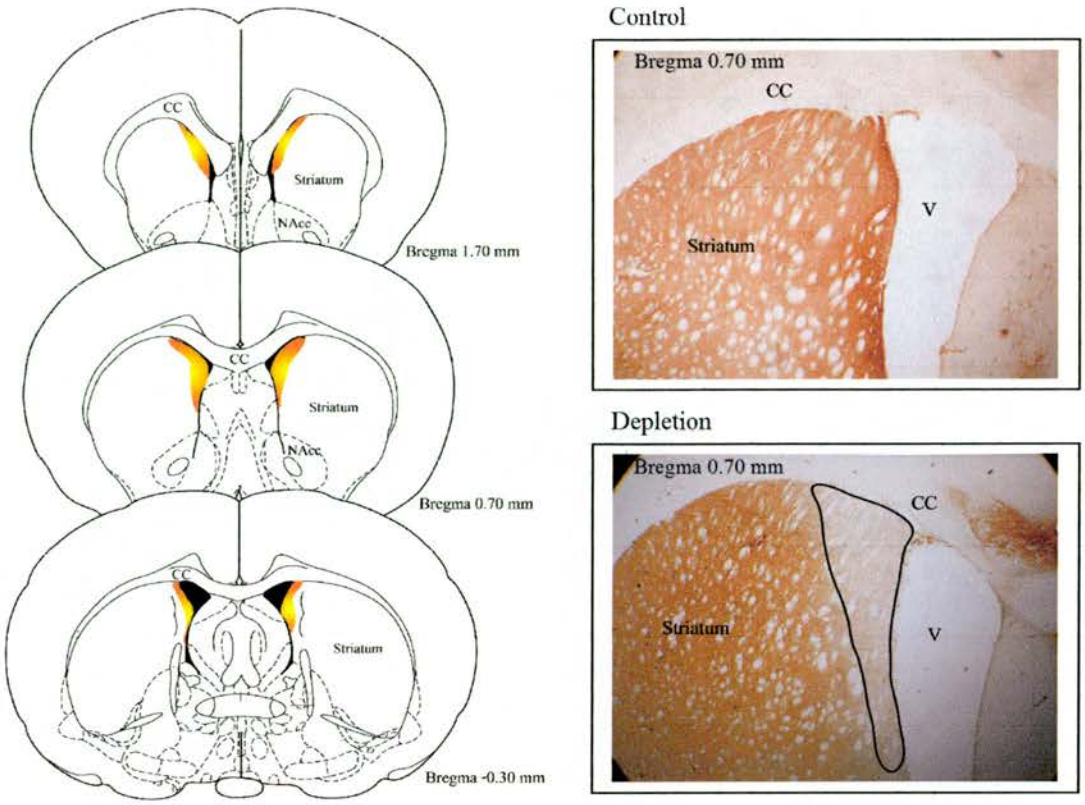

Figure 5.2 The extent of loss of tyrosine hydroxylase immunoreactivity common to all rats in both the KW-6002 and vehicle groups is displayed in the sections on the left. The pictures to the right show examples of typical tyrosine hydroxylase immunoreactivity in control and depleted rat brain sections.

5.3.2 SD/REV

Figure 5.3 shows the trials to criteria for the SD and reversal stages following 10 days of drug administration for both lesion and sham rats. The number of trials required to reach criterion decreased with each successive SD and reversal (main effect of order: $F_{(1, 40)} = 8.40$, $p = 0.001$), with the reversals requiring overall a greater number of trials than the SD (main effect of condition: $F_{(1, 20)} = 47.57$, $p < 0.001$). The lesion group made a greater number of errors on reversals compared to the control group (interaction of surgery with condition: $F_{(1, 20)} = 6.15$, $p = 0.022$; Figure 5.3). On average, KW-6002 increased trials to criteria at both the SD and reversal stages (main effect of drug: $F_{(1, 20)} = 4.51$, $p = 0.046$) and the effect of KW-

6002 was additive with the effect of the lesion. That is to say, KW-6002 did not ameliorate a lesion-induced deficit in reversal learning.

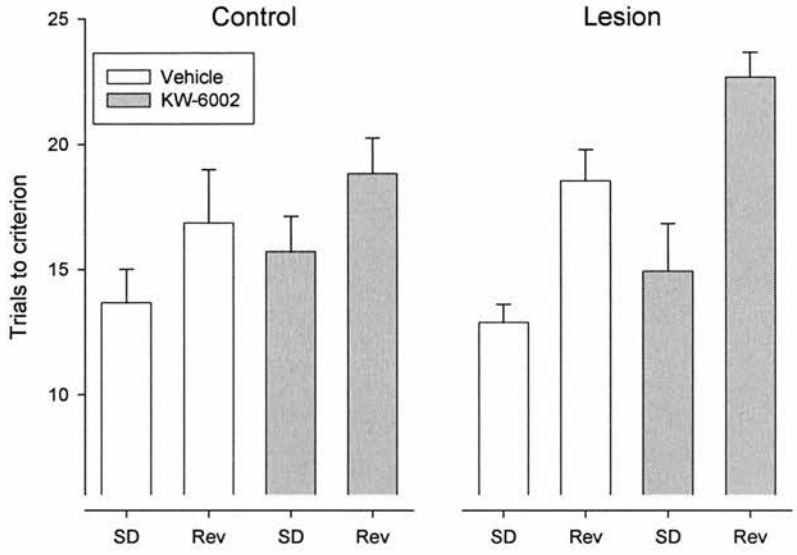

Figure 5.3 Trials to criteria for the simple discriminations (SD) and reversal stages (Rev) following 10 days of drug administration for both control (left panel) and lesion (right panel) rats. Reversals required more trials to criteria than SD. Surgery had a greater effect on reversals than SD (see text for discussion).

5.3.3 Set-shifting

Figure 5.4 shows the trials to criterion for all the discriminations in the set-shifting protocol following 15 days of drug administration. There was a significant main effect of shift on trials to criteria ($F_{(6, 126)} = 4.26$, $p = 0.001$). There was no effect of the lesion ($F_{(1, 21)} = 0.21$, ns) or drug ($F_{(1, 21)} = 0.34$, ns) across all discriminations. None of the interactions were significant.

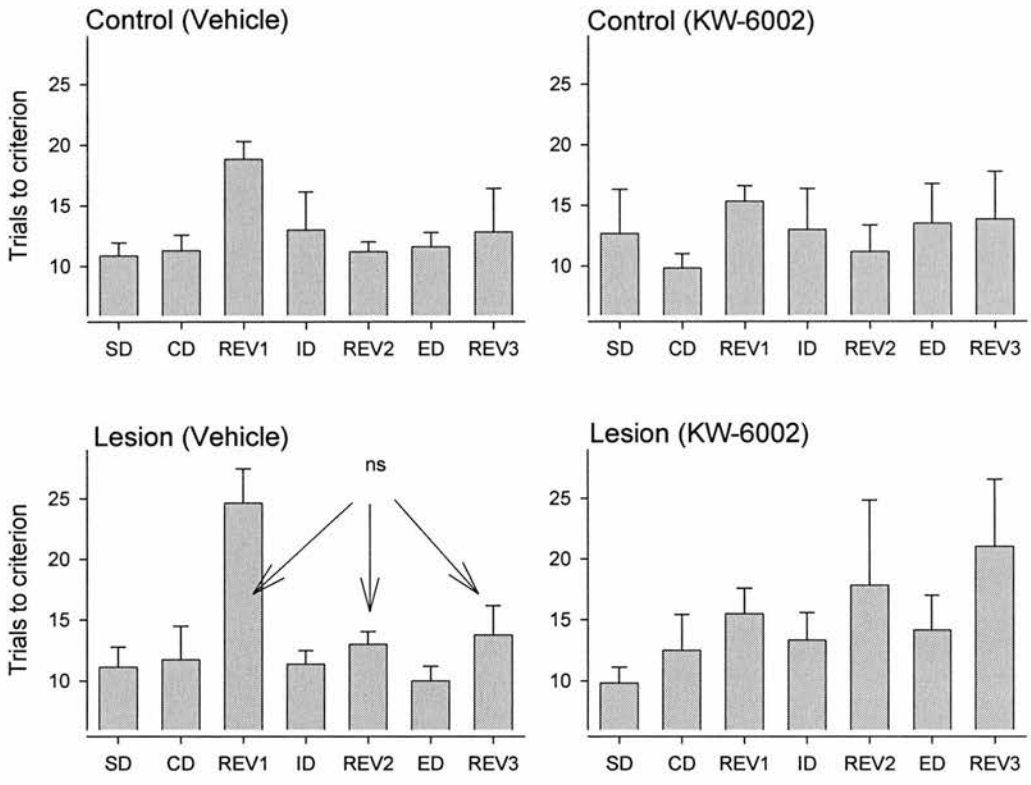

Figure 5.4 Trials to criteria at all discriminations for each drug and lesion group of rats. Overall, neither lesion nor drug had a significant performance effect.

5.4 Discussion

Rats with striatal dopamine depletion required more trials to criteria in reversal trials compared to simple discrimination trials. Following 10 days of chronic KW-6002 administration, rats generally required more trials to criterion in both acquisition and reversal. Therefore, KW-6002 had no beneficial effect on the reversal impairment induced by striatal dopamine depletion.

Following 15 days of chronic KW-6002 administration, rats were retested in a variation of the post-10 day task format, which incorporated an ID/ED shift (as used in Birrell and Brown, 2000). Typically, at the ED stage, in which attention must be shifted from a relevant dimension to a previously irrelevant dimension, the subject (whether human, monkey or rat) makes a greater number of errors compared to the ID stage. It is this difference in the rate of acquisition of a new discrimination that is used to infer that an 'attentional set' has been formed. Here, there was no

evidence of an advantage in acquisition of an ID discrimination compared to an ED discrimination, and therefore it can only be concluded that there was no evidence for the formation of an attentional set. This might have been an effect of modifying the testing procedure to include the additional days of learning new discriminations and reversals. In contrast to previous work, in this study the rats had all been exposed to all three perceptual dimensions on two previous occasions (the training phase and the SD/REV phases) and so were perhaps less ready to learn that a dimension was not relevant.

The habituation and SD/REV phases were comprised of a series of three simple discriminations and reversals for each perceptual dimension within one session. This may have had a priming effect in terms of switching responding to a 'novel' dimension – dimensions which were of course no longer novel in the final test as rats' had been previously exposed to each perceptual dimension - rendering the ED shift in the final set shifting test more accessible. This may explain why attentional set was not established. The present experimental design could incorporate an extended series of ID shifts and reversals (Dias et al. 1997) in order to ensure acquisition of attentional set.

However, in common with all previous 'failures' to show an ID/ED difference that I am aware of, the rats made an abnormally high number of errors at the SD/CD/ID stages. It is particularly worthy of comment as they continued to require more than 10 trials to acquire a new simple discrimination even after 'training'. For this reason it is not possible to draw any conclusions about the effects of either the lesion or the drug treatment on attentional set-shifting as it is not possible to confirm that an attentional set had been formed. It is interesting, and of potential significance, that the effect of KW-6002 observed at 10 days in the SD/REV testing was not observed in the final stage of testing at 15 days. It is impossible from these data to make anything other than speculative remarks about why this might have been so – for example, the chronicity of administration was greater and this might have affected the results. However, the performance of the rats was variable and therefore perhaps not too much emphasis should be placed on this non-significant result, given the low number of subjects.

Nonetheless, an fMRI investigation in healthy humans has provided evidence supporting a role of ventral frontostriatal circuitry in reversal learning (Clark et al. 2004; Cools et al. 2002). In addition, studies have implicated the ventral striatum as crucial in mediating reversal learning in rats (Annett et al. 1989; Taghzouti et al. 1985). The current data, replicating the unpublished observations of J.M. Phillips et al., suggests a role for more dorso-medial striatal regions in the reversal of reward contingencies.

In conclusion, rats with dopamine depletion of the dorso-medial striatum were impaired in reversal learning. KW-6002 resulted in a general increase in the number of trials for the rat to achieve criterion performance. The fact that this was additive with the effects of a lesion on reversal learning – neither exacerbating nor ameliorating the lesion impairment – suggests that the increase seen with KW-6002 is not mediated by the dorsomedial striatum. Obviously, data from this study, in which KW-6002 was administered systemically, cannot further test this point.

Chapter 6 General Discussion

6.1 Experimental summary

This thesis focused mainly on identifying factors that contribute to processes of motor preparation and the underlying neuropharmacological constituents that affect these processes. Drugs that affect dopamine transmission are widely implicated in motor preparatory processes. Also, a number of preclinical studies implicate the adenosine system is closely related to the dopamine system. Thus, the investigations in this thesis addressed the issue of motor preparation by analysing operant behaviour and the contribution of drugs that affect the dopamine and adenosine systems to any changes in the behaviour.

In collating the findings from Chapters 2 and 3 the following conclusions can be drawn. Chapter 2 supports the well established effect of increasing foreperiod length on responding; speeding of reaction times with an increase in anticipatory responses. In addition, the adenosine A_{2A} antagonist KW-6002 enhanced the foreperiod effect, particularly on anticipatory responding. In Chapter 2, the effect of temporal probability and duration of the foreperiod *per se* on responding could not be dissociated pre-empting any conclusion regarding the role of these factors on the observed behaviour.

Thus, Chapter 3 was designed to further investigate the effect of variable foreperiods on responding. Mean reaction times were influenced by the amount of preparation time available in the foreperiod, whereas movement time and anticipatory responding were more strongly influenced by the conditional probability of stimuli. Most notably, when rats had the same amount of opportunity to commit an anticipatory response (particularly the case with the 0.4 s foreperiod) the percentage of anticipatory responses reflected the conditional probability of the imperative stimulus rather than the amount of time available to make an anticipatory response. KW-6002 enhanced the effect of conditional probability more so than the effect of available preparation time, whereas there was no such predominant effect

of amphetamine on either factor. Interestingly, there was an opposite effect of foreperiod on mean movement time in Chapters 2 and 3.

Movement time co-varied positively with reaction time in Chapter 2, decreasing as a function of lengthening foreperiod whereas movement time increased as a function of increasing foreperiod in Chapter 3. The most likely explanation for this effect is the additional requirement for a nose-poke in an adjacent side-hole following the imperative signal in Chapter 3. In Chapter 2 rats were required to withdraw from the centre nose-poke hole when the imperative signal sounded then retrieve reward with the time from nose-poke withdrawal to activation of the micro-switch at the food hopper constituting movement time. Therefore, there was no choice element involved in the initiation (reaction time) and execution (movement time) of the response. In Chapter 3, rats were required to make a choice to which side they were to respond following the imperative signal. This appears to have had a considerable effect on the pattern of movement times as a function of increasing foreperiod. This effect might be attributable to an increase in the subjective uncertainty of the cue light location as the foreperiod preceding the imperative signal increases. If this is the case, the decision for which side to respond may have been withheld until after the imperative signal sounded. Indeed, rats were occasionally observed during training in Chapter 3 to withdraw and visually assess both side holes before responding. The probability of a cue signal to the left or right adjacent hole was equal in Chapter 3 and this was reflected by the lack of a significant effect of side on reaction times and movement times. There was no significant effect of foreperiod on mean movement time in Chapter 4.

In Chapter 4 a modified procedure was used to investigate whether additional requirements on attention would affect motor preparation. Modal reaction times were quicker when rats were able to predict and prepare a more likely response. Selective blockade of dopamine D_2 receptors by raclopride had a more profound effect on responding in this task then blockade of dopamine D_1 receptors by SCH-23390. As expected, raclopride and SCH-23390 reduced anticipatory responses, especially in conditions when they were high in the baseline (at the longer foreperiods). The hypothesis that selective blockade of dopamine transmission at the

D_2 receptor would attenuate the foreperiod-dependent speeding of reaction time, as observed in rats with striatal dopamine depletion (Brown and Robbins 1991), was not confirmed. Surprisingly, although raclopride increased reaction times there was a concomitant enhancement of the foreperiod-dependent speeding of reaction time. SCH-23390 generally increased reaction times, an effect which was the same across all foreperiods.

Finally, the therapeutic potential of KW-6002 on Parkinsonian motor impairments has been well documented. Chapter 5 investigated whether KW-6002 would have cognitive as well as motor benefits. The effect of chronic KW-6002 treatment on reversal impairments in dopamine depleted rats was examined and no restoration of reversal deficit was observed.

6.2 General considerations

6.2.1 Role of adenosine and dopamine

The findings from Chapters 2 and 3 imply that adenosinergic transmission plays an important role in mediating goal-directed behaviour. The proximity of these findings to the many studies of the effects of dopaminergic transmission on goal-directed behaviours gives rise to several possible roles of the adenosine system in behavioural control. The role of dopamine is not solely confined to motor control, as dopamine neurons and dopaminergic drugs have been shown to be involved in processes not directly related to motor output, such as cognitive flexibility (Cools et al. 2001) and the coding of stimuli predictive of future reward (Schultz 1998). Is it possible that the adenosine system also contributes to such processes?

A microdialysis study of extracellular adenosine efflux in the nucleus accumbens following presentation of novel rewarding and aversive stimuli, which had previously been shown to increase accumbens dopamine efflux, found no change in adenosine efflux (Nagel and Hauber 2002 and references therein). An investigation of direct blockade of accumbens adenosine A_{2A} receptors showed an increase in locomotion and disruptions of sensorimotor gating and feeding behaviour (Nagel et al. 2003). Also, central reward processing in a self-stimulating task has been shown to be unaffected by central blockade of the A_{2A} receptor in the nucleus

accumbens (Baldo et al. 1999). The absence of an effect of KW-6002 on reversal learning in the baseline or reparation of the reversal deficit induced by striatal dopamine depletion in Chapter 5 suggests that tonic adenosine does not contribute to flexible cognitive function. These findings in cohort with the findings from Chapters 2 and 3 imply that adenosine operates as an active tonic modulator of striatal function through the A_{2A} receptor and primarily mediates sensorimotor integration and motor control. A_{2A} antagonism has been shown to increase glutamate release in the striatum, possibly due to an activation of presynaptic receptors on glutamate afferent projections (Rodrigues et al. 2005). Moreover, A_{2A} antagonists increase glutamate release in the dopamine depleted striatum in the rat (Corsi et al. 2003). Thus, the general increase in trials to criteria observed in Chapter 5 could be due to an enhancement of a cortical or thalamic input rather than an effect on the dopamine system *per se*. This may also explain the lack of effect of A_{2A} antagonism in the aforementioned processes associated with dopamine activity.

In Chapter 3, antagonism of the A_{2A} receptor by systemic KW-6002 resulted in an increase in the effect of the conditional probability of stimuli on anticipatory responding and movement times. This suggests a possible role of tonic adenosine in modulating behavioural control through encoding the likelihood of stimuli based on the integration of current sensory information with previous likelihood (Carpenter and Williams 1995). Another question relevant to the observed effects of KW-6002 is whether or not these effects are independent of an interaction with the dopamine system.

6.2.2 Are the effects of adenosine blockade mediated via the dopamine system?

The functionally antagonistic relationship of A_{2A} receptors and dopamine D_2 receptors is widely cited (Ferre et al. 1997; Ferre et al. 1993; Ferre et al. 1999; Ferre et al. 1991; Franco et al. 2000; Le Moine et al. 1997). Also, following chronic systemic KW-6002 administration in dopamine depleted rats, the reparative cellular effects of KW-6002 were specific to the striatopallidal neurons (Aoyama et al. 2002), where D_2 receptors are also present. However, A_{2A} effects on locomotion have been

reported independent of dopamine D_2 receptors (Aoyama et al. 2000; Chen et al. 2001). Thus, it would be of interest to define whether the effects of blockade of A_{2A} receptors observed here are mediated via an increased efficacy of dopamine D_2 receptors or are independent of dopamine. Of course, this presumes that the observed behavioural effects are mediated at the level of the striatopallidal neurons. The alternative is a cortical mechanism, as A_{2A} receptors are also expressed in cortical areas (Sebastiao and Ribeiro 1996) and prelimbic-infralimbic cortex has been shown to mediate motor readiness in the rat (Risterucci et al. 2003). The distribution of KW-6002 in the rat brain following oral administration has been shown to become more globally distributed with increasing doses, particularly to cortical areas, notably so at the most effective dose (3 mg/kg) used in Chapters 2 and 3 (see Aoyama et al. 2002). However, the relationship between A_{2A} and D_2 receptors in the cortex is less well characterised, although a recent pharmacologic MRI study provides evidence that the dopamine-adenosine antagonistic relationship occurs mainly in the basal ganglia and not cortex (Chen et al. 2005).

6.2.3 Application to an information processing model of basal ganglia function

Although the effects of amphetamine in Chapter 3 were not as isolated to either the processing of conditional probability or time perception, a similar effect on conditional probability processing has previously been attributed to amphetamine (Brown et al. 1996). Amphetamine increases catecholamine release throughout the brain, including dopamine release in the striatum. Systemic amphetamine has been shown to have a bi-directional effect on striatal activity.

Striatal neurons express motor-related excitation or inhibition, with low doses (0.5-1.0 mg/kg) of amphetamine generally facilitating or inhibiting these neurons, respectively (Rebec et al. 1997; West et al. 1997). These findings have led to the proposal that amphetamine, possibly by enhancing dopaminergic neuromodulation, has the capacity to increase the gain of neuronal information processing (Haracz et al. 1998). This increase in neuronal processing may explain the speeding of reaction times by amphetamine in Chapter 3, whereby the selection

of the desired motor program is enhanced. Moreover, at higher doses of amphetamine stereotypy is induced (Rebec et al. 1997), which may be the result of an enhanced facilitation and inhibition of selective groups of striatal neurons. Activation of a subset of striatal neurons resulting in repetitive engagement of a limited category of responses may produce the stereotypical behaviour typically observed with high doses of amphetamine (Wickens and Kötter 1995). The early argument by Wickens and Kötter has received more recent empirical support. They argued that such behaviour may result from a strengthening of sensory input through potentiation of corticostriatal inputs (Centonze et al. 2001) and through competitive interactions between nearby striatal neurons (Czubayko and Plenz 2002), whereby the most active neurons inhibit less active neurons through collateral inhibition. The concept of intra-striatal collateral inhibition networks has received a lot of recent attention, but studies are confined to striatal organotypic cultures and slice preparations with the relevance of such networks in behavioural modulation as yet unclear (see Plenz 2003 for review and ideas on the behavioural relevance).

Nonetheless, the idea of enhanced neuronal information processing may explain the speeding of reaction times and the increase in anticipatory responses following amphetamine. Also, blockade of adenosinergic transmission at the A_{2A} receptor by KW-6002 may also enhance neuronal information processing. Moreover, the effects of amphetamine on conditional probability and time perception in Chapter 3 were difficult to disentangle, whereas KW-6002 predominantly enhanced the effect of conditional probability. Although this may be due to a dose-effect, it might represent a more selective neural substrate for the processing of stimulus conditional probability information. Indeed, the pharmacological profiles of both drugs are very different.

Amphetamine increases dopamine release resulting in a non-selective activation of D_1 and D_2 receptors in the striatum whereas the effects of KW-6002 are selective to the adenosine A_{2A} receptor and are most likely mediated at striatopallidal neurons, which constitute the indirect pathway of the basal ganglia (Aoyama et al. 2002). In support of the anatomical localisation of the adenosine A_{2A} receptor and the selective distribution of KW-6002 to the striatum following oral administration

(Aoyama et al. 2002), is the similarities in the behavioural effects of KW-6002 and lesions of the STN, particularly on anticipatory responding (Baunez et al. 1995b; Phillips and Brown 1999; 2000).

Reaction time deficits resulting from loss of striatal dopamine are thought to be predominantly due to a decrease in dopaminergic tone primarily at the dopamine D_2 receptor (Aoyama et al. 2000; Smith et al. 2000). Moreover, in anaesthetised animals with striatal dopamine depletion, the STN shows an elevated firing rate (Kreiss et al. 1997). This implies a key function of the indirect pathway in motor control. Indeed, unilateral STN lesions reverse the reaction time deficits in rats with striatal dopamine depletion. However, this also
results in an additional behavioural deficit manifest as an increase in anticipatory responding (Baunez et al. 1995b, Phillips and Brown, 1999). Anticipatory responses have also been shown to increase following lesions of the STN in isolation (Baunez et al. 1995b; Phillips and Brown 1999; 2000). Note that these studies differed in several methodological factors including the laterality of the lesions. Following bilateral STN lesions, reaction times were reported to decrease (Baunez et al. 1995b) whereas there was no effect on reaction times following unilateral lesions (Phillips and Brown 1999; 2000). Thus, bilateral lesions likely result in additional deficits that impact on reaction times. Like reaction times, anticipatory responses also show a robust relationship with foreperiod; as foreperiod increases anticipatory responding increases. This effect was enhanced by the unilateral STN lesions in the Phillips and Brown (2000) study and has also been shown to be enhanced by amphetamine (Brown et al. 1996), although amphetamine also enhanced the effect of foreperiod on reaction times.

Taken together, these studies implicate intact striatal dopamine transmission is essential for efficient reaction time performance and motor preparatory processes (Brown and Robbins 1991), whereas the STN seems to play a more important role in response inhibition processes. This suggests that separate components of the basal ganglia circuitry mediate different components of motor action.

The similar behavioural profile of KW-6002 and STN lesions (enhanced anticipatory responding) suggests that these effects may be mediated via the indirect

pathway (A_{2A} receptors are predominantly located on striatopallidal neurons of the indirect pathway that project to the STN), in support of the model of inhibition and facilitation of desired motor programs suggested by Mink (1996). Thus, it would of interest to test the effects of KW-6002 on operant responding in a Parkinsonian model in order to assess whether KW-6002 results in similar additional response inhibition deficits, which would have implications for the use of adenosine A_{2A} antagonists in the treatment of Parkinson's disease.

Also, a recent study implicated the importance of the interaction between adenosine A_{2A} and glutamatergic mGluR5 receptors in motor control; particularly in benefit to Parkinsonian-type deficits (Coccurello et al. 2004). Such treatment may be capable of improving reversal deficits following striatal dopamine depletion.

6.2.4 Conditional probability and interval timing

In Chapters 3 and 4, attempts were made to tease apart the relative contribution of the conditional probability of stimuli from the temporal characteristics of the stimuli. By temporal characteristics, I refer to the amount of time the rat had to wait (foreperiod) until the stimulus was presented. The rationale for this approach is nicely outlined in a recent review by MacDonald and Meck (2004), who present a comprehensive overview of the conceptual and empirical basis for a combined study of reaction time and interval timing mechanisms.

Chapter 3 begins to address the relationship of motor preparatory mechanisms with internal timing processes, however the time range incorporated in Chapter 3 is different from most interval timing studies (by a magnitude of 10). Foreperiods were in the millisecond range so the effect of preparation time would require an internal clock capable of monitoring the passage of time in milliseconds. Preparation time did contribute to responding in the task, suggesting that rats were able to monitor time intervals in the millisecond range. Perhaps a more direct examination of a millisecond 'internal clock' could be provided by a temporal bisection task (see Section 1.2.2) redesigned for the 9-hole operant box, with a nose-poke in the side holes instead of lever-pressing and intervals in the 0.2-0,8 s range,

rather than the conventional 2-8 s range. If the psychophysical functions from both time ranges were similar, then this would provide evidence of interval timing mechanisms in both the millisecond and second range. An advantage of this design would be that other behavioural measures (reaction times, movement times and anticipatory responses), as well as response rate, could be considered. The next step would be to investigate whether these timing mechanisms are mediated by the same or different 'internal clocks'. Indeed, there are suggestions that temporal processing of shorter durations (under 1 s) are mediated by the cerebellum with processing of longer durations (greater than 1 s) mediated by the basal ganglia (Ivry and Spencer 2004).

Moreover, there have been conflicting reports of different effects of the same drugs in interval timing tasks. Studies have reported conflicting findings of the effects of the D_2 receptor antagonist quinpirole on timing processes (Santi et al. 2001 and references therein). Also, one study highlighted methodological concerns by reporting differential effects of amphetamine contingent on discrete changes to the behavioural parameters in an interval timing task (Chiang et al. 2000). These reports do not disregard effects on timing processes but rather call for a reassessment of the specific effects on timing, suggesting that perhaps different neural mechanisms may be involved in different timing tasks, with attentional processes possibly playing a key role (Buhusi and Meck 2002; Chiang et al. 2000).

The contribution of attention and timing was addressed in Chapter 4. Rats were quicker to respond to the side that was most likely as indicated by the amount of time elapsed in the foreperiod. This suggests that rats were able to monitor the passage of time elapsed in the foreperiod and allocate response preparation to the suitable motor program required for a response to the desired target location. The dopamine D_2 receptor antagonist slowed modal reaction times with a concomitant enhancement of the foreperiod-effect. Although at the highest dose of raclopride it appeared as though rats were responding equally to both sides regardless of spatial probability, this effect was not significant. Therefore, there was a suggestion that blockade of the dopamine D_2 receptor may (at a higher dose than used here perhaps)

interact with attentional effects on timing during millisecond intervals but this requires further investigation.

6.3 Conclusion

Systemic administration of compounds affecting the dopamine and adenosine systems has a considerable effect on the intricate processes underlying response selection in the rat. Dopamine and adenosine receptors are present in areas of the brain known to be intimately involved in motor preparation. Combining the behavioural approach adopted in this thesis with discrete drug administration and recording techniques, such as intracerebral injections and electrophysiology and/or voltammetry, will advance our knowledge of the specific contributions of the components of these systems to processes of response selection, which may ultimately provide insightful clues into the underlying neuropathological alterations that result in debilitating motor impairments in neurodegenerative disorders such as Parkinson's disease.

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