THE NEURAL BASIS OF ATTENTION

by

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Submitted to the University of St. Andrews for the degree of PhD

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DECLARATION

i) I, Kerry McAlonan, hereby certify that this thesis, approximately 40,000 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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Especially for our brother Keiran – gone but not forgotten.

PUBLICATIONS

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ABSTRACT

Sensory information is routed to cortex via thalamus but despite this sensory bombardment, animals attend selectively to stimuli that signal danger or opportunity. Sensory input must be filtered allowing only behaviourally relevant information to capture limited attentional resources. The thalamic reticular nucleus receives collaterals from thalamocortical and corticothalamic fibres (Jones, 1975; Ohara and Lieberman, 1985) and therefore it is in a position to gate the flow of information between thalamus and cortex. Although specific roles for Rt in the control and generation of thalamocortical activity have been demonstrated during sleep the precise function of this nucleus in awake, behaving animals remains to be determined. By virtue of its anatomy and physiological properties, the thalamic reticular nucleus has been implicated in selective attention (Skinner and Yingling, 1977; Yingling and Skinner, 1977; review see Guillery et al., 1998). Crick (1984) said that if the thalamus is the gateway to cortex, then the thalamic reticular nucleus is the "guardian of the gateway". Selective attention involves the filtering of aspects of the environment to allow the processing of any given stimulus, dependent on its intrinsic nature or its position in space (Triesman, 1969). Preliminary behavioural evidence suggests that the thalamic reticular nucleus operates as an attentional gate or 'filter' at both sensory (Montero, 1997, 1999, 2000) and higher cognitive levels (Vann et al., 2000; Wilton et al., 2000). This thesis examines the possibility that the thalamic reticular nucleus of the rat is involved in subcortical mechanisms of attentional processing and control.

Chapter 1 highlights the neuroanatomical and physiological bases for ascribing functional roles, such as selective attention and attentional control, to the thalamic reticular nucleus. Chapters two and three attempt to elucidate the mechanisms underlying Fos immunoreactivity in Rt following focussed attention to task relevant cues (Montero, 1997, 1999, 2000) because there is considerable doubt as to whether Fos immunoreactivity following exploration of a novel complex environment represents attentional rather than sensory or motor task requirements. Chapter two investigates Fos immunoreactivity in attentional paradigms with differential response requirements. In Chapter three, the hypothesis that an attended stimulus would activate Rt more than an unattended stimulus is tested. It has been suggested that rostral and caudal divisions of Rt have distinct cognitive functions. Caudal 'sensory' sectors of Rt are thought to mediate selective attention to behaviourally relevant stimuli, while rostral Rt is thought to mediate more complex cognitive processes, including spatial processing. However, the behavioural evidence bearing on this issue is inconclusive. In an attempt to explain these discrepant behavioural results it has been suggested that rostral Rt might be involved not in spatial processing per se, but in focussed attention to contextually significant stimuli. This is investigated in Chapter 4, using an attentional set shifting task that requires focussed attention to and abstraction of contextually significant stimuli. The role of rostral Rt lesions in attentional set-shifting is explored using this task. In Chapter 5, the neurochemical modulation of selective attention is investigation by systemically altering cholinergic neurotransmission. The cholinergic system was chosen for investigation as this system is known to modulate the relay of sensory information through thalamus.

These results are discussed in terms of a dissociation of function between rostral and caudal divisions of Rt in the rat. The mechanisms by which Rt, in conjunction with cholinergic afferents from the basal forebrain and brainstem, might mediate selective attention are discussed.

Chapter 1: General Introduction

The thalamic reticular nucleus: from arousal to cognition

Thalamus

Figure 1.1 illustrates a sagittal section through rat thalamus. The thalamus (shown shaded) can be divided into two distinct regions: dorsal and ventral portions (Rose, 1942).

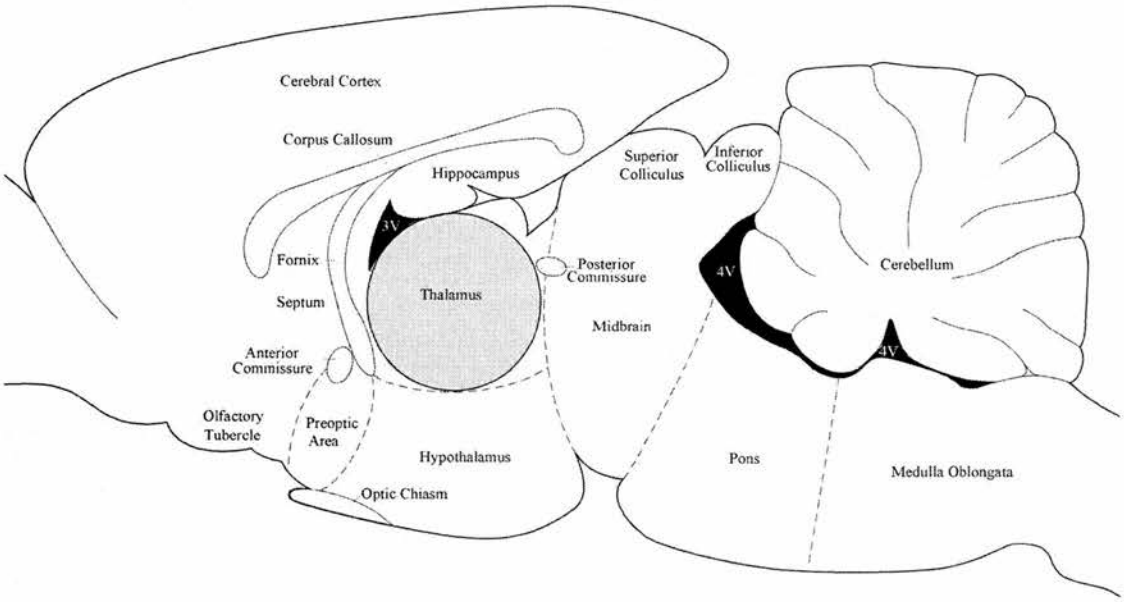


Figure 1.1. A sagittal view of the cerebral hemispheres of a rat (adapted from Paxinos and Watson, 1997). This section (approximately 0.4mm lateral to the midline) shows the position and size of thalamus (indicated by shading) relative to other central nervous system structures. Based on Sherman and Guillery (2001).

The thalamus has been divided into these regions according to the relationship of each with the cerebral cortex (see Castro-Alamancos and Connors, 1997). Dorsal thalamus (from now on referred to as 'thalamus') can be divided into several distinct nuclei or nuclear groups, each concerned with transmitting a characteristic type of signal (visual,

auditory, somatosensory, cerebellar, etc.) to cytoarchitectonically distinct and functionally corresponding area or group of areas of the cerebral cortex (Rose and Woolsey, 1949; Jones, 1985; Guillery, 1995; Sherman and Guillery, 1996; for a review see Sherman and Guillery, 2001). All dorsal thalamic nuclei send and receive fibres from the cerebral cortex. Ventral thalamus, which includes the thalamic reticular nucleus (Rt), zona incerta (ZI) and the ventral lateral geniculate (vLGN), does not project to the cortex (Jones, 1985). The principal output of these ventral thalamic nuclei, particularly Rt, is to dorsal thalamus (see Jones, 1975). Ventral thalamus can nonetheless regulate thalamocortical transmission through its interconnectivity with the dorsal thalamic nuclei.

Sensory transmission

All senses are routed to cortex through thalamus[1]. Thus thalamus has been referred to as the 'gateway to cortex' (Crick, 1984). Despite being faced with sensory bombardment, animals selectively attend to stimuli that signal danger or opportunity. Somehow, sensory input is filtered, allowing only behaviourally relevant information to capture limited attentional resources. Between thalamus and cortex lies Rt; see Figure 1.2. A derivative of ventral thalamus, Rt consists of a thin elongated sheet of GABAergic (inhibitory) neurons (rodent: Houser et al., 1980; Barbaresi et al., 1986; De Biasi et al., 1986; primate: Hendrickson et al., 1983; cat: Oertel et al., 1983, Fitzpatrick et al., 1984; Montero and Singer, 1984; Yen et al., 1985; Clemence and Mitrofanis, 1992) that surround most of the anterior and lateral aspects of thalamus (Jones, 1975). The primary source of excitation within this nucleus comes from ascending thalamocortical and

[1] This statement is somewhat oversimplified. While the central division of the mediodorsal thalamus receives a substantial input from the olfactory cortex (see Powell, 1965), olfactory afferents represent the only pathway of a sensory system that does not have to go through thalamus before it can reach cortex (see Sherman and Guillery 2001 p. 2).

descending corticothalamic axons that branch off and collateralize within Rt en-route to their respective targets (Jones, 1975; Carman et al., 1964). In turn Rt projects exclusively to thalamus, thus forming an essential component of the circuitry mediating thalamocortical transmission.

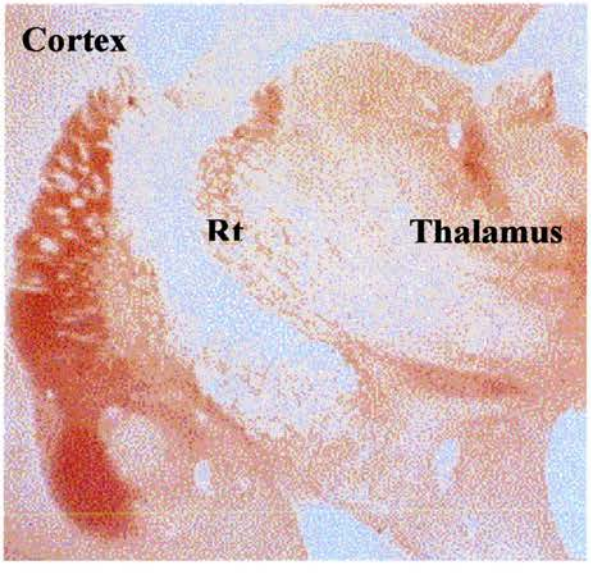


Figure 1.2. A coronal section through rat thalamus (at approximately 2.56 mm posterior to Bregma, taken from Paxinos and Watson, 1997). This section is stained with an antibody against parvalbumin, which is found in the GABAergic cells and terminals of Rt neurons. Rt lies immediately between thalamus and cortex and is innervated and activated by both. In turn, Rt projects back to thalamus.

By virtue of it's position and it's dense, exclusive GABAergic innervation of thalamus (Jones, 1975), Rt has been implicated as playing a critical role in the relay of sensory information from the thalamus to the cortex. Crick (1984) suggested that "*if the thalamus was regarded as the gateway to the cortex, then the Rt might be considered the Guardian of that gateway*". Numerous physiological studies have since confirmed

that, via its inhibitory interactions with the thalamus, Rt controls sensory transmission through the thalamus (Schlag and Waszak, 1971; Frigyesi and Schwartz, 1972; Steriade and Wysinski, 1972; Sumitomo et al., 1976; Yingling and Skinner, 1976; Hale et al., 1982; French et al., 1985).

Rt is thought to represent the major source of GABA-mediated inhibition within the thalamus (Thomson, 1988; Pinault and Deschenes, 1992; Kim et al., 1997). During slow wave sleep Rt's inhibition generates "spindle" oscillations that hyperpolarize and synchronise thalamocortical circuits, and consequently disrupt the processing of sensory signals (Steriade et al., 1985; Steriade et al., 1987; Steriade et al., 1991; Marks and Roffwarg, 1993; Hartings et al., 2000; for review see Steriade et al., 1993). During the transition from sleep to wakefulness, thalamic relay and reticular cells both depolarise and change their firing patterns from rhythmic bursts to single spikes. Recent physiological findings suggest Rt might also play a more specific role in processing ascending sensory signals. For example, the tonic activity of Rt neurons have been shown to sharpen both the receptive field properties and response times of thalamic relay neurons in anaesthetised animals (Salt, 1989; Simons and Carvell, 1989; Lee et al., 1994a b; Hartings and Simons, 2000; Hartings et al., 2000). Although specific roles for Rt in the control and generation of thalamocortical activity have been demonstrated during sleep and anaesthesia, the precise function of Rt mediated inhibition in awake, behaving animals (especially with respect to attention) remains to be determined. Crick (1984) has proposed that during wakefulness Rt mediates selective attention, by intensifying a particularly active thalamic input to the cortex. The functional basis of this 'amplification' is the arhythmic 'burst' firing expressed by hyperpolarized thalamic relay cells (Crick suggested this was mediated by Rt's inhibitory influence). Indeed

recent neurophysiological demonstrations have demonstrated that the tonic activity of Rt neurons contributes to both thalamic (Warren and Jones, 1994) and cortical responsiveness (Hartings and Simons, 2000; Hartings et al., 2000). These findings are fully consistent with a role for this region (Rt) in selective attentional mechanisms. The idea that Rt might be performing this function is not new: in 1977, Yingling and Skinner proposed that Rt is "*an obvious candidate for inclusion in any proposed control mechanism in the brain that underlies functions such as 'attention'*".

Selective attention

Selective attention has been referred to as the behavioural expression of a neural mechanism that selects relevant information (usually sensory) for additional processing, and simultaneously suppresses or inhibits the processing of irrelevant information. That information is then used to control performance in a task that is driven by the organism's specific goals (Wenk, 1997). Any structure that contributes to selective attention must carry a topographic map of the various sensory surfaces (Sherman and Guillery, 1996). Topographic maps exist when one set of neurons project to another so that the order of the neurons is mirrored by the order of synaptic connections. The mapping of one set of neurons onto another allows successive levels of neural processing to occur throughout the central nervous system whilst maintaining the integrity and localisation of the original input. These maps enable a specific point within any given sensory field to be enhanced relative to other points. Characterised by overlapping terminal arbours and dendritic fields (the axons of which projected non discriminatory to thalamus) Rt was considered far too diffuse to sample and modulate thalamocortical activity with any degree of precision (Scheibel and Scheibel, 1966; Jones, 1975). This seemed to preclude Rt's involvement in any specific functions such

as selective attention. However this notion has proved impossible to maintain. Contemporary neuroanatomical studies have subsequently divided Rt into modality specific sectors (visual, auditory and somatosensory) each principally related to one sensory modality or to one group of thalamic nuclei and their cortical connections (for comprehensive reviews see Guillery et al., 1998 and Crabtree, 1999).

Rt: general architecture and topography of major afferents and efferents

General architecture

Rt neurons vary with respect to size (somal diameters range between 10-20μm in rodents), shape (cell bodies are typically ellipsoidal) and dendritic morphology (bitufted and bipolar most common) (Ohara and Leiberman, 1985; see Lübke, 1993 for a review). Intranuclear (i.e. cell-cell) communications within Rt occur via inhibitory synaptic mechanisms (Bal et al., 1995; Huguenard and Prince, 1992; Ulrich and Huguenard, 1995, 1996). It is currently believed that such intranuclear communication is achieved via a network of symmetric, inhibitory dendrodendritic synapses in cats (Ide, 1982; Montero and Singer, 1984; Deschenes et al., 1985; Yen et al., 1985) and via a network of symmetric axoaxonic synapses in rodents (Ohara and Leiberman, 1985, Spreafico et al., 1988; Cox et al., 1996). However dendrodendritic synapses have been observed in rodents (Scheibel and Scheibel, 1972; Pinault et al. 1996, 1997). Figure 1.3 shows schematic representations of the many different types of synaptic contacts potentially formed between Rt neurons. Irrespective of its exact morphological substrate (dendrodendritic, axoaxonic, axosomatic, axodendritic) this inhibitory network may represent the basis by which Rt prevents unnecessary information from capturing limited attentional resources.

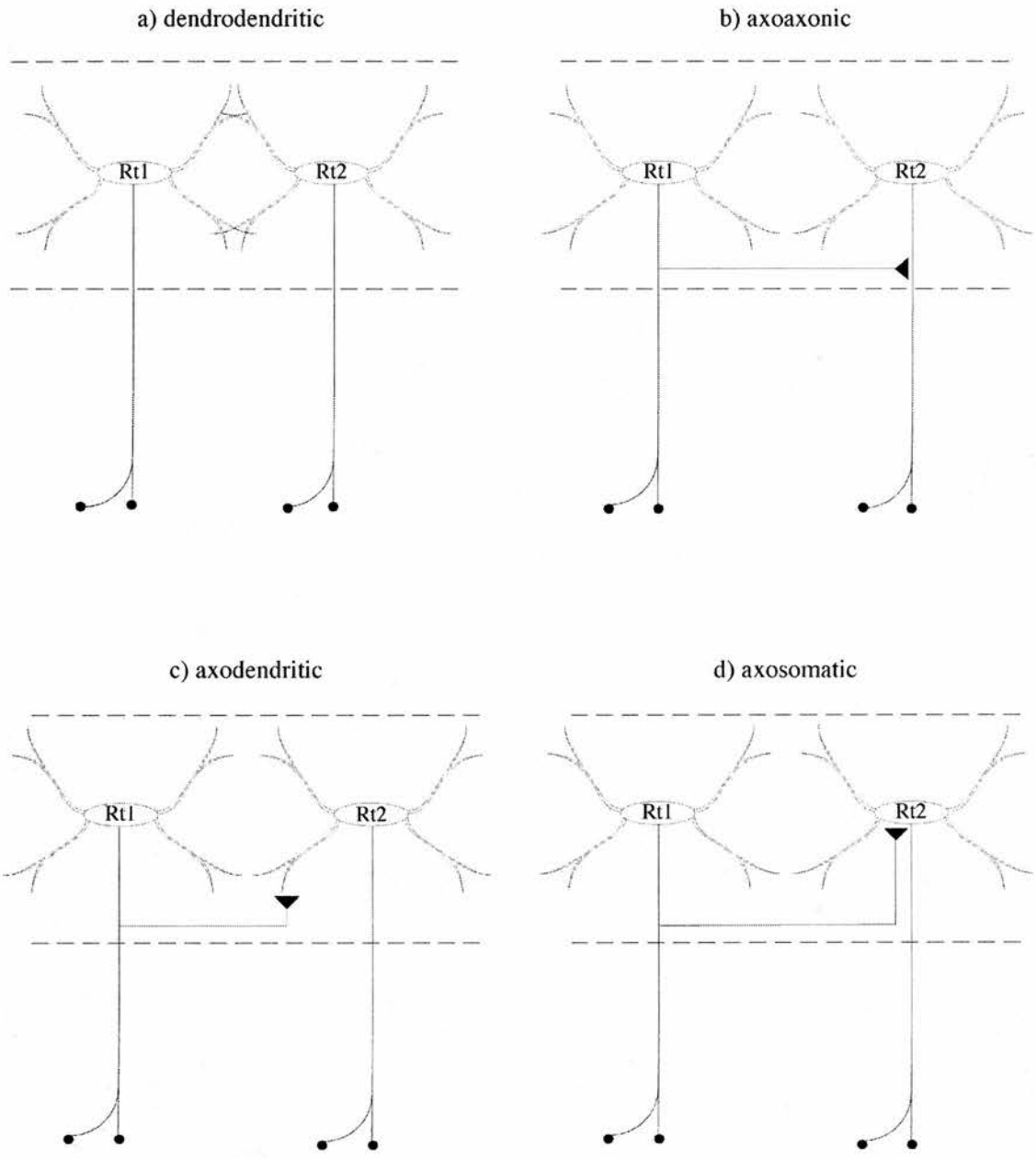


Figure 1.3. Schematic representation of the various synaptic connections potentially formed between thalamic reticular (Rt) neurons, (a) dendrodendritic, (b) axoaxonic, (c) axodendritic and (d) axosomatic. Abbreviations: Rt1, thalamic reticular cell 1; Rt2 thalamic reticular cell 2.

Through such intranuclear communication, a locus of excitation within Rt, could effectively eliminate activity in adjacent regions (potentially sectors), allowing the relay of behaviourally relevant information at the expense of irrelevant information.

Rt: Afferents and Efferents

Thalamocortical and corticothalamic axons traverse Rt, en route to their respective targets, imparting glutamatergic, presumably excitatory (Salt and Eaton, 1996 although see Cox and Sherman, 1999), collaterals that form synaptic contacts with the proximal portion of the somatodendritic complex of Rt cells (Ohara and Lieberman, 1985, Montero and Scott, 1981, Montero, 1983) and possibly also with the hillock and initial segment of their axon (Pinault et al., 1997). In turn, Rt projects to ipsilateral thalamus (Jones, 1975; Ohara and Leiberman, 1985; Pinault and Deschenes, 1998). Although Rt has also been shown to project to contralateral thalamus (Raos and Bentivoglio 1993; Lizier et al., 1997), these projections (especially those from rostral Rt to the contralateral midline and intralaminar thalamic nuclei) are thought to be negligible in rodents (Kolmac and Mitrofanis, 1997; Hazrati et al., 1995). These synapses are inhibitory reflected by their F-type terminals (flattened synaptic vesicles, Gray type 2 synaptic contacts) that form contacts with the cell bodies and dendrites of thalamocortical neurons (Houser et al., 1980; Ohara et al., 1980; Peschanski et al., 1983). The cell bodies and dendrites of Rt are oriented parallel to the oblique rostro-caudal plane of the nucleus, perpendicular to the traversing corticothalamic and thalamocortical fibres, lending the structure it's 'reticulated' appearance and name.

Rt: Topographic organisation

This section considers some of the contemporary neuroanatomical studies that have proved invaluable in mapping the complex connectivity between Rt, thalamus and cortex. Recent investigations of Rt sectors (using more refined neuroanatomical techniques) have revealed the presence of topographic maps, that mimic those found in modality specific thalamic nuclei (Adams et al., 1997; Crabtree and Killackey, 1989). These perceptual maps imply Rt might execute localised modulation of thalamocortical activity (as opposed to global non-specific actions) a requirement of any 'attentional searchlight' (Crick, 1984) that amplifies particularly active regions of the sensory environment.

Caudal Rt

Anatomical studies in rats, cats and monkeys have revealed Rt's division into several modality specific sectors. Each sector (somatosensory, visual and auditory) receives axon collaterals from reciprocally connected thalamic relay nuclei and their respective cortical areas and projects back only to the corresponding thalamic nuclei (Jones, 1975; Ohara and Leiberman, 1985). This is illustrated schematically in Figure 1.4. Early anatomical methods exemplified a rather widespread, diffuse projection for the reticulo-thalamic axons (Jones, 1975; O'Hara and Lieberman, 1985; Yen et al., 1985), however there is now compelling evidence for topographically organised pathways to and from the reticular nucleus, at least for 'first-order' sensory regions (see especially Crabtree and Killackey 1989; Crabtree 1992a, b; Conley and Diamond, 1990; Conley et al., 1991; Coleman and Mitrofanis, 1996; Hartings et al., 1991; Pinault and Deschenes, 1998; for comprehensive reviews see Guillery et al., 1998 and Crabtree, 1999).

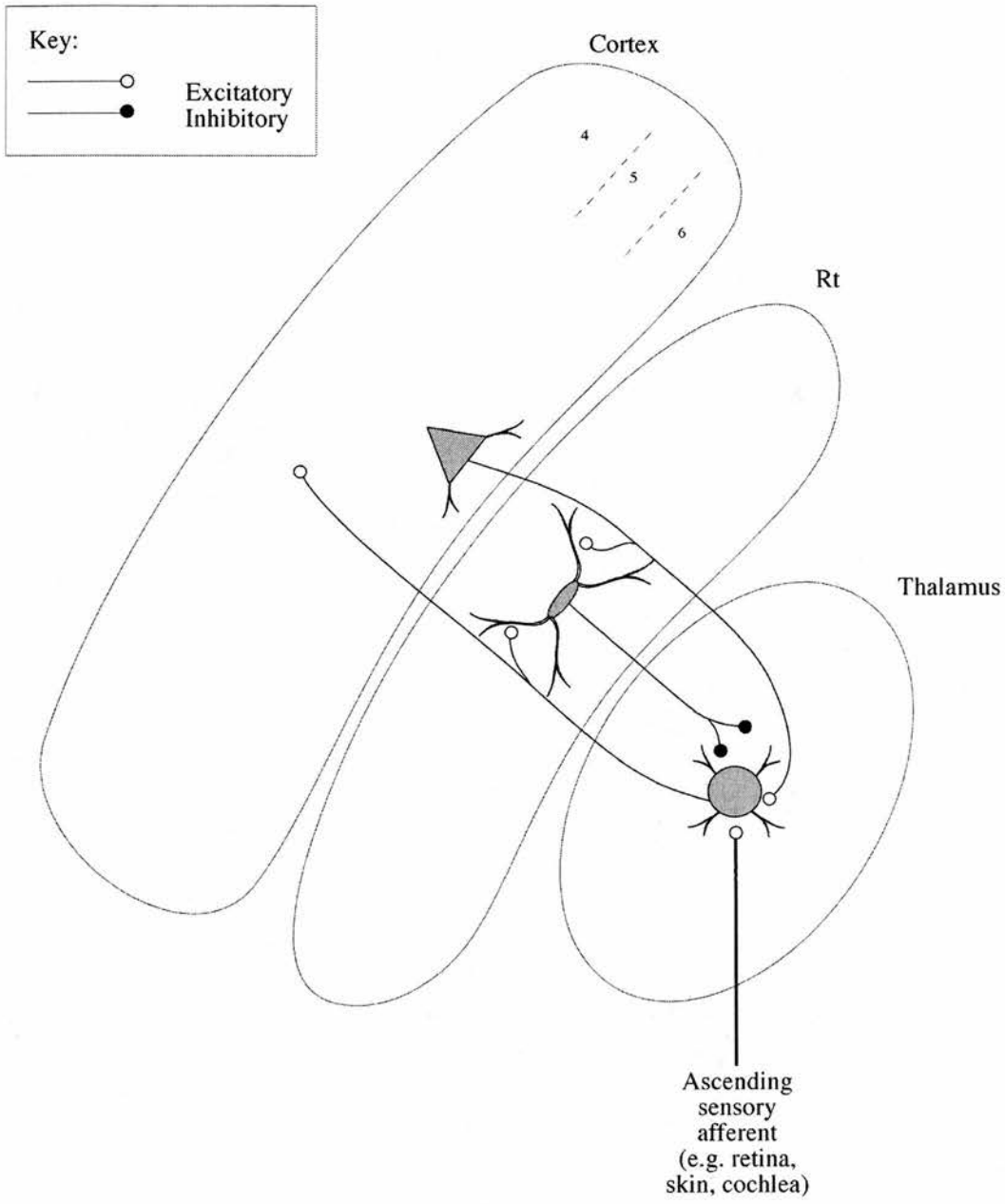


Figure 1.4. A schematic coronal section through rodent thalamus showing the major connections between thalamic relay cells, cells of the thalamic reticular nucleus (Rt) and the cerebral cortex. Cortical layers are indicated by numbers. Based on Guillery et al., (1998).

Sensory transmission involves two types of modality specific thalamic nuclei: first- and higher-order groups (Guillery, 1995; Sherman and Guillery, 1996; Guillery et al., 1998). First-order nuclei receive their primary 'driving' inputs from ascending sensory pathways (i.e. retina, skin and cochlea), while higher-order nuclei receive their main 'driving' inputs from descending pathways from cortical layer V. Table 1.1 illustrates the major first- and higher-order nuclei of central sensory pathways in rodents.

Central Sensory Pathway	'First-order' nucleus	'Higher-order' nucleus
Visual system	Dorsal lateral geniculate nucleus	Lateral posterior nucleus
Auditory system	Ventral nucleus of the medial geniculate complex	Dorsal nucleus of the medial geniculate complex
Somatosensory system	Ventrobasal complex	Medial division of the posterior thalamic complex

Table 1.1 Classification of modality specific thalamic relay nuclei (in rodents) according to 'first' and 'higher' order groups. 'First order' thalamic nuclei receive their 'driving' afferents from the various sensory surfaces (ascending afferents). 'Higher order' thalamic nuclei receive their 'driving' afferents from layer 5 of modality specific cortices (descending afferents). Both first- and higher-order thalamic nuclei receive 'modulatory' (descending) afferents from layer 6 of modality specific cortices (See Sherman and Guillery, 1996 for a comprehensive review).

First-order nuclei transmit information about stimuli in the extrapersonal environment to cerebral cortex (i.e. primary areas), whilst higher-order nuclei transmit information about sensory processing from one cortical area to another (see Sherman and Guillery, 1996). Collectively these nuclei play a pivotal role in the relay of information to the sensory cortices. Both first- and higher-order thalamic nuclei send efferents to cortex forming reciprocal connections with Rt en route to their respective targets (for a review see Guillery et al., 1998). The sensory sectors of Rt can be sub-divided into lateral and medial 'tiers' according to their connectivity with the first- and higher-order thalamic nuclei. 'Lateral' tiers receive axon collaterals from 'first-order' thalamic nuclei and their respective cerebral cortices (e.g. dorsolateral geniculate nucleus and primary visual cortex) while medial tiers, receive axon collaterals from the 'higher-order' thalamic nuclei and the cortical areas to which they project (e.g. lateral posterior nucleus of thalamus and secondary visual cortex). These connections are illustrated schematically in Figure 1.5. Typically, reticular tiers projecting to first-order nuclei (i.e. ventrobasal complex) display a well-defined topography, whereas those projecting to higher-order nuclei (i.e. the medial division of the posterior complex) seem not to (see especially Crabtree, 1996; but also see Pinault et al., 1995). The neurons within lateral but not medial tiers display a distinct 'slab-like' organisation. These slabs are present within visual (rat: Lozsádi et al., 1996; Coleman and Mitrofanis, 1996; rabbit: Crabtree and Killackey, 1989; bushbaby: Conley and Diamond, 1990; Hartings et al., 1991; Symonds and Kaas, 1978), auditory (Galago: Conley et al., 1991; cat: Crabtree, 1998) and somatosensory sectors of Rt (rat: Pinault et al., 1995; cat: Crabtree, 1996). These slabs have been identified as the organisational component of Rt (see especially Crabtree and Killackey, 1989).

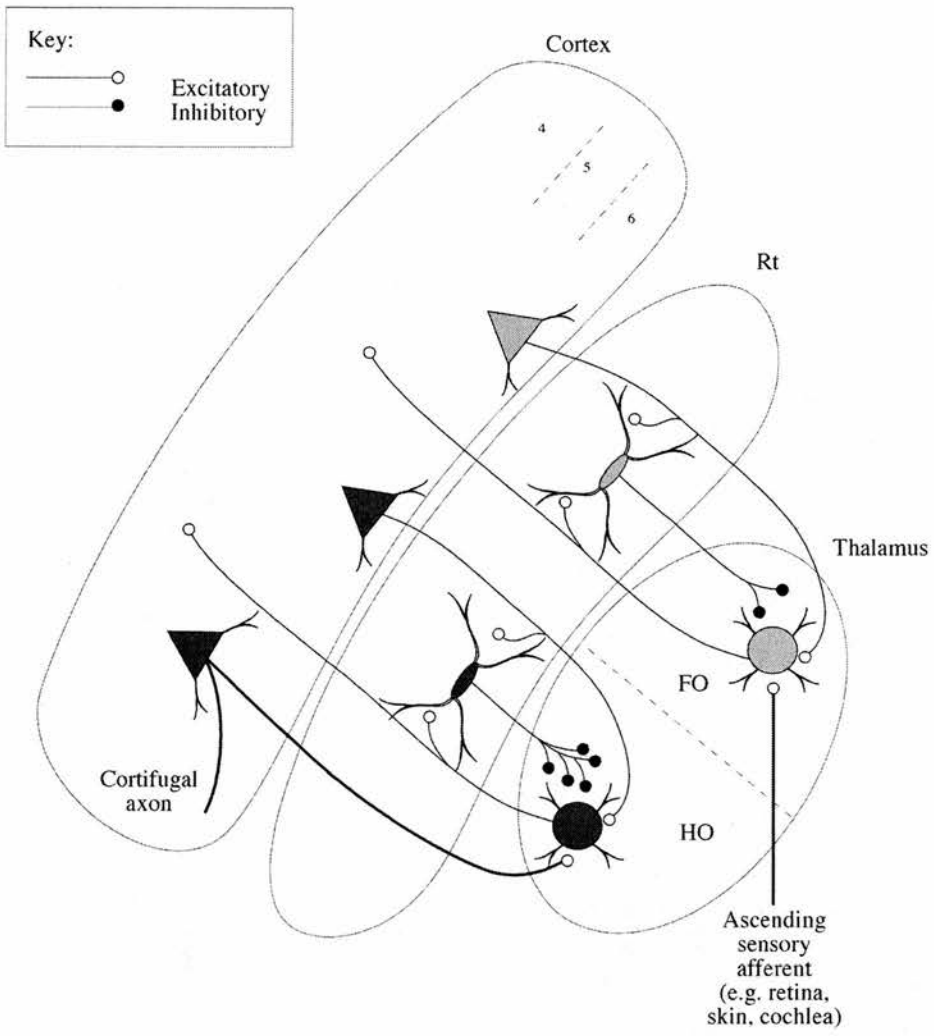


Figure 1.5. A schematic coronal section through rodent thalamus showing the major connections between cells of the thalamic reticular nucleus (Rt), first- (FO) and higher-order (HO) nuclei and their respective cerebral cortices. 'First-order' thalamic nuclei receive their main 'driving' afferents (thicker lines) from ascending sensory specific afferents whereas other 'higher-order' nuclei receive their 'driving' afferents from layer 5 of the cerebral cortex. Both first- and higher-order thalamic nuclei send efferents to cortex forming reciprocal connections with Rt en route to their respective targets. Based on Guillery et al., (1998).

These 'slabs' run parallel to the borders of the nucleus and are defined by the terminal zones of input (imparted through collaterals) from discrete regions of modality specific cortices (i.e. visual, auditory or somatosensory). 'Slabs' have also been described as zones of retrogradely labelled Rt cells (Crabtree, 1996, 1998, 1999). Thus, each 'slab' represents a small area of any given sensory surface (e.g. retina, skin, cochlea). This is illustrated schematically in Figure 1.6 using the rabbit visual system as an example (based on data from Crabtree and Killackey, 1989).

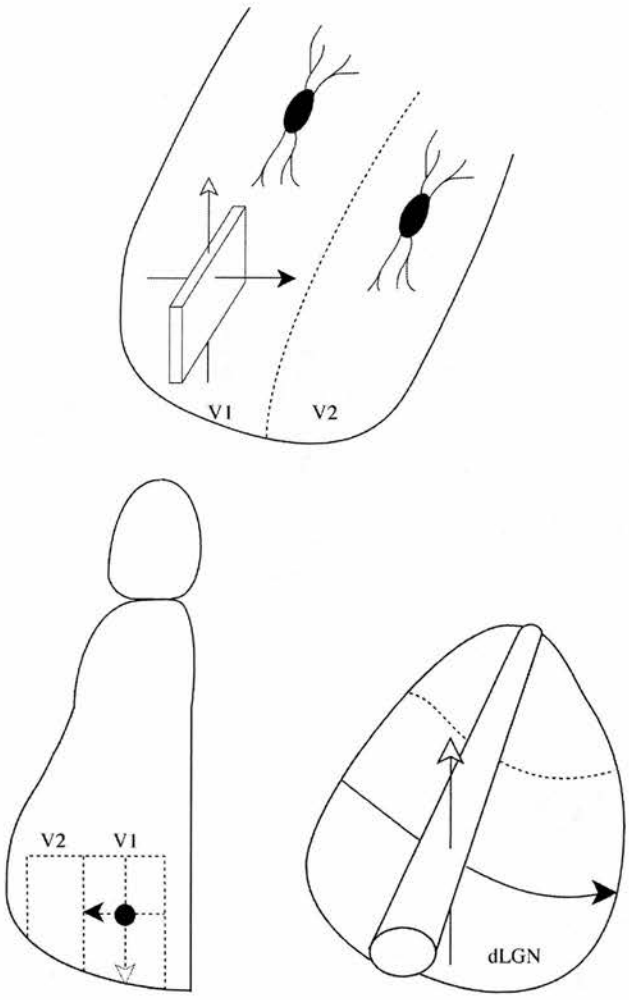


Figure 1.6. A schematic illustration of the 'slab-like' organisation within Rt and how it corresponds to the topographic maps in cortex and thalamus. A small area of visual cortex (V1), representing a discrete region of the visual field (bottom left) relates to a single column in the dorsal lateral geniculate nucleus (dLGN) (bottom right) which corresponds to a single slab within the lateral tier of the visual sector of the thalamic reticular nucleus (top). This slab like organisation is not found in the medial tier of the visual sector of the thalamic reticular nucleus that receives projections from secondary visual cortex (V2)(bottom left) and the lateral posterior nucleus (not shown) (Based on Mitrofanis and Guillery, 1998, using data from Crabtree and Killackey, 1989).

In summary, Rt sectors can be compartmentalised into distinct tiers (lateral and medial), each receiving afferents from functionally related although anatomically distinct thalamic nuclei (first-order versus higher-order) and their respective (reciprocally connected) cerebral cortices (see Guillery et al., 1998 for a review). However the degree of overlap between medial and lateral tiers can differ extensively both within and across species. For example, only a single tier can be recognised in the somatosensory sectors of cats (Crabtree, 1992a, Crabtree, 1996) and rabbits (Crabtree, 1992b), while a bilaminar organisation is found in the corresponding region in rodents (Pinault et al., 1995 – although see Crabtree et al., 1998). Three tiers can be recognised in the auditory Rt of Galago (Conley et al., 1991), which contrasts with the bilaminar organisation found in the auditory sector of cats (Crabtree, 1998). Using a thalamic slice preparation, Crabtree et al., (1998) have demonstrated that this overlap between medial and lateral zones of Rt represents the neuroanatomical basis by which neurons in separate (although functionally related) sensory nuclei of thalamus (first- and higher-order) interact and influence each other's activity. The stimulation of neurons in the ventrobasal nucleus (VB, a first-order somatosensory nucleus of dorsal thalamus), by local application of glutamate, inhibits the activity of neurons in the medial part of the posterior complex (POm, a higher-order somatosensory nucleus of dorsal thalamus) and vice versa. These two nuclei must interact through intrathalamic connections because other parts of the brain, including somatosensory cortex, are removed from the slice. Because cells projecting to VB or POm occupy overlapping territories within Rt, Crabtree et al., (1998) have interpreted their results as evidence for reticular mediated inhibition between functionally related, although anatomically discrete thalamic nuclei. Furthermore, because GABAergic interneurons are notably absent in the VB and POm of rats (Arcelli et al., 1997), Rt cells represent the only

source of intrathalamic inhibition. Collectively, these results provide compelling *physiological* and *morphological* support for reticular mediated intrathalamic pathways. The connections formed between first- and higher-order nuclei and Rt are illustrated in Figure 1.7.

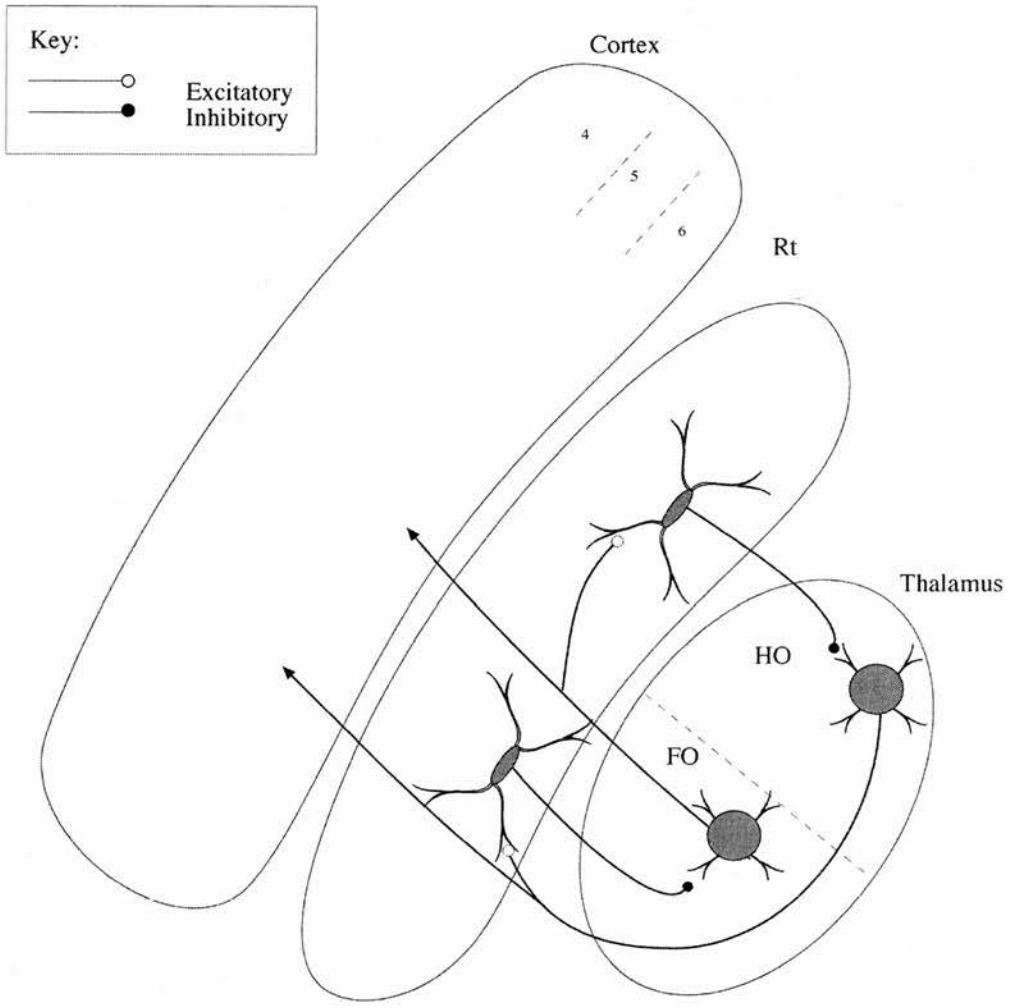


Figure 1.7. A schematic representation of a coronal section through the thalamus showing intrathalamic pathways linking a first-order (FO) nucleus e.g. ventrobasal nucleus and a higher-order nucleus (HO) e.g. medial part of the posterior complex. These are disynaptic pathways mediated by the thalamic reticular nucleus (Rt). The cells in the first- and higher-order nuclei project to cortex (indicated by arrows). Cortical layers (4,5,6) are indicated by numbers. Based on Crabtree et al., (1998).

It has been suggested that each sector of Rt represents a 'nexus' where functionally related pathways can interact and modulate each other's activities (Guillery et al., 1998). The results of Crabtree et al., (1998) are exciting because they imply that Rt is not only a convenient location for such interactions to take place, but that individual sectors of Rt actively participate in these interactions. Thus, reticular mediated thalamic processing is not limited to the interactions between a given sensory sector of Rt and a single nucleus of dorsal thalamus. Rather, in conjunction with inputs from cortex and brainstem, modality related first- and higher-order nuclei influence each other's sensory processing through their connectivity with Rt (Crabtree et al., 1998). While the precise functional significance of these intrathalamic pathways remains to be determined, these results suggest that Rt mediated inhibition is far more complex and interesting than previously thought.

Rostral Rt

While distinct 'slabs' (Crabtree, 1992 a, b) and 'tiers' have been demonstrated for the various sensory sectors of Rt, these organisational components are apparently absent in 'limbic' (Cornwall and Phillipson, 1988; Cornwall and Phillipson, 1988a, b, c; Cornwall et al., 1990; Lozsádi 1994,1995) and 'motor' (Cicirata et al., 1990) sectors (at least in rodents). This notwithstanding, a loose (albeit crude) topography exists within these regions.

'Limbic' Rt

In rodents the most rostral portion of Rt, categorised as 'limbic' by Jones (1975), forms complex reciprocal connectivity with anterior (Lozsádi, 1994, 1995; Gonzalez-Ruiz et al., 1997), mediodorsal (Cornwall and Phillipson, 1988; Cornwall and

Phillipson, 1988a; Cornwall et al., 1990), midline and intralaminar thalamic nuclei (see especially Cornwall and Phillipson, 1988 b, c and Kolmac and Mitrofanis, 1997, but also see Cornwall et al., 1990).

Rostral Rt and the anterior thalamic nuclei

The anterior thalamic nuclei are comprised of three distinct nuclei (anterodorsal anteromedial and anteroventral) that receive their principal afferents from the hippocampal complex: directly through the subicular complex (Swanson and Cowan, 1977; Sikes et al., 1977; van Groen and Wyss, 1990 a, b) and indirectly through a relay in the mammillary nuclei (Seki and Zyo, 1984; Shibata, 1992). In turn, the anterior thalamic nuclei are interconnected with the cingulate cortex and in particular the retrosplenial granular cortex (Domesick, 1969; Kaitz and Robertson 1981: Robertson and Kaitz, 1981). All three regions – anterior thalamus, hippocampus and retrosplenial cortex - are critical for the performance of spatial learning and memory tasks in rats (Aggleton et al., 1996; Byatt and Dalrymple, 1996; Morris et al., 1982; O'Keefe and Nadel, 1978; Vann et al., 2000). Because all of the anterior thalamic nuclei project to and receive topographically organised fibres from the most rostral and dorsal parts of Rt (Lozsádi, 1994, 1995; Gonzalez-Ruiz et al., 1997) it has been suggested that this sector of Rt contributes to spatial learning and memory in rodents (M'Harzi et al., 1991; Collery et al., 1993; Vann et al., 2000; although see Wilton et al., 2001).

Rostral Rt and mediodorsal thalamus

Mediodorsal thalamus (MD) in rodents is comprised of central, lateral and medial subdivisions (Leonard, 1972; Krettek and Price, 1977; Ray and Price, 1992). Lateral

MD receives projections from the superior colliculus and projects to the frontal eye fields. Medial and central divisions of MD receive their principal afferents from the primary olfactory cortex, amygdala and ventral pallidum (Price and Slotnick, 1983; Groenewegen, 1988; Kuroda and Price, 1991a, 1991b; Ray and Price, 1992). In turn, medial and central divisions of MD are interconnected with medial and orbital parts of the prefrontal cortex (respectively) (Leonard, 1972; Krettek and Price, 1977; Groenewegen 1988; Ray and Price, 1992). The prefrontal cortex, which forms dense reciprocal connections with MD, has recently been implicated in the ability to shift attention between perceptual features of complex stimuli in rodents (Birrell and Brown, 2000). All three segments of MD project to and receive topographically organised fibres from the most rostral, lateral, intermediate and medial parts of rostral Rt (Cornwall et al., 1990). Correspondingly, projections from the prefrontal cortex, en-route to MD, also collateralize within rostral Rt (Cornwall and Phillipson, 1988). By virtue of these connections rostral Rt might contribute to extradimensional set-shifting in rodents. Indeed the neuropsychological mechanisms underlying extradimensional set-shifting are not the property of a single brain area, such as the prefrontal cortex, but represent the concerted effort of a network of brain regions (for a review see Rogers et al., 2000), of which rostral Rt may represent an important, if not crucial, component.

Rostral Rt and intralaminar/midline thalamus

The intralaminar nuclei of thalamus constitute a heterogeneous group of structures that include the parafascicular, centromedial, centrolateral and paracentral nuclei. The midline nuclei include the parataenial, paraventricular, intermediodorsal and rhomboid nuclei. Like Rt (Jasper, 1960), the intralaminar nuclei were once considered

'non-specific' because their stimulation gave rise to widespread changes in cortical activity (Dempsey and Morison, 1942; Morison and Dempsey, 1942). Morphological demonstrations of widespread cortical connections seemed to provide further support of a role for these nuclei in relatively global functions (Herkenham, 1986; Royce et al., 1989). However, the notion of these nuclei mediating widespread cortical arousal has largely been abandoned. Contemporary neuroanatomical investigations have demonstrated that the individual intralaminar/midline nuclei have far more restricted projections to the cortex (and striatum) than previously thought (see Groenewegen and Berendse, 1994 for a review). Furthermore, numerous physiological investigations have since implicated the intralaminar/midline nuclei in various functions including gaze control (Schlag and Schlag-Rey, 1984; Schlag-Rey and Schlag, 1977), nociception and visceral functions (Albe-Fessard and Besson, 1973, Dong et al., 1978; Peschanski et al., 1981).

The intralaminar thalamic nuclei receive multi-modal (visual, auditory and somatosensory) sensory afferents from the spinothalamic and spinoreticulothalamic pathways (Chaouch and et al., 1983; Giesler et al., 1979; Kevetter and Willis, 1982; Peschanski and Besson, 1984) and from the deep intermediate layers of the superior colliculus (Chevalier and Deniau, 1984; Huerta and Hartings, 1984; Yamasaki et al., 1986). In turn individual intralaminar nuclei project back to the cortex and striatum *"with a restricted terminal field that overlaps minimally with the projection fields of adjacent nuclei"* (Groenewegen and Berendse, 1994). The inputs to intralaminar thalamus from the spinothalamic and spinoreticulothalamic pathways are thought to be involved in the transmission of pain through thalamus (Peschanski et al., 1981; Peschanski and Besson, 1984) while the inputs to intralaminar thalamus from the

superior colliculus, (in conjunction with outputs of these nuclei to striatum) are thought to be central to attention related orienting movements (Schlag-Rey and Schlag, 1977; Schlag and Schlag-Rey, 1984; Grunwerg and Krauthamer, 1992; Krauthamer et al., 1992). The superior colliculus, which projects to the intralaminar complex has also been implicated in the organization of visual and bodily orienting movements associated with attention i.e. the orientation of gaze or body towards and object of interest (Wurtz and Goldberg 1971; Sparks, 1988; Westby et al., 1990; Sprague and Meikle, 1965; Posner and Presti, 1987). Because rostral Rt forms dense reciprocal interconnectivity with the intralaminar thalamic nuclei (Kolmac and Mitrofanis, 1997; Hazrati et al., 1995), collectively, these regions (the superior colliculus, intralaminar thalamus and rostral Rt) might represent components of an interconnected network that mediates attention related orienting movements in response to behaviourally relevant external events. Indeed lesions to Rt (Friedberg and Ross, 1993), superior colliculus (Kirvel, 1975) or intralaminar thalamus (Orem et al., 1973; Hunsperger and Roman, 1976; Heilman and Watson, 1977; Ahlenius et al., 1982; Zainos et al., 1984) all lead to multi-modal sensory neglect and gross orientation deficits, supporting a role for these regions in the execution of attention guided behavioural responses. Rt probably contributes to sub-cortical stages of attentional processing, where currently significant sensory information is selected for further processing at the expense of currently irrelevant input, while intralaminar thalamus probably acts as an interface (between sensory input and motor output) translating behaviourally relevant sensory input into goal directed behaviour. While these investigations have revolutionised the role played by intralaminar thalamus, the idea of these nuclei mediating arousal has not been completely abandoned. An important role for the intralaminar thalamic system in global shifts in the sleep-wake

cycle is still advocated (Llinás and Ribary, 1993; Steriade et al., 1993). Furthermore, it has been suggested that intralaminar nuclei may contribute to the arousal that accompanies pain (Cechetto et al., 1985). Irrespective of the exact functions of the midline/intralaminar nuclei (and there may be many) Rt is indirectly implicated in their regulation by virtue of it's interconnectivity with this region.

Collectively these results imply that individual nuclei of anterior, mediodorsal and midline/intralaminar thalamus are connected to rostral Rt in a precise, topographic manner, similar to that found between caudal (modality specific) sectors of Rt and thalamus. Rostral Rt's reciprocal interconnectivity with these functionally diverse nuclei implicate this sector not only in relatively global 'non-cognitive' functions such as alertness and arousal (as originally proposed by Steriade and Deschenes, 1984), but also in specific 'cognitive' processes such as learning, memory, spatial processing and extradimensional set-shifting (M'Harzi et al., 1991; Collery et al., 1993; Tenas-Huerga et al., 1998, Vann et al., 2001; Wilton et al., 2000).

Motor Rt

The ventrolateral nucleus transmits information from cerebellum to motor cortex (Angaut et al., 1985). In turn, motor cortex projects back to ventrolateral nucleus via two parallel efferent projection systems: a direct projection from motor cortex to the ventrolateral nucleus (Kunzle, 1976, Asanuma et al., 1983, Niimi et al., 1963, Kawana and Kusama 1968, Jones and Burton, 1974, Hendry et al., 1979, Tanaka et al., 1983) and an indirect projection via Rt. Using neuroanatomical tracers (wheat germ-agglutinin horseradish peroxidase), combined with electrophysiological identification of motor cortical sites (where stimulation of discrete regions of primary motor cortex

produces overt skeletal responses in anaesthetised subjects), Cicirata et al., (1990) has identified the organization of this 'indirect' pathway from the motor cortex to the ventrolateral nucleus (via motor Rt). Remarkably, corticothalamic collaterals from primary motor cortex synapse on lateral and medial tiers within motor Rt in a dorso-ventral pattern, which mirrors the caudorostral body representation found in the direct pathway. 'Hindlimb', 'forelimb' and 'head' regions of cortex and ventrolateral thalamus project topographically and coincidentally to dorsal, central and ventral regions of motor Rt respectively. Motor Rt can be divided into medial and lateral tiers, according to their connections with discrete somatopically defined areas of the body surface. Corticothalamic and thalamocortical axons from motor regions representing 'forelimb 'and 'head' areas project to both tiers, while the 'hindlimb' area projects exclusively to the lateral tier. In turn, Rt neurons project back topographically to the area from which they receive afferents. Thus despite an absence of 'slabs', motor Rt displays a complex topography similar to that shown by the adjacent 'sensory' sectors. Such topography implies Rt may 'gate' or 'sharpen' signals being transmitted from the cerebellum (via ventrolateral thalamus) to primary motor cortex.

Collectively, anatomical and physiological studies imply that Rt can be divided into several distinct nuclei or 'sectors', each concerned with gating a characteristic type of signal (visual, auditory, somatosensory, cerebellar, etc.) through cytoarchitectonically distinct and functionally corresponding area (Jones, 1975) or group of areas of thalamus (Crabtree et al., 1998). This relationship between Rt and thalamus is primarily modulated by the neurotransmitter acetylcholine, the principal neurotransmitter released by the terminals of cholinergic neurons.

Brainstem and basal forebrain afferents to the thalamic reticular nucleus

Rt receives additional innervation from several brainstem (pedunculopontine and laterodorsal tegmental nuclei) and basal forebrain nuclei (this is illustrated in Table 1.2). Noradrenergic inputs from the locus coeruleus (Asanuma, 1992) provide a diffuse innervation that is distributed throughout Rt. By contrast, cholinergic afferents from the basal forebrain and the mesopontine nuclei project to Rt with marked regional and species variation (for a review see Semba, 1999). This contrast is most apparent when comparing felines and rodents (see Table 1.2). The cholinergic mesopontine nuclei of rodents project to central and caudal Rt, with fewer than 5% of these neurons projecting to rostral Rt (Spreafico et al., 1993; Jourdain et al., 1989). In contrast, cholinergic (Hallanger et al., 1987; Levey et al., 1987; Jourdain et al., 1989) and GABAergic afferents from the basal forebrain (Asanuma and Porter, 1990; Chen and Bentivoglio 1993) disproportionately innervate the rostral region of rodent Rt. Conversely, retrograde studies of the relative densities of forebrain and brainstem inputs to Rt in cats indicate dense, disproportionate innervation to rostral Rt from the cholinergic brainstem nuclei (Steriade et al., 1987; Parent et al., 1988). GABAergic afferents to feline Rt arise from the substantia nigra (Pare et al., 1990) innervating the portion of Rt which corresponds to the 'motor' region defined by Jones (1975).

Species	Source and references	Innervates	Neurotransmitter Identification
Rat	*Basal forebrain:*		
	Hallanger et al., (1987)	Rostral Rt	Cholinergic
	Jourdain et al., (1989)	Rostral Rt	Cholinergic
	Levey et al., (1987)	Rostral Rt	Cholinergic
	Asanuma and Porter (1990)	Rostral Rt	GABAergic
	Chen and Bentivoglio (1993)	Rostral Rt	GABAergic
	Brainstem afferents:		
	Mesopontine (PPTg and LDTg)		
	Spreafico et al., (1993)	Central and Caudal Rt	Cholinergic
	Jourdain et al., (1989)	Central and Caudal Rt	Cholinergic
	Locus coureleus (Asanuma, 1989)	Distributed throughout Rt	Noradrenergic
Cat	*Basal forebrain:*		
	Parent et al., (1988)	Caudal Rt	Cholinergic
	Steriade et al., (1987)	Caudal Rt	Cholinergic
	Bickford et al., (1994)	Caudal Rt	GABAergic
	Brainstem nuclei:		
	Mesopontine (PPTg and LDTg)		
	Steriade et al., (1987)	Rostral Rt	Cholinergic
	Parent et al., (1988)	Rostral Rt	Cholinergic

Table 1.2. Basal forebrain and brainstem inputs to Rt. Abbreviations: LDTg, laterodorsal tegmental nucleus; PPTg, pedunculopontine tegmental nucleus; Rt, thalamic reticular nucleus.

Additional GABAergic afferents from the basal forebrain (Bickford et al., 1994) and the pretectum (Cucchiaro et al., 1991) project exclusively to the visual sector of feline Rt. Ultrastructurally, cholinergic, glutamatergic, and noradrenergic axon terminals form asymmetric synapses with reticular dendrites, whilst GABAergic axon terminals from the basal forebrain and adjacent Rt neurons form symmetric contacts with both the soma and dendrites of reticular neurons. Figure 1.8 summarises the typical inputs received by Rt neurons, their principal neurotransmitters and their physiological effects (inhibitory versus excitatory).

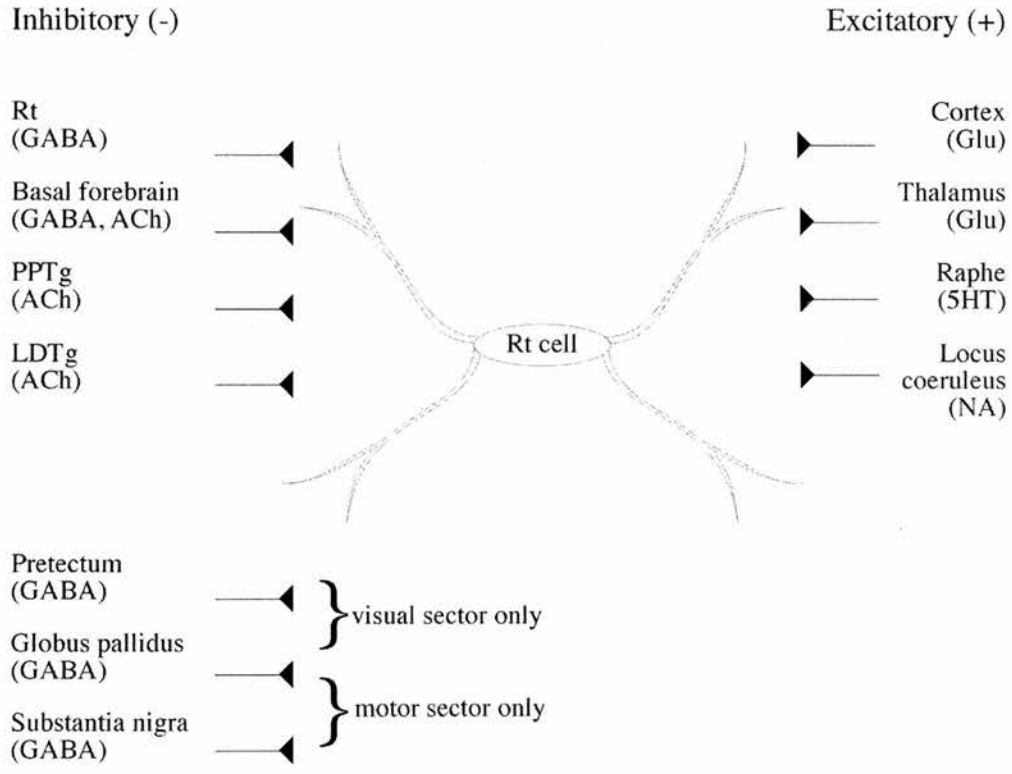


Figure 1.8. Typical inputs to cells of the thalamic reticular nucleus, their neurotransmitters and their post-synaptic effects. Based on Sherman and Guillery (2001). Abbreviations: ACh, Acetylcholine; GABA, Gamma-aminobutyric acid; Glu, Glutamate; NA, Noradrenaline; Rt, thalamic reticular nucleus; 5HT, Serotonin.

The neurotransmitters released by these afferents mediate diverse physiological effects within Rt. Glutamate[2] (De Curtis et al., 1989 although see Cox and Sherman, 1999), serotonin (McCormick and Wang, 1991) and noradrenaline (Kayama et al., 1982; although see McCormick and Wang, 1991) exert excitatory, depolarizing effects, while GABA (McCormick and Prince, 1986b) and acetylcholine (in vivo: Ben Ari et al., 1976; Godfraind, 1978; Sillito et al., 1983; in-vitro: McCormick and Prince, 1986; Marks and Roffwarg, 1991) mediate inhibitory, hyperpolarizing effects within Rt. With the exception of acetylcholine (see below) these neurotransmitters exert the same effects on thalamic relay cells. Glutamate (McCormick and von Krosigk, 1992) serotonin and noradrenaline (McCormick and Pape, 1990; Pape and McCormick, 1989) have excitatory effects on thalamic neurons while GABA has inhibitory, hyperpolarizing effects (Crunelli et al., 1988; Hirsch and Burnod, 1987; Thomson, 1988b). While the inputs listed in Figure 1.8 probably collectively determine Rt function and it's subsequent modulation of thalamocortical activity, acetylcholine (the principal neurotransmitter released by basal forebrain and mesopontine afferents) is particularly involved in the transfer of sensory information through thalamus (Ben-Ari et al., 1976; Dingledine and Kelly, 1977; Singer, 1977; McCormick and Prince, 1986; Francesconi et al., 1988; Hu et al., 1989; McCormick, 1989; Funke and Eysel, 1993; Marks and Roffwarg 1991). Single cholinergic fibres from the brainstem (mesopontine region) are known to innervate multiple thalamic structures, which may include Rt and the reciprocally connected relay nuclei (Shiromani et al., 1990; Uhlrich et al., 1988). Acetylcholine has been shown to depolarize thalamic relay neurons (in vivo: Eysel et al., 1986; Phillis, 1971; Sillito et al., 1983; in vitro: McCormick and Prince 1987; McCormick, 1991), whereas it hyperpolarizes GABAergic neurons of Rt

[2] Glutamate has also been shown to mediate inhibitory, hyperpolarizing effects on Rt neurons, see Cox and Sherman, 1999.

(in vivo: Ben Ari et al., 1976; Godfraind, 1978; Sillito et al., 1983; in-vitro: McCormick and Prince, 1986; Marks and Roffwarg, 1991). Brainstem cholinergic inputs might regulate sensory transmission by way of their inhibitory projections to modality specific regions of Rt and disinhibitory projections to functionally related dorsal thalamic nuclei. It is of great interest that cholinergic cells of the basal forebrain project to the rostral sector of Rt (Hallanger et al., 1987, Levey et al., 1987; Jourdain et al., 1989). Electrophysiological studies of rostral Rt, have concluded that neurons within this sector have generally diffuse, global actions on the thalamic neurons to which they project (Steriade and Deschenes, 1984). As there is strong evidence that basal forebrain neurons play an important role in cortical arousal (for a review see Semba, 1999), it may be parsimonious to assume that basal forebrain afferents to Rt form part of an interconnected network which regulates general arousal. In summary, it appears that regional variation of cholinergic afferents to Rt might underlie or contribute to the distinct cognitive processes thought to be carried out by rostral and caudal sectors of Rt.

Rt and Thalamus: the functional consequences of closed versus open loop connectivity

Rt appears to contribute to the control of sensory transmission through the thalamus (Schlag and Waszak, 1971; Frigyesi and Schwartz, 1972; Steriade and Wysinski, 1972; Sumitomo et al., 1976; Yingling and Skinner, 1976; Hale et al., 1982; French et al., 1985). This relationship between Rt and thalamus is primarily modulated by acetylcholine (Ben-Ari et al., 1976; Dingledine and Kelly, 1977; McCormick and Prince, 1986; Francesconi et al., 1988; Hu et al., 1989; McCormick, 1989; Funke and Eysel, 1993; Marks and Roffwarg, 1991). Most views of Rt function are based on the

idea that it provides a feedback inhibitory input to thalamus (i.e. being excited by glutamatergic relay cell axons and projecting back to these relay cells) and a feedforward inhibitory input from cortex (i.e. being excited by glutamatergic corticothalamic axons and projecting to thalamic relay cells) (Sherman and Guillery, 2001). However, both electrophysiological and anatomical studies have provided conflicting results. Cross correlation analyses of the simultaneous ongoing activities of Rt and thalamocortical, vibrissae-responding neurons revealed that a pair of such cells with reciprocal relations represented rather a minority of cases (Shosaku, 1986). However, there is evidence from intracellular recording from geniculate relay cells of cat and ferret that action potentials in a relay cell are sometimes followed disynaptically by an inhibitory post-synaptic potential (Lo and Sherman, 1994; Kim and McCormick, 1998) suggestive of a direct feedback. Correspondingly, extracellular and intracellular recording techniques in slices of ferret thalamus have demonstrated that reciprocal interactions between subsets of thalamocortical and Rt neurons are responsible for generating burst firing within thalamus (von Krosigk et al., 1993). Figure 1.9 is a schematic representation of the possible patterns of connectivity between Rt and relay cells.

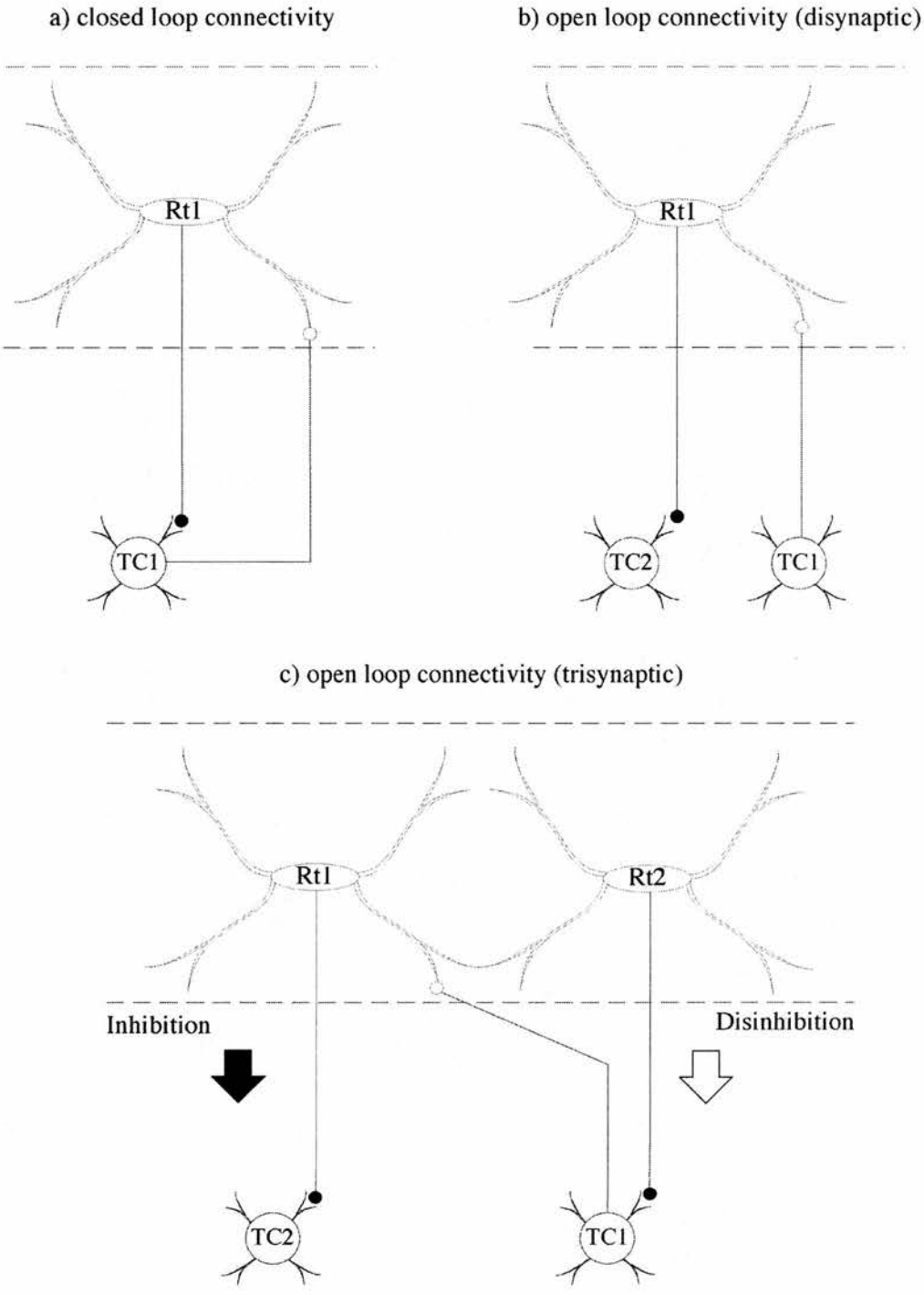


Figure 1.9. Schematic representation of the patterns of connectivity formed between thalamic reticular and relay cells. Based on Pinault and Deschenes (1998). Abbreviations: Rt 1, thalamic reticular cell 1; Rt 2, thalamic reticular cell 2; TC1, thalamocortical cell 1; TC2, thalamocortical cell 2.

The effect of Rt on thalamic relay cells (and ultimately thalamocortical transmission) depends on the details of such connectivity at the single cell level (Sherman and Guillery 2001). Subtle differences in connectivity could radically alter how Rt modulates thalamocortical transmission. For example, say Rt neurons project only to those neurons that provide them with an excitatory input, as in Figure 1.9 (a). The resultant inhibition will be feedback or true recurrent inhibition. Thus, activity in a given thalamocortical neuron (TC1) would induce activity in its respective Rt cell (Rt1) which would feedback onto the relay cell. It has been suggested that during wakefulness, such feedback inhibition initiates 'arhythmic' burst firing in thalamic relay cells, which may serve to alert cortex of behaviourally relevant input (Sherman, 1996). If however Rt neurons project only to adjacent thalamocortical neurons, as in Figure 1.9 (b), the resultant inhibition will be lateral or surround. In this case, activity in any given thalamocortical neuron (TC1) will activate the Rt neuron to which it projects (Rt 1). This neuron will inhibit adjacent (neighbouring) thalamocortical neurons (TC 2), creating a halo of inhibition around the active thalamocortical loop, thus elevating the signal to noise ratio and improving the discriminability and detectability of the thalamocortical signal. Such lateral inhibition may function to make some information more salient or conspicuous by precluding the relay of irrelevant information. Pinault and Deschenes (1998b) have examined, at the single cell level, the relationship between single thalamocortical and reticular neurons using the anterograde/retrograde marker biocytin (in conjunction with the extracellular and/or juxtacellular tracing technique). During this procedure, single neurons have positive nanocurrents applied to their membranes using tracer-filled micropipettes under continuous electrophysiological control. The labelling of cells within Rt was accompanied by the retrograde marking - 'back filling' - of thalamocortical

somatodendritic arbours that lay outside the anterogradely stained Rt axon terminal fields. These results suggest that Rt neurons typically do not form inhibitory synaptic connections with the thalamocortical neurons from which they receive excitatory synaptic contacts. Thus, reciprocal connections between Rt and thalamocortical neurons represent a relatively small proportion of cells, both anatomically and physiologically (see Shosaku, 1986). Such 'open-loop' connectivity may represent an anatomical substrate of a lateral inhibition mechanism within thalamus (Pinault and Deschenes, 1998b). This is illustrated schematically in Figure 1.9 (c). Activity in thalamocortical cell 1 (TC1) will activate Rt cell 2 (Rt2), which inhibits thalamic cell 2 (TC2) creating an inhibitory or centre surround, which improves the contrast and discriminability of behaviourally relevant information. Furthermore, given that Rt cells communicate via dendrodendritic synapses (Deschenes et al., 1985; Pinault et al., 1997 although see Ohara and Lieberman 1985; Cox et al., 1996), firing in cell Rt2 may also decrease the output of cell Rt1, reducing the level of inhibition on thalamic neuron 1 (TC1). This trisynaptic loop could form the anatomical substrate of a feedback *disinhibiton* mechanism, which could enhance the centre-surround contrast generated by lateral *inhibition*. These anatomical data are consistent with physiological data implicating Rt in both feedback and/or feedforward thalamic inhibitions and disinhibitions (for review see Sherman and Guillery, 1996 and Shosaku et al., 1989). Pinault and Deschenes (1998b) argue that lateral inhibition and feedback disinhibition operate conjointly producing a functional modularization of thalamus, which may underlie the selection of salient stimuli during selective attention (Crick, 1984).

Interim synopsis: Rodent Rt – functional anatomy

In the rat, specific sectors of Rt related to different sensory modalities - somatosensory, visual and auditory - have been identified by anatomical connectivity (Pinault et al., 1995; Bourassa and Deschenes 1995; Lozsádi et al., 1996) and physiological properties (Sugitani, 1979; Hale et al., 1982; French et al., 1985; Shosaku and Sumitomo 1983). Figure 1.10 illustrates the remarkable degree of correspondence between anatomical and physiological investigations of Rt organisation in rodents. The topographic representations (sensory maps) within Rt 'mimic' those found in their respective thalamic nuclei and, although this does not prove that Rt is involved in attention, it certainly implies the topographic modulation of thalamocortical activity by Rt – a fundamental requirement of any 'attentional searchlight' which samples and highlights discrete portions of the perceptual environment (Crick, 1984). Such topography highlights the potential for investigating Rt involvement in attention using the rodent.

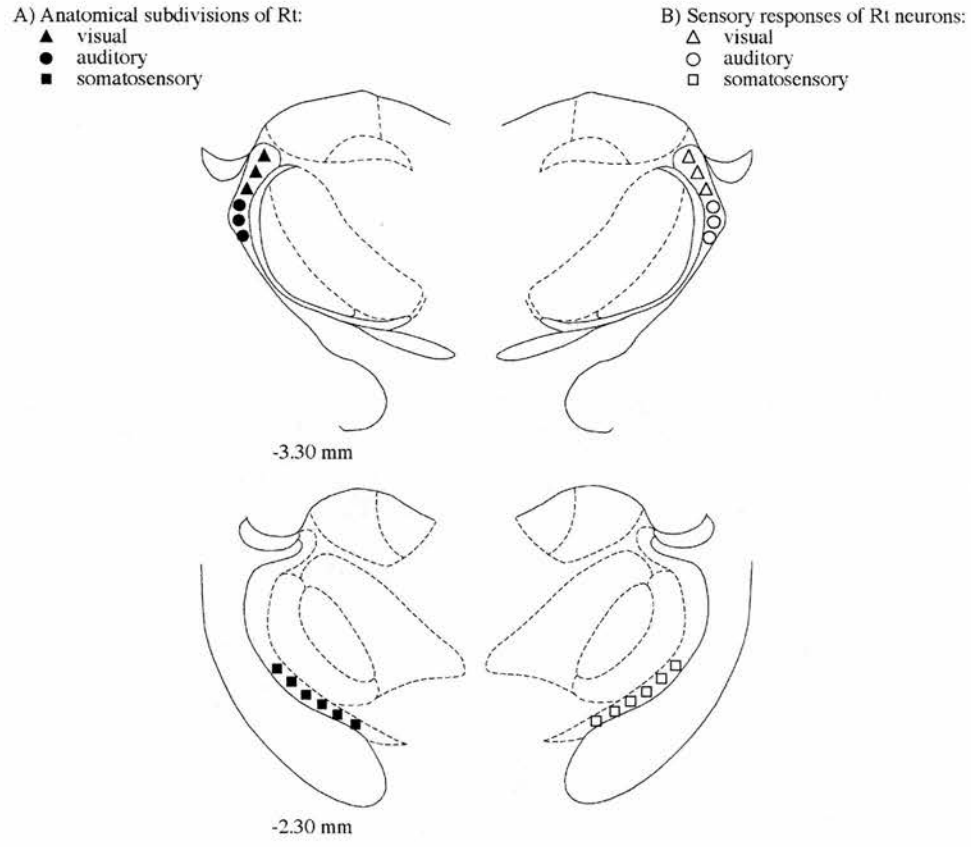


Figure 1.10. Coronal sections through Rt (adapted from Paxinos and Watson, 1997) showing the location of modality specific sectors, based on anatomical and physiological mapping of Rt in rodents. (A) Anatomical methods using neuroanatomical tracers have divided Rt into several sectors, each sending axons to a particular thalamic relay nucleus (visual, auditory and somatosensory) and receiving axon collaterals from the same nucleus and it's respective cerebral cortex. This pattern of distribution corresponds to that shown by physiological studies in rodents (B) which have categorised Rt neurons according to their response to stimulation of central sensory pathways. Numbers correspond to distance from Bregma.

Neurophysiology of the thalamic relay

Anatomical (see Guillery, 1998 and Crabtree, 1999 for a review) and physiological evidence (Suigitani, 1979; Hale et al., 1982; French et al., 1985; Shosaku and Sumitomo, 1983) has converged supporting Rt's role in the topographic modulation of thalamocortical transmission. The mechanisms by which this is achieved is still a subject for speculation, but recent insights into the dynamic nature of the thalamic relay in both thalamic slice (in vitro) preparations (Jahnsen and Llinás 1984) and awake, behaving animals (McCarley et al., 1983; Guido et al., 1992; 1995; Guido and Weyand 1995; Albrecht et al., 1998; Mukherjee and Kaplan, 1995; Ramcharan et al., 2000) provides a clue.

Tonic versus burst firing: In vitro investigations

In vitro investigations in guinea pig slice preparations have demonstrated that thalamic relay neurons respond in one of two different firing modes, which are known as burst and tonic (Jahnsen and Llinás 1984). Activation of these modes depends on thalamic relay cell's membrane potential, as they are based on a *voltage* and *time* dependent Ca^{2+} conductance that underlies a low threshold spike. This low threshold spike is inactivated by membrane depolarization more positive than -60mV, but is deinactivated at hyperpolarized levels, whereby it can be activated by a suitably large depolarization. When the cell membrane is more positive than -55mV, the neuron responds to injected current by firing at a linear rate, between 25 and 100 spikes per second - tonic activity. When the cell membrane is hyperpolarized i.e. more negative than -65mV for about 100msecs, the low threshold spike is activated with a burst of conventional Na^{+}/K^{+} action potentials riding the crest of the spike. During burst firing the neuron responds to an injected current with a succession of rapid spikes, firing at

approximately 300 spikes per second - a burst response. These firing patterns, a ubiquitous feature of thalamic relay cells, are illustrated schematically in Figure 1.11 (based on Lu et al., 1992).

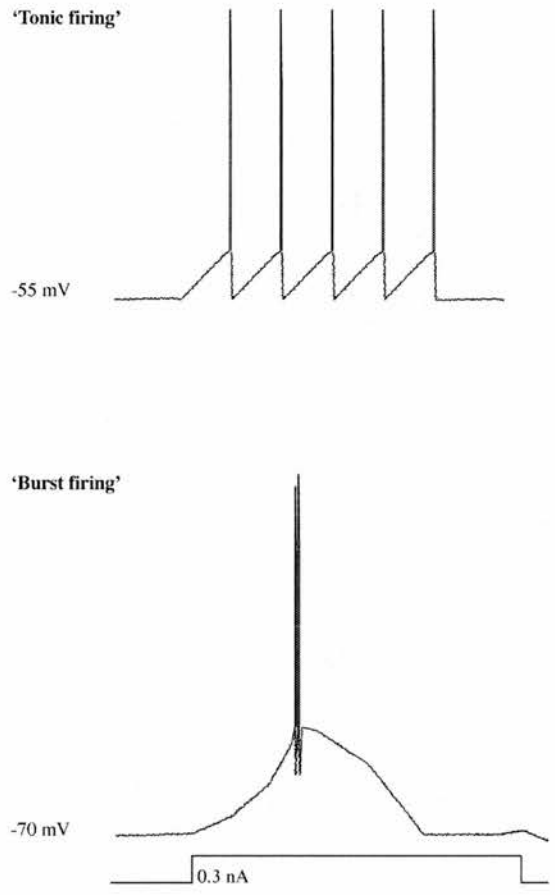


Figure 1.11. Tonic and burst firing modes for a relay cell from the cat's lateral geniculate nucleus recorded intracellularly in an in vitro slice preparation. The cell was held at different initial membrane voltages by adjusting the current injected through the recording electrode. Both recording traces show cellular responses to the same depolarizing 0.3 nA current pulse (shown under the bottom recording trace). When the cell is relatively depolarized (-55m V) the current injection evokes a stream of conventional action potentials, a tonic response. When the cell is relatively hyperpolarized (-70 mV), the same depolarizing impulse now triggers a low threshold Ca^{2+} spike. Riding the crest of this spike is a burst of conventional action potentials. Based on Lu et al., (1992).

Tonic versus burst firing: In vivo investigations

Subsequent in vivo investigations have demonstrated that thalamic relay cells can respond to sensory stimuli in either 'burst' or 'tonic' mode. It was originally believed that 'tonic' firing occurred only during wakefulness providing a linear, faithful relay of sensory information. Rhythmic 'burst' firing occurred only during periods of sensory detachment and consequently was thought to interrupt or scramble the relay of sensory information. These conclusions were drawn from early neurophysiological recordings which demonstrated the prevalence of 'bursting' in the thalamic relay during periods of behavioural quiescence i.e. slow wave sleep, general anaesthetic or during absence epileptic seizures and the presence of tonic firing in the thalamic relay neurons of alert animals (for a review see Steriade et al., 1993). Recent demonstrations in lightly anaesthetised and awake, behaving animals indicate that burst firing not only serves as an effective relay mode in the awake state (indeed, relay cells in burst mode can transmit slightly more information than the same cells in tonic mode (Reinagel et al., 1999)) but also facilitates the detection of subthreshold stimuli (Guido et al., 1992). Thalamic response mode also has an effect on temporal tuning (Mukherjee and Kaplan, 1995, Smith et al., 2000). In tonic mode, geniculate cells respond optimally to the lowest temporal frequencies of visual stimulation and continue to respond as temporal frequency increases, until the resolution limit is approached. In contrast, cells in burst mode respond poorly (indeed rarely) to low (<1 Hz) or high temporal frequency stimulations (>10 Hz), responding optimally to middle frequency visual stimulations (~4 Hz). Functionally, this means that dorsal lateral geniculate cells, when in burst mode, respond more readily to sudden changes in the visual environment and react poorly to static or gradual changes (Sherman and Guillery 2001). Collectively, these studies suggest that arhythmic bursting within

thalamus may provide a form of signal amplification that enables a hyperpolarized relay cell to alert cortex to the presence of potentially important, currently unattended stimuli (Sherman, 1996). Burst responses have also been recorded from somatosensory thalamus of awake monkeys (Ramcharan et al., 1999) and rodents (Fanselow et al., 2001). In rodents, thalamic bursting both precipitates and occurs throughout 'whisking' behaviours. Such whisking typically occurs during periods of alert immobility when animals are anticipating potential incoming, particularly novel or subthreshold, stimuli. This contrasts with the quiet immobile state, in which animals are awake, but less alert and not anticipating an impending stimulus. Crucially, burst firing is substantially reduced during periods of quiet immobility, supporting the idea that burst firing represents a event related 'wake-up-call' that alerts cortex to potentially interesting or dangerous stimuli (Sherman, 1996). From these results it appears that the basic principles abstracted from studies of the dorsal lateral geniculate nucleus (that burst firing represents a 'wake-up call' to cortex) can be extended to other categories of sensory information.

Control of response mode

Burst firing of thalamic relay cells appears to be associated with attentive, vigilant behaviours in rats (Fanselow et al., 2001), cats (Guido and Weyand, 1995) and primates (Ramcharan et al., 1999). Thalamic relay cells receive both 'driving' and 'modulatory' inputs (see Sherman and Guillery, 2001 for a comprehensive review). 'Drivers' (ascending sensory afferents) determine the receptive field properties of relay cells while 'modulators' determine non-receptive field properties such as firing rate. Electron microscope analyses of the dorsal lateral geniculate nucleus (dLGN), the first order thalamic nucleus by which visual information from the retina reaches cortex, have

revealed that 'driving' synapses, from the retina, constitute only 5-10% of synaptic input to dLGN neurons. Modulators which include GABAergic (i.e. from local interneurons and cells of the adjacent Rt), corticogeniculate and subcortical inputs from the mesopontine nuclei, constitute the other 90-95%. These non-retinal 'modulatory' afferents probably collectively determine whether visual thalamic relay cells operate in burst or tonic mode (see Sherman and Guillery, 2001). The tonic, possibly burst discharge of Rt neurons during wakefulness, and their powerful post-synaptic effects, may be influential in providing the proper priming actions - namely membrane hyperpolarization - on thalamocortical neurons necessary for the voltage dependent induction of event related burst discharge. Activation of the low threshold spike is voltage and time dependent. A lengthy, sustained inhibitory post-synaptic exceeding $\geq$ 100 msecs is required to de-inactivate the low threshold spike. Gamma-aminobutyric acid (GABA), the principal neurotransmitter released by Rt neurons, mediates its hyperpolarizing effect on thalamic relay neurons through activation of $GABA_A$ and $GABA_B$ receptors (Cox et al., 1997). The activation of metabotropic $GABA_B$ receptors, through G-protein coupled second messenger pathways, produces lengthy, stereotyped changes in thalamic neuron excitability lasting $\geq$ 100 msecs. This contrasts with the short lasting actions following activation of the ionotropic $GABA_A$ receptors (see Crunelli and Leresche, 1991 for a review of the literature). Because of the voltage and time dependence of the low threshold conductance, $GABA_B$ mediated inhibition is much better suited for eliciting the low threshold calcium conductance and its associated burst firing (Crunelli et al., 1988). The idea that Rt contributes to such $GABA_B$ mediated induction of the low threshold conductance is supported by the elimination of $GABA_B$ inhibitory post-synaptic potentials in thalamocortical relay cells following lesions of Rt (Steriade et al., 1985). This finding, coupled with the fact that

Rt terminals and calcium channels are disproportionately represented on the dendrites of thalamocortical relay neurons (see Wang et al., 2001) support a role for Rt in the initiation of event-related burst firing in awake, behaving animals.

Rt function in awake, behaving animals

Anatomical and electrophysiological evidence is clearly pointing to a role for Rt in attention. Anatomically, Rt appears suitably organised to modulate thalamocortical activity with topographic precision, a fundamental requirement of any attentional searchlight that samples and amplifies 'to-be-attended' thalamocortical activity, whilst simultaneously precluding the relay of irrelevant information (Crick, 1984). Contemporary electrophysiological investigations also support Crick's originally speculative hypothesis. Specifically, Rt has been shown to contribute to the responsiveness of both thalamus (Warren and Jones, 1994) and cortex (Hartings and Simons, 2000; Hartings et al., 2000) to afferent sensory input. These findings are fully consistent with a role for Rt in the amplification of behaviourally relevant information. Furthermore, there is now a growing body of behavioural evidence in support of this hypothesis. Weese et al., (1999) showed that lesions of Rt that included the visual sector did not render rats blind and did not result in an impairment in the ability of rats to detect and respond appropriately to visual targets. The lesions did, however, remove the processing advantage or 'priming effect' conferred by orienting cues preceding the target. Montero (1997, 1999, 2000) showed that Rt was involved in exploratory behaviour in a novel complex environment. Fos protein (a marker of neuronal activation) was elevated in the modality specific sector of Rt as a function of the cues (visual or somatosensory) to which rats attended during exploration. Remarkably, 'non-attending' sectors of Rt were devoid of Fos despite robust labelling in functionally

related cortical and sub-cortical regions. These results indicate that a primary function of Rt in awake, behaving animals is to optimize and enhance the transmission of essential, relevant cues from the environment. These results are consistent with the attentional deficits to visual, acoustic and somatic stimuli observed after domoic acid lesions of Rt in rats (Friedberg and Ross, 1993). These animals orient very slowly, or not at all towards stimuli, which in normal subjects, elicits rapid head turning in the direction of the stimulus. However, it should be borne in mind that results based on such overt orienting tasks cannot unequivocally demonstrate an attentional, as opposed to a sensory or motor role, for Rt. In strictly observational methods such as those employed by Friedberg and Ross (1993) and Montero (1997, 1999, 2000) the process being examined - attention - is simply defined by the procedure and no evidence is provided to characterise it (see Bushnell, 1998). Only by using more sophisticated paradigms where attention to modality specific stimuli are manipulated and quantified, can we attempt to understand the mechanisms underlying either Fos immunoreactivity within Rt or the behavioural dysfunction following Rt lesions.

Aims and hypotheses of thesis

The neural basis of attention

Although often discussed as though a unitary construct, 'attention' refers to multiple component functions that are important in the selection and processing of information (Gallagher and Rapp, 1997). These include the ability to discriminate relevant from irrelevant stimuli (selective attention), the ability to distribute attentional resources across multiple behaviourally relevant stimuli (divided attention), the ability to focus or maintain cognitive activity on these stimuli (sustained attention) and the ability to use

attended information to select and perform appropriate motor responses (attentional/executive control). Current views of attention suggest it is mediated by a network or networks of brain areas that perform different but interrelated functions (Posner and Petersen, 1990). Many neuropsychological theories postulate the existence of at least two separate, but interrelated attentional networks. A 'posterior' attentional network (which includes the parietal cortex, pulvinar of thalamus and superior colliculus) is thought to deal with the sensory aspects of attention i.e. the enhanced representation of behaviourally relevant stimuli and the orienting of attention to a location in space, while an 'anterior' attentional network is thought to be involved in various aspects of motor and/or attentional control i.e. the selection and execution of appropriate motor programs (see Posner and Petersen, 1990 for a review). Preliminary behavioural evidence implicates Rt in both selective (Montero, 1997, 1999, 2001) and executive attentional mechanisms (Wilton et al., 2001).

Rt and selective attention

Attentional selectivity involves the designation of some elements of a stimulus array for further processing at the expense of others. Selective attention necessitates the filtering of aspects of the environment to allow the processing of any given stimulus, dependent on its intrinsic nature or its position in space (Triesman, 1969). Preliminary behavioural evidence suggests Rt contributes to selective attention by operating as an attentional gate or 'filter' (Montero, 1997, 1999, 2000). Montero (1997) demonstrated selective Fos activation in the modality specific sector of Rt as a function of the cues (visual or somatosensory) with which subjects (normal or functionally blind rats, respectively) explored a novel environment. Such restricted activation is highly suggestive of a filtering process within Rt, where the transmission of behaviourally

relevant cues is enhanced relative to irrelevant cues. However, there are a number of shortcomings in Montero's task (1997, 1999, 2000) that this thesis seeks to address. First of all, exploratory behaviour alone represents a relatively crude measure of attentional function. Secondly, the sensory, motor and attentional prerequisites of this task are inextricably linked as rats explore their surroundings. The hypothesis that Rt represents an attentional substrate can only be adequately tested using a sophisticated paradigm in which the ability to orient attention can be distinguished from overt head and body movements (e.g. Weese et al., 1999). Because of the limitations of Montero's procedure, Fos activity within Rt cannot unequivocally be held to represent attentional as opposed to sensory or motor processing. With this in mind, Chapters 2 and 3 attempt to elucidate the mechanisms underlying Fos labelling within Rt. In Chapter 2, different groups of rats are trained to detect brief, unpredictable visual stimuli (presented to one side of the head only) in attentional paradigms with different response selection requirements. Provided Rt is involved in the enhanced processing of behaviourally relevant stimuli, visual Rt contralateral to attended visual stimuli should be disproportionately labelled with Fos protein irrespective of response direction. This would provide compelling evidence of a role for this nucleus in focussed attention to task relevant cues. In Chapter 3, a conditioned blocking paradigm (Kamin 1969) – in which prior conditioning to a stimulus 'blocks' conditioning to a second stimulus presented coincidentally with the first – is used to manipulate attention to stimuli in different modalities. This phenomenon provides an opportunity to test whether an attended, behaviourally relevant stimulus activates Rt more than an unattended stimulus. Different groups of rats are given conditioning sessions with a single stimulus, a light or a tone, and then given conditioning sessions with compound (light and tone) stimuli. Provided Rt mediates selective attention to task relevant cues,

Fos-immunoreactive neurons should be more numerous in the sector of Rt associated with the attended conditioned stimulus than in the sector associated with the unattended stimulus. Selective activation in the sector of Rt associated with attended stimuli would support the view that this structure acts as an attentional gate or filter, which modulates the flow of information between thalamus and cortex according to its behavioural relevance.

Rt and attentional/executive control

The rostral sector of Rt forms complex interconnectivity with the anterior (Lozsádi, 1994, 1995; Gonzalez-Ruiz et al., 1997), mediodorsal (Cornwall and Phillipson, 1988a, Cornwall et al., 1990) and midline/intralaminar nuclei (Cornwall and Phillipson, 1988b,c; Cornwall et al., 1990; Hazrati et al., 1995; Kolmac and Mitrofanis, 1997). These nuclei have been implicated in various cognitive functions including learning, memory (Beracochea et al., 1989, Markowitsch, 1982), and spatial processing (Aggleton et al., 1995, Aggleton et al, 1996). Because Rt forms interconnectivity with the anterior thalamic nuclei, it was originally suggested that this sector might be involved in spatial learning and memory (M'Harzi et al., 1991; Collery et al., 1993). As predicted, lesions to rostral Rt were found to selectively impair reference, but not working spatial memory (M'Harzi et al., 1991; Collery et al., 1993). However recent attempts to replicate these original findings, using more precise, circumscribed lesions have proved unsuccessful (Wilton et al., 2001). The lack of evidence supporting a role for rostral Rt in spatial processing, coupled with transient learning impairments following Rt lesions has led to the suggestion that rostral Rt might be involved not in spatial learning and memory per se, but in attention to (and abstraction of) task relevant features (Wilton et al., 2001). This idea

is at least anatomically plausible, because rostral Rt receives overlapping connections from the mediodorsal thalamus and the prefrontal cortex (Cornwall and Phillipson, 1988, 1988a; Cornwall et al., 1990). Medial prefrontal cortex in rodents mediates the ability to shift attentional set between perceptual features of complex stimuli in (Birrell and Brown, 2000). The term 'attentional set' refers to an acquired predisposition to direct attentional resources towards contextually significant aspects of the perceptual environment. The concept of 'attentional set' assumes that subjects analyse stimuli in terms of dimensions, that they can only optimally process a limited number of dimensions at any one time, and that reinforcement will strengthen attention and responses to particular dimensions. The formation of an 'attentional-set' requires that subjects filter sensory input from complex multi-dimensional stimuli, allowing the abstraction and processing of behaviourally relevant features (i.e. those contingent with reward) at the expense of irrelevant features. While set shifting capacities are vulnerable to medial prefrontal lesions, overall this capacity remains intact, as animals do form attentional set and do eventually re-direct their attentional resources towards the newly rewarded perceptual dimension (Birrell and Brown, 2000). This implies the neuropsychological mechanisms underlying extradimensional set-shifting are not the property of a single brain area, such as the prefrontal cortex, but represent the concerted effort of a network of functionally and anatomically related brain regions (see Rogers et al., 2000 for a review), of which rostral Rt may represent an important, if not crucial, component. The hypothesis that Rt might form a functional component of this network is investigated in Chapter 4 using an attentional set-shifting task that is formally the same to that used in humans and primates. The acquisition, maintenance and shifting of attentional set is examined in rats with neurotoxic (ibotenate) lesions centred on the rostral sector of Rt.

Although specific roles have been proposed for Rt in selective attention (Crick, 1984) and attentional/executive functions (Wilton et al., 2001), Rt might contribute not only to these functions, but to the entire collection of processes subsumed under the heading of 'attention'. Rt probably contributes to very early stages of attentional processing where interactions between Rt neurons, possibly sectors (although as of yet there is no compelling neurophysiological or anatomical evidence in adult rodents to support communication between sectors), provide the mechanism by which currently behaviourally irrelevant information is discarded and behaviourally relevant information is transferred to adjacent attentional sub-systems (i.e. those involved in selective, divided sustained and executive attentional mechanisms) for further processing. This thesis proposes that Rt plays a crucial role in early sub-cortical stages of attentional processing where significant information is selected for further processing at the expense of irrelevant input. This information may then be used to guide appropriate, efficient behaviour.

The cholinergic system and selective attention

The midbrain cholinergic system (which includes the PPTg and LDTg) operates in conjunction with Rt (see especially Ben Ari et al., 1976 and Dingledine and Kelly, 1977) regulating thalamocortical transmission. This is achieved by way of PPTg's inhibitory projections (Ben Ari et al., 1976; Godfraind, 1978; Sillito et al., 1983; McCormick and Prince, 1986; Marks and Roffwarg, 1991) to modality specific regions of Rt and disinhibitory projections (Eysel et al., 1986; Phillis, 1971; Sillito et al., 1983; McCormick and Prince 1987; McCormick, 1991b) to functionally related dorsal thalamic nuclei (Shiromani et al., 1990; Uhlrich et al., 1988). Although the cholinergic system, in conjunction with Rt, is known to modulate the transmission of sensory

information in anaesthetised animals (Ben-Ari et al., 1976; Dingledine and Kelly, 1977) the precise role played by this neurotransmitter, with respect to attention (especially covert orienting of attention), remains to be determined in awake, behaving animals. Specifically, the extent to which the cholinergic system contributes to 'cognitive' factors such as attention rather than 'non-cognitive' factors such as arousal or alertness remains to be determined. First of all it was necessary to demonstrate that acetylcholine was involved in selective attention as opposed to sensory or general alerting mechanisms. This was achieved using an attentional orienting task that allows cognitive processes such as attention to be studied separately from non-cognitive factors processes such as alertness and general arousal. Although somewhat tangential to the central theme of the thesis - Rt's contribution to subcortical attentional processes - this experiment represents the first of many ongoing experiments attempting to determine the relative roles played by acetylcholine and Rt in sensory-attentional phenomena.

As noted, the behavioural evidence bearing on a role for the cholinergic system in attentional orienting, as opposed to general alertness or arousal, is inconclusive and controversial. In humans and primates nicotine, a cholinergic agonist that operates at the nicotinic receptor, has been shown to enhance specifically the orienting of attention, having no effect on general alerting to visual stimuli (Murphy and Klein, 1998; Witte et al., 1997). This facilitatory effect is manifest as shortened reaction times to invalidly cued targets. Thus, nicotine is thought to facilitate the disengagement of attention from an invalid attentional focus. Pharmacological manipulations of the muscarinic cholinergic system however, have proved less consistent. Manipulation of the muscarinic cholinergic system, via systemic administration of scopolamine (a cholinergic antagonist which operates at muscarinic

receptors) was found to influence specifically the orienting of attention (manifest as increased reaction times to valid cued targets, decreasing the validity effect) (Davidson et al., 1999), whilst atropine (a muscarinic agonist) was found to mediate alerting to visual stimuli (Witte et al., 1997). As atropine and scopolamine act on the same neurotransmitter system, one might have expected atropine to have the opposite effect, namely shorted reaction times to validly cued targets. Furthermore, cholinergic blockade is typically associated with an increase in reaction times to invalid and not validly cued targets (Voytko et al., 1994; Parasuraman et al., 1992). In chapter 5, experiments are conducted to clarify the role of the cholinergic neurotransmitter system in the arousal and orienting to peripheral visual targets. Using a cued target detection task that is formally the same to that used in humans (Posner, 1980; Murphy and Klein, 1998; Fernandez-Duque and Posner, 1997) and primates (Bowman et al., 1993; Witte et al., 1997; Davidson et al., 1999) rats were required to fixate a central visual stimulus and respond to the onset of visual targets presented randomly in two visual field locations. The location of the target's appearance is preceded by a cue that is either valid (cue and target at same location) or invalid (cue and target at opposite locations). In order to test the hypothesis that nicotine mediates specifically orienting and not alerting to visual targets, a second group of subjects will receive additional trials where the cues are spatially uninformative (bilateral cues) or omitted altogether. The subtraction of reaction times to bilateral cued trials from non-cued trials provides an index of general alertness. It is predicted that nicotine will selectively facilitate the orienting of attention and have no effect on alerting to cued targets. This would provide evidence that the neural mechanisms mediating orienting and alertness are subserved by distinct neurotransmitter systems in rodents, thus supporting a role for the midbrain cholinergic system in focused attention to task relevant cues. Chapter 5 also intends to

contribute to the debate over the effect of muscarinic cholinergic blockade, which has been less consistent in primates. In order to clarify the role of the muscarinic cholinergic system in attentional orienting, the muscarinic antagonist scopolamine is administered to rats trained to perform a cued target detection task. It is predicted that scopolamine will increase the validity effect, manifest as a disproportionate lengthening of reaction times to invalidly cued targets. Although somewhat tangential to the central theme of this thesis, Rt's contribution to subcortical attentional processes, this chapter nonetheless represents the first of many ongoing experiments attempting to determine the relative roles played by Rt and acetylcholine in sensory-attentional mechanisms in awake, behaving animals. The results are discussed in terms of the possible conjoint roles played by Rt and the basal forebrain and midbrain cholinergic systems with respect to sensory-attentional processes.

Concluding remarks

By virtue of its anatomical connectivity and physiological characteristics, Rt has been ascribed various functional roles: non-specific modulator of thalamocortical transmission (Scheibel and Scheibel, 1966; Jones, 1975); mediator of selective attention (Yingling and Skinner, 1976; Crick, 1984) and thalamic pacemaker (Steriade and Deschenes, 1984). Although specific roles for Rt in the control and generation of thalamocortical activity have been demonstrated during sleep (Steriade and Deschenes, 1984), the precise function of this nucleus in awake, behaving animals remains to be determined. While it has been suggested that Rt mediates selective attention by gating thalamocortical transmission, the behavioural evidence bearing on this issue is inconclusive (Weese et al., 1999; Montero, 1997, 1999, 2000). Using a multi-methodological approach - functional immunohistochemistry, lesion studies - in

conjunction with robust behavioural paradigms - this thesis attempts to understand the functional significance of Rt in awake, behaving rodents.

Chapter 2: Thalamic reticular activation in a task of attentional orienting

Abstract

All senses are routed through the thalamus to cerebral cortex. Thus, the thalamus is often referred to as the sensory gateway to cortex. Located between thalamus and cortex is a thin lamina of neurons called the Rt, which may function as an attentional gate. In this study, we examine Fos protein expression – a marker of neuronal activation – in rats trained to perform a visual reaction time task. The brief, unpredictable target stimuli to which the rat was required to respond were preceded by cues, which served to direct attention toward the target location. The visual cues and targets were presented to one side only. Different groups of rats were trained to respond towards or away from the side of the target. Fos protein was observed, bilaterally and uniformly, in the dorsal lateral geniculate. There was Fos protein in the visual sector of Rt, which was non-uniform, being greatest contralateral to the visual stimuli, regardless of the direction of the response. These data support the suggestion that the visual sector of Rt is selectively activated by the allocation of visual attention.

Introduction

Attention involves the selection of stimuli for processing, at the expense of others. Attentional selectivity implies a mechanism that filters sensory information, enhancing the transmission of some, while attenuating the transmission of other sensory information to brain areas for further analysis. Anatomical (Jones, 1975; Ohara and Lieberman, 1985) and electrophysiological (Yingling and Skinner, 1977; Skinner and Yingling, 1977; Sumitomo et al., 1976; Shosaku and Sumitomo, 1983) investigations of Rt suggest that this nucleus may act as a filter of information between thalamus and cortex. The primary source of excitation within Rt comes from glutamatergic thalamocortical and corticothalamic axons, which send collaterals to Rt en-route to their respective targets. Rt is divided into modality specific sectors, with topographic mapping of projections from sensory and motor cortices (for comprehensive review see Guillery et al., 1998 and Crabtree, 1999). In the rat, specific sectors of Rt related to different sensory modalities - somatosensory, visual and auditory - have been identified both physiologically (Sugitani, 1979; Hale et al., 1982; French et al., 1985; Shosaku and Sumitomo, 1983), and anatomically (Pinault et al., 1995; Bourassa and Deschenes, 1995; Lozsádi et al., 1996). Rt sends inhibitory projections back to thalamus, both reciprocally to areas from which it receives input (Jones, 1975) and in some cases to other functionally related thalamic nuclei (see Crabtree et al., 1998). In addition, neurons within Rt can inhibit each other (Cox et al., 1997; Sanchez-vives et al., 1997). As far as current electrophysiological evidence indicates, this lateral inhibition within Rt could improve the relay of relevant information whilst attenuating the relay of competing stimuli within the same modality. However, given that the dendritic fields and axon collaterals of Rt neurons

often extend beyond the modality specific sectors (Scheibel and Scheibel, 1966; Cox et al., 1997), it is possible that one sector of Rt could actively inhibit another sector. However, the anatomical findings of Scheibel and Scheibel (1966) and Cox et al., (1997) have to be interpreted cautiously as they were performed on the brains of neonatal rats. Contemporary investigations have since demonstrated that the lengthy dendritic profiles and axon collaterals found in the Rt of neonatal rats (a potential neural substrate of inter sector communication) are subsequently lost or truncated during development (see especially Pinault et al., 1997).

Using an open field paradigm in conjunction with Fos immunohistochemistry, Montero (1997, 1999) demonstrated selective Fos labelling in the modality specific sector of Rt as a function of the cues (visual or somatosensory) with which subjects (normal or amblyopic rats, respectively) explored a novel environment. This restricted activation is highly suggestive of a filtering process within the Rt, whereby behaviourally relevant cues are selectively relayed to cortex at the expense of irrelevant cues. However, it must be noted that the rats engaged in tactile exploration of the environment were amblyopic, so one cannot rule out the possibility that there was abnormal processing of visual information. Moreover, it is not possible to separate the exploratory and attentional components of this behaviour.

In the present study, rats were trained to perform a visual reaction time task. In order to distinguish between the sensory/attentional and the motor components of performance, different groups of rats were trained to respond either towards the visual targets or away from the targets (Carli et al., 1989). If Rt is involved in selective attention to stimuli, then irrespective of which direction rats respond, neurons in

visual Rt contralateral to attended visual stimuli should be disproportionately labelled with Fos protein.

Experimental Procedures

Subjects

Eleven experimentally naïve, male Lister Hooded rats (Charles River, U.K), two months old at the start of behavioural training (weight range 210-290gm), were used. The rats were housed in pairs and given controlled access to food, comprising 15-20g a day of standard laboratory chow, in addition to earned sucrose pellets. Water was freely available in the home cage. The colony room was on a twelve-hour light/dark cycle, with lights on at 7am. The procedures were licensed under the UK Animals (Scientific Procedures) Act 1986.

Apparatus and behavioural testing

Training was conducted in four nine-hole operant boxes (CeNes Ltd., Cambridge, U.K), each enclosed in a sound-attenuating chamber, ventilated by fans, which provided a steady stream of background noise (50 decibels). The operant box was made of aluminium with the exception of the front wall, which had an outward opening Plexiglas door, through which the rat was introduced into the chamber. A magazine tray, with an inward opening Plexiglas panel, was recessed into the front wall of the chamber beneath this door. A quiet-operation automatic pellet-dispenser delivered single 45mg sucrose pellets (Noyes precision food pellets, Bioserv Inc., New Jersey) into the magazine tray. Entries to the magazine tray were recorded by a micro-switch. The rear wall was curved and nine holes (1.5 cm^2 opening) were situated, 1 cm above the metal grid floor, in a horizontal array on this wall. At the rear of each of the nine holes was a light bulb. Photocells detected breaks in an infrared

beam projected vertically across the front of each hole. For this experiment, only the middle three holes were used; the others were capped.

Lateralized Target Detection Task

The hole central in the array was designated the 'fixation' hole. One of the adjacent holes was the 'stimulus hole' and one was the 'response hole'. For five rats, the stimulus hole and the response hole were the same. For the other six, the stimulus and response holes were on opposite sides, as shown in Figure 2.1.

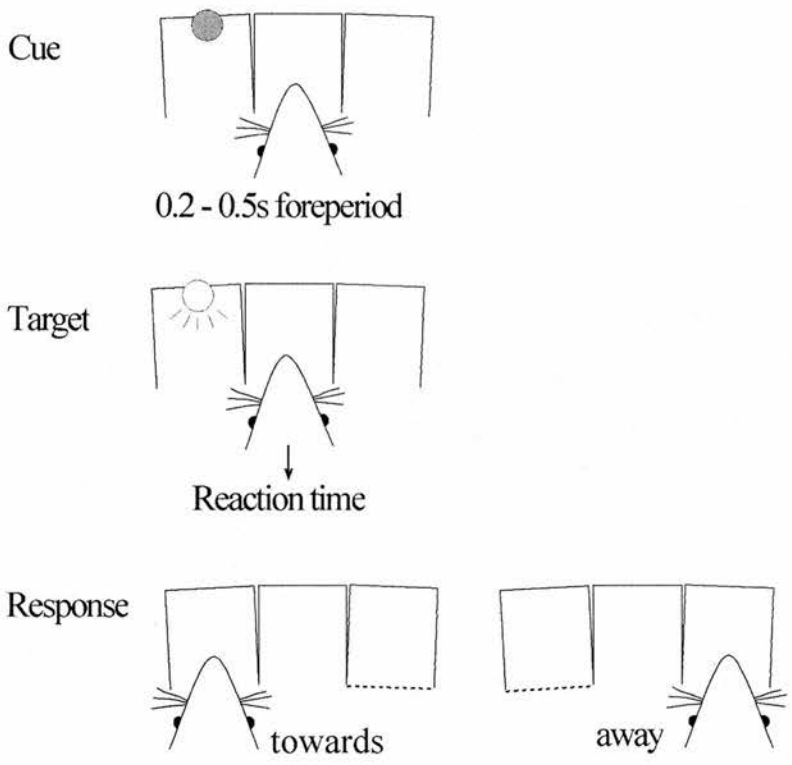


Figure 2.1. Schematic representation of the task requirements. Rats were trained to make a sustained nose-poke and wait (0.2 – 0.5 secs) for the target. A brief, dim, visual cue was presented in the left or right aperture at the beginning of the foreperiod. Different rats reported the target by responding either towards or away from it.

The fixation hole was illuminated at the start of the trial. Subjects were required to initiate a sustained nose poke in the fixation hole, which turned off the light in the fixation hole and lit the stimulus hole dimly for 100 msecs. This cue served to attract attention to the target location. Previous work has demonstrated that attentional orienting to such visual cues is automatic and involuntary (Ward and Brown, 1996). Even when cues do not faithfully predict target location (as they do here), attention is nevertheless drawn to cue location. The fixation position had to be maintained for a variable and unpredictable foreperiod (0.2, 0.3, 0.4 or 0.5 secs) preceding the target, which was the bright illumination, for 200 msec, of the stimulus hole. The rat was then required to make a nose-poke in the response hole to earn a single sucrose pellet. Anticipatory errors (withdrawal from the central hole prior to, or within 100 msecs of, the imperative) or late errors (failure to respond within 1.5 secs) resulted in a 1 sec time-out period of darkness in the chamber and no food reward. Thus, if the animals were not attending to the target, they would not be able to complete the trial to obtain a reward. Each training session lasted until successful completion of 120 correctly completed trials or until 60 minutes elapsed. Three days prior to the final test session, rats were double-run (for 2 hours or until they had completed 240 correct trials) in preparation for the final test session. The final test session lasted 1 hour and 45 minutes, regardless of the number of completed trials. This time period was used in accordance with the temporal kinetics of Fos induction (Dragunow and Faull, 1989; Herrera and Robertson, 1996; Sagar et al., 1988) and successful experimental methodology adhered to by Montero (1997, 1999, 2000).

Fos immunohistochemistry

Repeated stimulation of certain brain regions leads to activation of the *c-fos* nuclear proto-oncogene and subsequent production of Fos protein, which can be used as a high-resolution marker of neuronal activation (Dragunow and Faull, 1989; Herrera and Robertson, 1996; Sagar et al., 1988). Fos protein has been used to identify neurons responsive to visual, tactile and auditory stimuli in the cortical and sub-cortical centres of rats (Bullitt, 1990; Friauf, 1992; Mack and Mack, 1992; Montero and Jian, 1995). In order to prevent non-specific Fos induction, rats were held overnight in darkness with minimal exposure to auditory and visual stimuli. Rats were carried to the darkened testing room in covered cages and placed immediately in operant chambers. Once in the operant chambers, rats performed the unilateral cued target-detection task for 1 hour 45 minutes and were then anaesthetised before being transcardially perfused with saline, followed by 4% paraformaldehyde. Brains were removed immediately and 1 in 4, 40 μm, coronal sections were cut on a freezing microtome and placed into phosphate buffer solution (pH 7.4). The sections were then rinsed twice, for 3 minutes, in 0.1M phosphate buffer saline (PBS) and incubated in blocking solution (100ml PBS, 20ml normal goat serum, 1ml 0.1% Triton X-100) for 60 minutes at room temperature. Afterwards, the sections were rinsed for 5 minutes in PBS and incubated for 48 hours in goat anti-*c-fos* (dilution 1:10,000) at 4°C on a shaker. Following a 5 minute wash in PBS, the sections were incubated on a shaker for 1 hour in rabbit anti-goat biotinylated secondary antibody. This was proceeded by five PBS rinses and a 45 minute incubation in ABC complex (antibody diluting solution, substrate A and Substrate B) on a shaker. To visualise the Fos protein, sections were rinsed five times in PBS and developed using Sigmafast 3'3

Diaminobenzidine Peroxidase substrate (Sigma, UK). Finally, sections were mounted on gelatin coated glass-slides, air dried and coverslipped with DPX.

Fos immunoreactivity: quantitative analysis

Holding the level of microscope illumination constant across sections and hemispheres, counts were made of positively stained neurons. Images of sections were projected onto a 19" screen with a superimposed 2"-square grid. For each section, cells in the nuclei of interest were counted twice, on different occasions, with the experimenter blind to condition. However, the repeat-counts never differed by more than 10% and so the first count was always used. A correction procedure (such as the Abercrombie correction) is generally applied to raw counts of cells in histological sections, to allow for sectioned particles ('lost caps') being counted more than once, so overestimating total counts. However, as the sections counted here were separated by 120 μm (1:4, 40 μm, sections), total counts are certainly underestimated and therefore no correction is applied. Of interest in the present study, was the inter-hemisphere non-uniformity and no conclusions were drawn concerning absolute number of neurons.

Counts of Fos immunoreactive neurons were made for both hemispheres in two areas of interest: the Rt, and the dorsal lateral geniculate nucleus (dLGN). The dLGN was selected because it is the thalamic first-order visual relay nucleus. Other visual centres – like visual cortex/superior colliculus – were intentionally not selected for comparison, because activity within these areas is known to be modulated by attention. The purpose of selecting the dorsal lateral geniculate was to demonstrate

that any non-uniformity within Rt could not be accounted for by non-uniformity of visual input but rather must be due to non-uniformity of attention.

For Rt, the dorsocaudal region (visual sector) was selected on the basis of electrophysiology (Sumitomo et al., 1976; Hale et al., 1982; French et al., 1985) and anatomy studies (Coleman and Mitrofanis, 1996; Lozsádi et al., 1996). Neurons were counted in the dorsal 50% of the nucleus, between bregma –4.1 and bregma –3.1mm, at 10x magnification. Inspection of the rest of Rt, throughout its rostral-caudal extent, revealed that there was no other consistent labelling. In the dLGN, only the monocular segment was sampled over one field per section at 25x magnification. This segment receives input from the contralateral eye (Hayhow et al., 1962; Montero et al., 1968). We took the average count over the eight sections on which dLGN was present.

Results

Fos activation patterns in attending and non-attending hemispheres

Fos immunoreactive neurons were observed in dLGN and Rt in both attending (contralateral to the stimulus hole) and non-attending (ipsilateral to the stimulus hole) hemispheres. Table 2.1 summarises the number of Fos immunoreactive neurons in dLGN and Rt (visual sector) across 'attending' and 'non-attending' hemispheres of rats responding either towards or away from cued target stimuli.

Group	Thalamic reticular nucleus (visual) (absolute number of Fos immunoreactive neurons)		Dorsal lateral geniculate nucleus (average number of Fos immunoreactive neurons/section)	
Respond towards:	'attending' hemisphere	'non-attending' hemisphere	'attending' hemisphere	'non-attending' hemisphere
99/051	136	106	51.4	52.2
99/052	225	179	58.6	54.8
99/328	349	156	69.2	67.7
99/329	506	451	66.3	66.0
99/333	229	101	63.6	65.2
Respond away:				
99/041	281	169	68.1	67.6
99/453	472	442	64.3	60.5
99/456	557	438	84.5	81.1
99/334	436	291	40.0	43.8
99/002	145	102	75.6	76.3
99/007	124	79	79.5	79.0

Table 2.1. Comparison of Fos immunoreactive neurons in the visual thalamic reticular and dorsal lateral geniculate nuclei across 'attending' and 'non-attending' hemispheres of rats responding either towards or away from cued target stimuli.

Fos activation: Dorsal lateral geniculate nucleus

In dLGN, there was no difference between hemispheres in the magnitude or distribution of Fos immunoreactive neurons. In particular, there was no differential activation as a function of either the side of the stimulus or the side of the response hole. Table 2.1 reveals that uniform Fos labelling was consistently seen in the dorsal lateral geniculate nucleus, across hemispheres, for every animal, irrespective of response direction. This is shown in Figure 2.2, a bar graph of the percent non-uniformity across groups, showing that for dLGN there is no systematic pattern of non-uniformity across hemispheres (t-test: $p > 0.05$). Figures 2.3 and 2.4, photomicrographs with schematics (adapted from Paxinos and Watson, 1997), illustrate attending and non-attending dLGN (top panel) in representative cases from both groups.

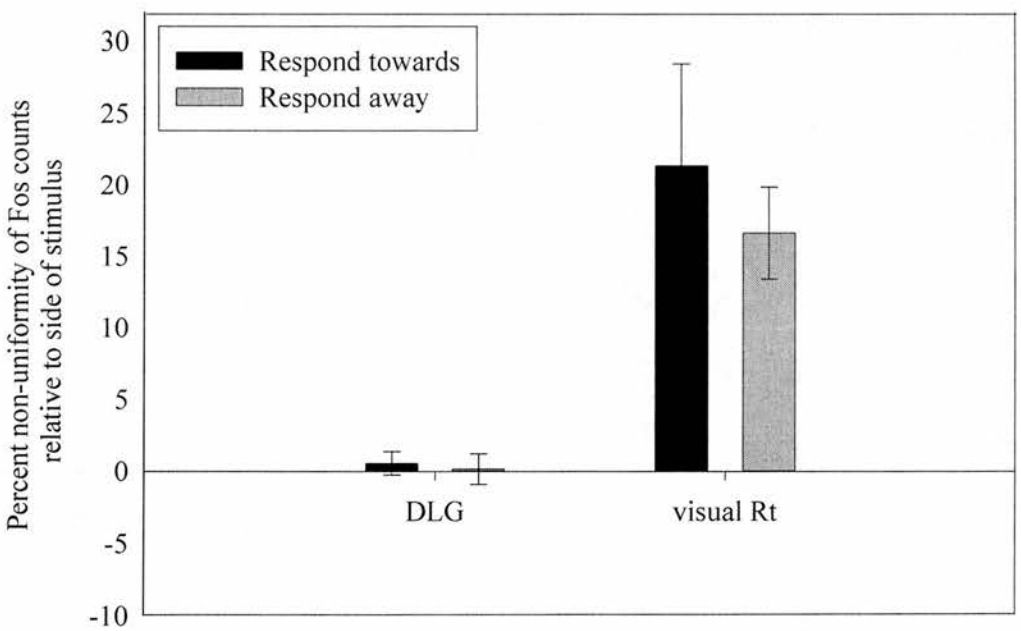


Figure 2.2. A bar graph showing percent non-uniformity of Fos counts (Mean +/- SE) in the dLGN and visual Rt of rats responding either towards or away from cued target stimuli. Positive values represent greater Fos counts in the hemisphere contralateral to the visual stimuli; negative values would represent greater ipsilateral activation.

Fos activation: Thalamic reticular nucleus (Rt)

Fos immunoreactive neurons were observed in dorsocaudal Rt in both 'attending' (contralateral to the stimulus hole) and 'non-attending' (ipsilateral to the stimulus hole) hemispheres. However, there was consistently greater Fos labelling in visual Rt contralateral to the attended stimulus, irrespective of the location of the response; see Table 2.1. Figure 2.2 shows the percent non-uniformity of Fos counts in Rt: irrespective of response direction, there were fewer Fos immunoreactive neurons in the ipsilateral compared to the contralateral Rt (*t*-test: $p < 0.05$). Figures 2.3 and 2.4, photomicrographs with schematics, illustrate attending and non-attending Rt (bottom panel) from representative cases across groups.

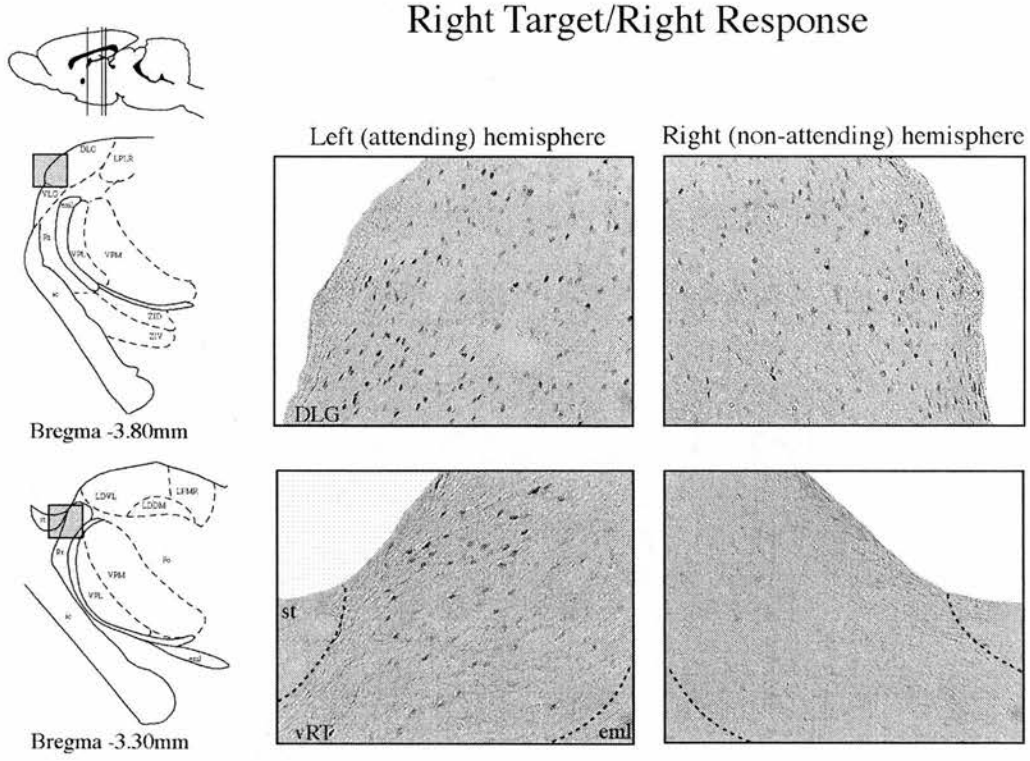


Figure 2.3. Coronal sections, with schematics (adapted from Paxinos and Watson, 1997) through the dorsal lateral geniculate nucleus, and visual sector of the thalamic reticular nucleus of a rat responding towards (Case 99/052) cued target stimuli; right target, right response. Uniform Fos labelling was seen in the dorsal lateral geniculate nucleus across hemispheres, whilst non-uniform Fos labelling was seen in the visual sector of the thalamic reticular nucleus across hemispheres (i.e. the sector contralateral to the attended stimulus was disproportionately labelled with Fos protein). Abbreviations: DLG, dorsal lateral geniculate nucleus; eml, external medullary lamina; ic, internal capsule; LDVL, laterodorsal nucleus, ventrolateral part; LDDM, Laterodorsal nucleus, dorsomedial part; LPLR, lateral posterior nucleus, laterorostral part; LPMR, mediorostral part of the lateral posterior nucleus; Po, posterior thalamic nuclear group; Rt, reticular thalamic nucleus; vRt, visual segment of the thalamic reticular nucleus; VLG, ventral lateral geniculate nucleus; VPL, ventral posterolateral thalamic nucleus; VPM, ventral posteromedial thalamic nucleus; ZID, zona incerta, dorsal part; ZIV, zona incerta, ventral part; (all abbreviations taken from Paxinos and Watson 1997).

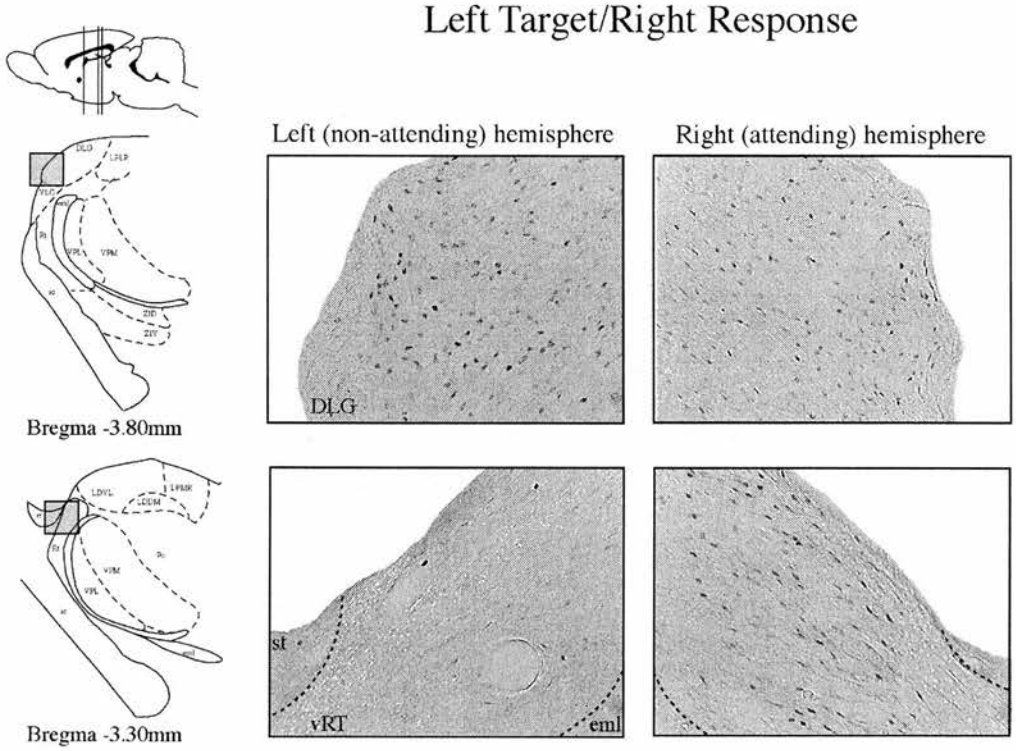


Figure 2.4. Coronal sections, with schematics (adapted from Paxinos and Watson, 1997) through the dorsal lateral geniculate nucleus and visual sector of the thalamic reticular nucleus of a rat (Case 99/334) responding away from cued target stimuli; left target, right response. Uniform Fos labelling was seen in the dorsal lateral geniculate nucleus across hemispheres, whilst non-uniform Fos labelling was seen in the visual sector of the thalamic reticular nucleus across hemispheres (i.e. the sector contralateral to the attended stimulus was disproportionately labelled with Fos protein).
Abbreviations: DLG, dorsal lateral geniculate nucleus; eml, external medullary lamina; ic, internal capsule; LDVL, laterodorsal nucleus, ventrolateral part; LDDM, Laterodorsal nucleus, dorsomedial part; LPLR, lateral posterior nucleus, laterorostral part; LPMR, mediorostral part of the lateral posterior nucleus; Po, posterior thalamic nuclear group; Rt, reticular thalamic nucleus; vRt, visual segment of the thalamic reticular nucleus; VLG, ventral lateral geniculate nucleus; VPL, ventral posterolateral thalamic nucleus; VPM, ventral posteromedial thalamic nucleus; ZID, zona incerta, dorsal part; ZIV, zona incerta, ventral part; (all abbreviations taken from Paxinos and Watson 1997).

Discussion

Fos labelling was measured in the brains of normal rats trained to respond towards or away from visual stimuli. Fos labelling is observed in the thalamic first-order visual relay nucleus, the dLGN, and this labelling is uniform across hemispheres. Non-uniform Fos labelling is seen in the visual sector of Rt across hemispheres, with greater Fos counts in the hemisphere contralateral to the visual stimulus. It should be emphasised that labelling in visual Rt is bilateral, but disproportionate, the greatest degree of non-uniformity (across hemispheres) being seen on sections immediately rostral to the dorsal lateral geniculate nucleus (as illustrated in Figures 2.3 and 2.4). Although the posterior third of Rt in rodents is categorised as 'visual' the region illustrated in Figures 2.3 and 2.4 corresponds to the region of Rt in rodents that responds to photic stimulation (French et al., 1985). However, these results are interpreted as reflecting non-uniform allocation of attentional resources, to a specific location in space, rather that non-uniform visual input, because no attempt is made to deprive the rats of visual input. Although the task requires attention to a lateralized stimulus, visual input is not restricted to one eye or one hemisphere. The chamber is lit throughout the task. The animal is required to move from the food-magazine, having retrieved a pellet, towards the illuminated fixation hole. After the target, the rat has to move to the response hole. Throughout the task, there are ample visual (as well as auditory and tactile) stimuli, some of them task-relevant, but also many irrelevant. Thus, it is reasonable to assume that both hemispheres are registering visual stimuli and engaged in visual processing (confirmed by the presence of Fos labelling in the dLGN, which was uniform across attending and non-attending hemispheres).

Nevertheless, the component of the task requiring selective attention is lateralized and thus restricted to one hemisphere. A brief visual cue precedes the target at the beginning of the foreperiod: previous work has shown that such cues attract visual attention to the cued location so speeding reaction time to targets at that location (Posner, 1980; Ward and Brown, 1996). Such attentional orienting has been described as a *"type of selective attention...which highlights stimuli in a particular portion of the visual field"* (Colby, 1991). Given that successful completion of this paradigm requires selective attention to task-relevant visual stimuli, specific labelling of visual Rt supports a role for this nucleus in the enhanced transmission of behaviourally relevant 'attended' information. Because the response is also lateralized, it would not have been surprising to see lateralized activation of Rt in motor sectors. However, as this was not seen, it can only be concluded that activation of the motor sector of Rt is dependent upon factors other than motor preparation and responding. In any case, activation of adjacent sectors would imply Rt regulates or gates sensory transmission in a non-specific manner, i.e. Rt facilitates or amplifies the transmission of both relevant and irrelevant cues irrespective of their behavioural significance (or insignificance). The visual sector of Rt is disproportionately labelled contralateral to attended stimuli, providing evidence that Rt is involved in attentional processing and specifically that activity is contingent on selective attention to task relevant cues irrespective of response direction (Montero, 1997, 1999, 2000). Not only does this result corroborate previous findings of active shifts in Fos labelling of Rt according to the attended modality (Montero, 1997), but extends them by showing that the resultant Fos labelling represents attentional as opposed to motor or exploratory task requirements. In this respect, this experiment has successfully disentangled and isolated the mechanisms mediating Fos labelling within Rt.

The present results also corroborate and extend those of Montero (1999) where unilateral amblyopia by visual deprivation, in rats exploring a novel complex environment, produced strong non-uniform Fos labelling in visual Rt contralateral to the deprived eye. Remarkably, the degree of non-uniformity across hemispheres was positively correlated with the degree of time spent in active exploration. Our results extend these findings by demonstrating that not only does such activation of visual Rt represent the attentional, as opposed to the exploratory, pre-requisite of behaviour, but also that the observed non-uniformity across hemispheres is not necessarily a consequence of an impaired visual system but also applies to the normal rat. Non-uniform Fos labelling in the thalamic first-order visual relay nucleus, dLGN, in spite of uniform immunoreactivity in Rt across hemispheres might represent intranuclear (cell-cell) inhibition within Rt (Cox et al., 1997) as opposed to internuclear (feedback) inhibition between cells of Rt and thalamus (Sanchez-Vives et al., 1997). Extensive inhibitory interactions between topographically organised Rt cells might preclude the relay of irrelevant sensory input and facilitate the amplification and relay of behaviourally relevant information. These results support a role for Rt in the gating of sensory information, which is modulated by attention. Thus rather than modulating the relay of sensory information irrespective of its behavioural relevance, Rt appears to represent an inhibitory interface or 'attentional gate', that regulates the flow of information between thalamus and cortex according to its behavioural significance.

One has to consider the possibility that disproportionate (greater) Fos expression contralateral to the attended stimuli represents not the up-regulation of Fos in the 'attending' hemisphere, but the down-regulation of Fos in the 'non-attending' hemisphere. If Fos is being down-regulated in the 'non-attending' Rt, relative to a

basal level in the 'attending' Rt, then surely one would expect to see comparable base levels of Fos in adjacent somatosensory and auditory sectors of Rt. However, this clearly isn't the case. From this observation, one can only conclude, or speculate, that Fos immunoreactive cells have been up-regulated in the 'attending' hemisphere relative to the 'non-attending' hemisphere. From, a behavioural perspective this question is probably irrelevant. Because Fos activity represents a marker of neural activity, either way the outcome remains that one Rt is activated significantly more than the other. Therefore, whether the present results reflect up or down-regulation of Fos immunoreactive neurons, the 'attending' Rt is still regulating thalamocortical transmission, and ultimately the contents of consciousness, to a greater extent than the 'non-attending' Rt.

Various models have been presented that attempt to explain the mechanisms by which Rt mediates selective attention (i.e. the enhanced transmission of behaviourally relevant cues) in the behaving animal. Recently, Montero (2000) demonstrated that Fos labelling in visual Rt is contingent on excitatory primary visual cortical (V1) input. Using a 'focal attention hypothesis' Montero argues that such 'top down' inputs from a focus of attention in V1 generate a core of excitation in the dorsal lateral geniculate nucleus via direct glutamatergic corticogeniculate pathways which, coupled with surround (lateral) inhibition from cortico-reticulo-geniculate (Glutamatergic-GABAergic) pathways, results in enhanced thalamocortical transmission of an attentional focus, relative to diminished transmission of surrounding regions of the visual field. Whilst we agree this cortico-reticulo-thalamic influence might serve to create a powerful 'top-down' inhibitory surround, thus improving signal discriminability and detectability, Rt's role in post excitatory

(feedback) inhibition (see Shosaku et al., 1989 for a review) must also be considered. Post excitatory inhibition may serve to initiate burst firing within thalamic relay nuclei. As noted in the introduction, thalamic relay neurons exhibit two modes of activity: 'burst' and 'tonic' activity. Whether a cell is in burst or tonic mode depends on the inactivation state of a low threshold calcium conductance or 'spike'. When the cell membrane is more positive than -55mV, the low threshold spike is inactivated and the neuron responds to an injected current by firing at a linear rate, between 25 and 100 spikes per second - tonic activity. When the cell membrane is hyperpolarized i.e. more negative than –65mV for about 100msecs, the low threshold spike is activated with a barrage of conventional Na^+/K^+ action potentials riding the crest of the spike – burst firing (Jahnsen and Llinás, 1984). In vivo investigations have demonstrated that tonic firing typically occurs during sensory transmission, whilst rhythmic burst firing serves to interrupt or disrupt the relay of sensory information. These conclusions are drawn from neurophysiological recordings which demonstrate the prevalence of bursting in the thalamic relay during periods of behavioural quiescence (i.e. slow wave sleep, general anaesthetic or during absence epileptic seizures) and the presence of tonic firing in the thalamic relay neurons of alert, behaving animals (Steriade et al., 1985; Steriade et al., 1987; Steriade et al., 1991; Marks and Roffwarg, 1993; for review see Steriade et al., 1993). However, arhythmic burst responses (although not nearly as frequent as tonic responses) have been recorded from the thalamic relay neurons of alert behaving animals in response to sensory input (see especially Guido and Weyand, 1995 and Ramcharan et al., 2000). These findings have proved controversial as burst firing was originally thought, somewhat dogmatically, to be unique to periods of sensory detachment. In the dorsal lateral geniculate nucleus (the first-order primary visual thalamic relay), the tonic

mode appears as a relatively faithful relay of sensory information from retina to cortex. The precise function of the burst mode however - especially in awake, behaving animals - is highly controversial (compare Ramcharan et al., 2000 and Weyand et al., 2001 and see general discussion). It has been suggested that burst firing may serve as a wake up call for novel, currently unattended sensory stimuli (Sherman, 1996) as burst responses (whilst infrequent) always comprise the initial cellular response to visual stimulation (Guido and Weyand, 1995; Ramcharan et al., 2000). Swadlow and Gusev (2001) recently provided evidence that burst firing represents a powerful way to get information into the cortex of awake, behaving rabbits. Fanselow et al., (2001) have extended these findings by demonstrating that burst activity within the somatosensory thalamic relay of rodents is restricted to periods of alert immobility, where subjects anticipate the presence of novel, potentially dangerous (or interesting) stimuli. Collectively, these results have revolutionised the prevalence and significance of burst activity during wakefulness. The exact mechanisms by which Rt might contribute to this phenomena and attention in general were considered earlier in the general introduction and are considered further in the general discussion. Drawing from recent insights into the dynamic nature of the thalamic relay in awake, behaving animals, a speculative account is presented in the of how Rt might regulate thalamocortical transmission and ultimately the contents of consciousness.

The present findings support the notion that a primary function of Rt in awake animals is to optimize and enhance the thalamocortical transmission of essential cues from the environment to guide appropriate, efficient behaviour (as suggested by Montero, 1997, 1999, 2000) and that Rt plays a critical role in attention mechanisms affecting early,

subcortical stages of sensory processing. However it could be argued that disproportionate Fos labelling contralateral to attended visual stimuli reflects not non-uniformity in the allocation of attentional resources, but non-uniformity of visual input. The present results might be parsimoniously explained on the basis of one hemisphere (and it's corresponding Rt) receiving significantly greater visual input than the other. However, such an interpretation implies Rt operates as a fairly low level, regulating the relay of sensory input irrespective of its behavioural valence. This seems unlikely as despite being bombarded with sensory information - relevant and irrelevant - Fos labelling was restricted to sectors associated with behaviourally relevant stimuli (i.e. visual). These results imply Rt receives 'top-down' information with respect to the behavioural significance of sensory stimuli and modulates their transmission through thalamus accordingly. However, confirmation of Rt's role in higher level processes such as attention (i.e. the enhanced processing of potentially significant stimuli at the expense of irrelevant stimuli) requires a behavioural task where multiple sensory stimuli are presented, but only one is attended. This is investigated in Chapter 3, where Kamin's (1969) conditioned blocking paradigm is used to test whether a conditioned, 'attended' stimulus (one associated with reward) activates Rt more than an unconditioned, 'unattended' stimulus.

Although we believe the present findings support a role for Rt in selective attention, a case can be made that performance of the attentional orienting task places additional demands on sustained attention. Sustained attention has been described as "*a fundamental component of attention characterised by the subject's readiness to detect rarely and unpredictable occurring signals over prolonged periods of time*" (Sarter et al., 2001). In the attentional orienting task, the requirement that subjects detect and

respond to temporally unpredictable stimuli over a protracted test session undoubtedly places demands on sustained attentional capacities. However, several characteristic, possibly defining, features of sustained attentional tasks, such as the interspersion of behaviourally relevant 'signal' events (i.e. those associated with reward) with irrelevant 'non-signal' events and the systematic variation of signal length and intensity, are neither manipulated or measured in the present paradigm (see Bushnell, 1998 for a review). For these reasons we believe attentional orienting principally measures selective and not sustained attentional performance. However, this notwithstanding, the potential involvement of sustained attention in this task represents a confound that should be investigated further. The pedunculopontine tegmental nucleus has recently been implicated in sustained attentional capacities in rodents (Inglis et al., 2001; Kozak et al., 2001). Because the pedunculopontine tegmental nucleus projects extensively to caudal sectors of Rt in rodents (Hallanger et al., 1987), a role for Rt in sustained attentional function is at least anatomically plausible. However, this remains to be determined behaviourally. The present results cannot unequivocally support Rt's involvement in sustained attention performance, however likely it seems. In order to demonstrate a role for Rt in sustained attention, a specific task (such as those employed by Inglis et al., (2001) and Kozak et al., (2001)) would have to be adopted in order to allow sustained attentional capacities to be isolated and measured separately from other potentially confounding variables (such as selective attention, response selection and execution etc.).

Chapter 3: Thalamic Reticular Nucleus Activation Reflects Attentional Gating During Classical Conditioning

Abstract

Evidence was presented in Chapter 2, which suggests Rt may function as an attentional gate in awake, behaving animals. Different groups of rats were trained to detect brief, unpredictable visual stimuli (presented to one side of the head only) in attentional paradigms with different response selection requirements. Irrespective of the direction of response, visual Rt contralateral to attended visual stimuli was disproportionately labelled with Fos protein, evidence of a role for this nucleus in focussed attention to task relevant cues. However, it could be argued that disproportionate (i.e. increased) Fos labelling contralateral to visual stimuli merely represents non-uniform visual input and not necessarily the selective allocation of attentional resources towards contralateral visual targets. The phenomenon of blocking in classical conditioning provides an opportunity to test whether an attended stimulus activates Rt more than an unattended stimulus: when a second stimulus is presented together with a previously conditioned stimulus, conditioned responding to the second stimulus is inhibited. Different groups of rats were given conditioning sessions with a single stimulus, a light or a tone, and then given conditioning sessions with compound (light and tone) stimuli. Blocking was confirmed using probe trials of single stimulus presentations. After a final test session of compound stimulus presentations, the brains were processed for the presence of Fos protein. Here we show that Fos immunoreactive neurons were more numerous in the sector of Rt associated with the attended conditioned stimulus than in the sector associated with

the unattended stimulus. Thus, we provide evidence for an involvement of Rt in selective attention.

Introduction

Functional sectors of Rt are defined by the origin of the cortical and thalamic collaterals they receive and by physiology. In the rat, a visual sector is located dorsocaudally in the nucleus (Sumitomo et al., 1976; Hale et al., 1982; Coleman and Mitrofanis, 1996; Lozsadi et al., 1996), while an auditory sector is ventral to this (Shosaku and Sumitomo, 1983). Lateral inhibition within sectors could enhance the relay of relevant (attended) information whilst attenuating the relay of irrelevant (unattended) information. It remains to be determined whether sectors might communicate with each other. The organization of Rt suggests that it may function as an attentional gate (see Chapter 1). Chapter 2 presents evidence which suggests a role for Rt in the allocation of limited attentional resources to task relevant stimuli. Two groups of rats were trained to detect brief, unpredictable visual stimuli in attentional paradigms with different response selection requirements. Animals were required to hold their heads in a central location for a variable delay and then respond to either the same (towards condition) or the opposite side (away condition) to where the visual stimuli were presented. Irrespective of response requirements, there was consistently more Fos labelling, a marker of neural activation, within visual Rt contralateral to the attended stimulus. Given that successful completion of this paradigm requires selective attention to visual stimuli, specific labelling of visual Rt supports a role for Rt in the regulation or enhanced transmission of behaviourally relevant 'attended' information. However it could be argued that disproportionate Fos labelling of visual Rt contralateral to attended visual stimuli merely represents non-uniform visual input, and not necessarily the disproportionate allocation of attentional resources. However, such an interpretation implies Rt operates at a relatively low-level, regulating the

transmission of sensory input, regardless of its behavioural significance. In order to demonstrate that the Fos labelling within visual Rt, following a lateralized attention task represents Rt's regulation of behaviourally relevant stimuli a task was required where multiple stimuli were presented but only one was attended. This was realised using a behavioural task in which attention is directed to one stimulus and not another, Kamin's (1969) blocking procedure. In this procedure, a stimulus that reliably predicts reward evokes a conditioned response. A second stimulus, introduced after conditioning but presented simultaneously with the first, is redundant and therefore results in no conditioned response. This second stimulus is referred to as the 'blocked stimulus'.

According to attentional theories of blocking limited attentional resources are directed to the conditioned stimulus. Since the blocked stimulus conveys no additional relevant information, it is unattended (Mackintosh, 1975; Solomon, 1977; Pearce and Hall, 1980; Holland and Gallagher, 1993). Although this task has conventionally been used to assess both normal and abnormal attentional function (for a review see Oades and Sartory, 1997), associative accounts have attempted to explain this phenomenon without recourse to attentional mechanisms (see Kamin, 1969 and Rescorla and Wagner, 1972). Associative theories explain conditioned blocking based on varying degrees of associative strength between conditioned responding and 'blocked' and 'unblocked' stimuli (the pre-exposed, 'unblocked' stimulus commanding the greatest associative strength). Using cytochrome oxidase as a metabolic marker, it has been demonstrated that secondary auditory cortex responds to an auditory conditioned stimulus predictive of footshock, but not if the stimulus is blocked by prior conditioning to a visual stimulus (Poremba et al., 1997). Metabolic differences

between the two conditions were observed in structures in which the convergence of auditory and somatosensory input is modulated by visual input. Thus, the authors concluded that blocking was not due to inattention to the blocked stimulus, supporting instead an associative explanation of blocking (see Kamin, 1969 and Rescorla and Wagner, 1972). Nevertheless, their data do not preclude the possibility that blocking has an attentional component. Indeed it seems inconceivable that associative and attentional mechanisms can ever be completely separated. Intuitively, animals must be initially attending to environmental stimuli in order to form an association between the unblocked stimulus and the reward (see Escobar et al., 2002 for a description of 'hybrid' theories which attempt to reconcile attentional and associative accounts of conditioned blocking). However, it should be borne in mind that the purpose of the present experiment is not to demonstrate whether the mechanisms underlying conditioned blocking are attentional or associative in origin, but whether Rt selectively enhances the relay of contextually significant (in this case, 'unblocked') stimuli.

Because conditioned blocking requires the greater allocation of processing resources to the 'unblocked' stimulus at the expense of the 'blocked' stimulus, this task can be used to measure selective attention or at the very least selective processing. If Rt mediates selective attention, labelling of Fos protein will be restricted to the sector of Rt associated with the attended conditioned stimulus and not in the sector of Rt associated with the blocked, unattended, stimulus. Such a demonstration would confirm Rt's role in attentional selectivity.

Experimental Procedures

Subjects and apparatus

Fifteen experimentally naïve male Lister hooded rats (Charles River, Margate, UK) were housed in pairs. The colony was maintained on a 12:12 hour dark-light cycle (lights on 07:00) with water freely available in the home cage and food restricted to 15-20g a day, provided after testing. Procedures were licensed under the UK Animals (Scientific Procedures) Act, 1986. Testing was conducted in standard Skinner boxes, with a food hopper between two fixed levers (Campden Instruments, Sileby, UK).

Behavioural Testing

An appetitive blocking procedure, with food reward, was used (see Table 3.1). After conditioning to a single stimulus and exposure to the compound stimulus, rats were given probe trials of alternating presentations of single stimuli under extinction, to verify blocking. Thus, there were 12 days of conditioning sessions, comprising 120 stimulus-reward pairings a day. The conditioned stimulus, presented for one second, was a light in the food hopper (Light, n=5) or an 800Hz, 70 decibel tone (Tone, n=6) from a speaker located in the ceiling of the chamber, or the light and tone together (Both, n=4). The reward was a 50% sucrose pellet (45mg; BioServ Inc., New Jersey), delivered into the hopper by automatic dispenser coincident with the offset of the conditioned stimulus. Inter-stimulus intervals were randomised between 1 and 30 seconds. On day 13 of training, there was a single session of 70 presentations of the compound-stimulus followed by reward. Blocking was assessed the following day by 30 alternating presentations of the light and tone alone, with no reward. Responses at the food hopper were recorded, and responses in the one second preceding the

stimulus were subtracted from responses during the stimulus, so that positive values would indicate greater responding during the stimulus and negative values would indicate greater responding prior to the stimulus. The final test session, which lasted 105 minutes, consisted of presentations of the compound stimulus followed by reward.

Group:	CS conditioning (12 x 120 trials)	Compound Conditioning (70 trials)	Probe trials (30 alternating CS – no reward)	Test (105 mins)
Light Tone Both	Light + reward Tone + reward Light/tone + reward	Light/tone + reward	Light, Tone, Light, Tone, Light, Tone, Etc	Light/tone + reward

Table 3.1. Outline of conditioned blocking procedure

Fos immunohistochemistry

Immediately after the final session, the brains were processed for the presence of Fos immunoreactive protein. In order to prevent non-specific Fos induction, for the twelve hours preceding the final test, rats were held in darkness with minimal exposure to auditory and visual stimuli. Rats were carried to the darkened testing room in covered

cages and immediately placed in the operant chambers. Research (Dragunow and Faull, 1989; Herrera and Robertson, 1996; Sagar et al., 1988) indicates that Fos synthesis follows mRNA expression approximately 30-45 minutes post stimulation and has a half-life of 2 hours. Previous work in the same laboratory using a >2-hour time frame has demonstrated a significant decay in the Fos protein, due to additional time incurred exporting rats to histology. This is especially problematic, as the immunohistochemical technique employed is highly specific to detection of the *C-fos* protein. Therefore, the rats were anaesthetised immediately after the final test session, of 1 hour and 45 minutes and transcardially perfused with saline followed by 4% paraformaldehyde.

Brains were removed immediately, and processed for Fos immunoreactivity as follows: One in four 50μm sections were cut using a freezing microtome and put into phosphate buffer solution (PBS). Sections were treated as follows: thirty minute wash in 20% sucrose solution (in PBS); two rinses in PBS followed by a 60 minute wash in blocking solution (100ml PBS, 20ml goat serum, 1ml triton); two PBS rinses; 48 hours incubation in primary *c-fos* antibody (Ab-5; 1:20000. Oncogene Research Products, Cambridge, MA); five PBS rinses; 45 minute incubation period on a shaker in Vector IgG solution (5μl per ml. Vectastain rabbit ABC Kit, Vector Laboratories, Peterborough, England); five PBS rinses; 45 minute incubation in ABC complex (antibody diluting solution, substrate A and substrate B, each 20μl per ml) on a shaker; five PBS rinses; incubated for 2 – 10 min in diaminobenzidine peroxidase substrate (Sigma Fast 3-3'- diaminobenzidine tetrahydrochloride with metal enhancer tablet sets; Sigma-Aldrich, Poole, England).

Fos immunoreactivity: quantitative analysis

Rt is comprised of large neurons that are easily distinguished by morphology from adjacent dorsal thalamic neurons. Positively stained neurons across sections or hemispheres displayed little, if any, variations with respect to size, orientation or level of stain intensity. Changes in the level of illumination across sections/hemispheres produces concurrent changes in cell density, allowing some neurons to appear lighter or darker, a procedure that may render profile counts unpredictable and susceptible to over or under estimations. Holding the level of microscope illumination constant across sections and rats, counts were made of positively stained neurons throughout the thalamic reticular nucleus. Images of sections were projected onto a 19" screen with a superimposed 2"-square grid. For each section, cells in the region of interest were counted twice on different occasions with the experimenter blind to condition. However, the repeat-counts never differed by more than 10% and so the first count was always used. The regions of interest were the visual sector and the auditory sector of Rt. Based on electrophysiology (Shosaku and Sumitomo, 1983; Hale et al., 1982) and anatomy (Pinault and Deschenes, 1998), these regions are located approximately between Bregma –3.1 mm and –4.1 mm, with the visual sector located dorsal to the auditory sector. Therefore, we counted all neurons stained positively for Fos immunoreactive protein in the dorsal 50% and ventral 50% of Rt on 15 sections between –2.7 mm and –4.1 mm, but used counts from the sections between approximately Bregma –3.1 mm and 4.1 mm to obtain means for visual (dorsal 50%) and auditory (ventral 50%) sectors.

Data analysis

During the probe session, conditioned responses to light, and tone stimuli were recorded for each rat. A repeated measures ANOVA was employed, with two factors, one within subjects (stimulus: light-CS, tone-CS,) and one between subjects (group: pre-training to light, tone, or compound stimuli). Following the final test session brains were processed for the presence of Fos protein, counts being made throughout the regions of interest (visual and auditory Rt). A repeated measures ANOVA was employed with two factors, one within subjects (Rt sector: visual and auditory) and one between subjects (group: pre-training to light, tone or compound stimuli).

Behavioural Analysis

The probe trials confirmed that the procedure resulted in a conditioned response to the stimulus presented during the conditioning sessions and not to the second stimulus. Rats conditioned to the compound stimulus showed intermediate levels of conditioned responding to either stimulus alone (Fig. 3.1; interaction of group and stimulus, $F_{(2,12)} = 24.7$, $p < 0.01$).

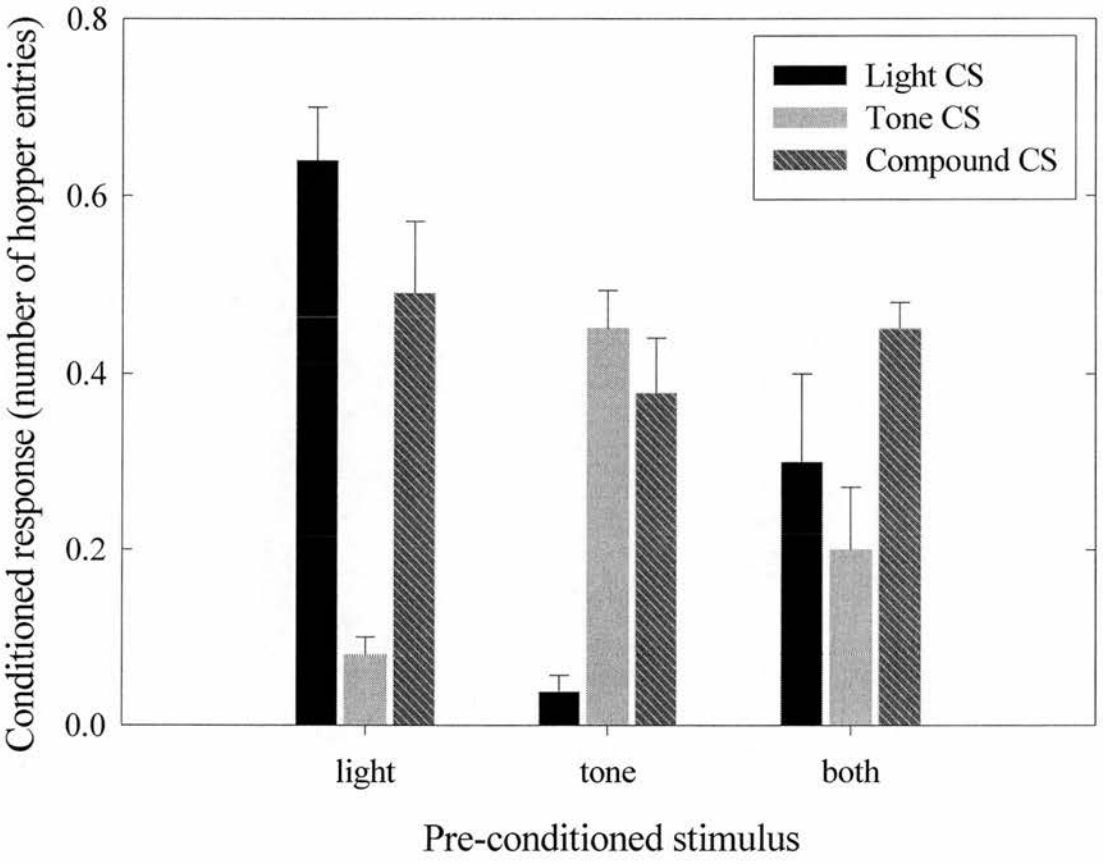


Figure 3.1. Conditioned responding was assessed in probe trials in which the stimuli – light or tones – were presented alone and not followed by food reward. Responses to compound stimuli (recorded during the preceding test session) are also shown. Responses in the one-second prior to stimulus onset were subtracted from responses

during the one second stimulus. The bars show responding to the light (black), tone (light grey) and compound stimuli (dark grey). The number of hopper entries was highest when the preconditioned stimulus was presented and lowest when the blocked stimulus was presented. Intermediate values were observed for rats trained with the compound stimulus.

Fos activation patterns across Rt sectors

Modality specific sectors of Rt were activated disproportionately according to the attended stimuli. Neurons stained for Fos protein were more numerous in the sector of Rt associated with the conditioned, attended, stimulus. Figure 3.2 shows that rats conditioned to light showed activation of visual Rt (dorsocaudal region), while rats conditioned to tone showed activation of auditory Rt (ventrocaudal region). Rats who had received prior conditioning to the compound stimulus showed activation of both visual and auditory thalamic reticular nucleus (interaction of group and thalamic reticular sector, $F_{(2,12)} = 4.0, p < 0.05$). Figure 3.3 shows photomicrographs with schematics (adapted from Paxinos and Watson, 1997) illustrating neural activation in regions of Rt (visual versus auditory) in representative cases from each group (light-CS, Tone-CS and Both).

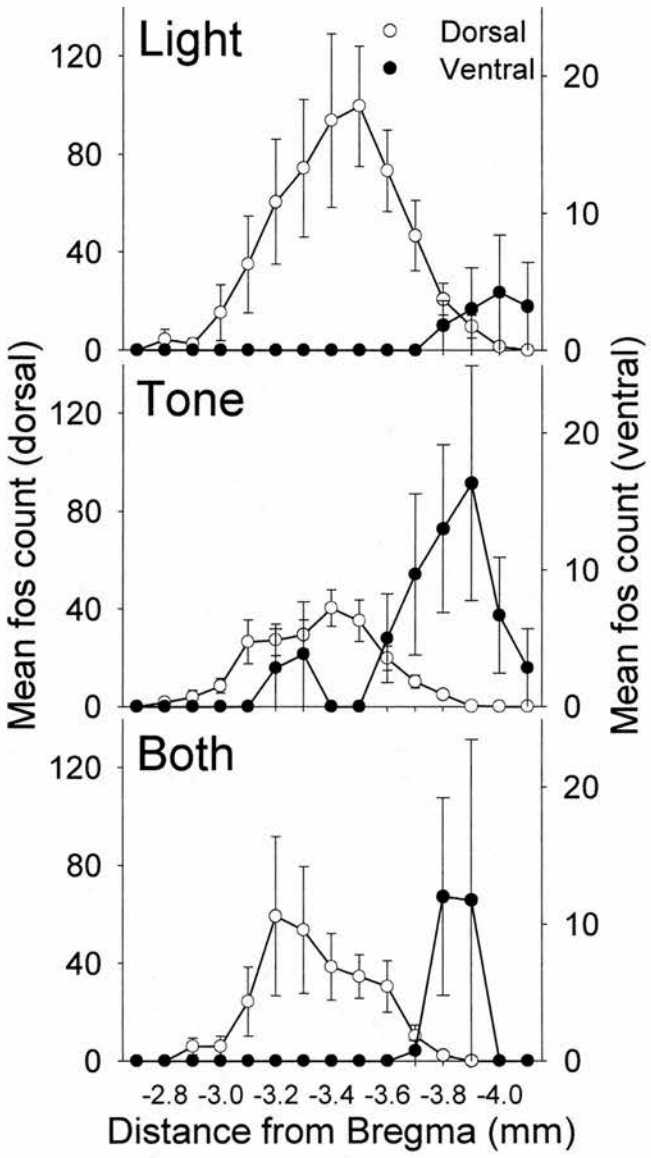


Figure 3.2. Mean counts of Fos immunoreactive neurons in the dorsal 50% and ventral 50% of the thalamic reticular nucleus on sections between Bregma –2.7 and –4.1mm for each group.

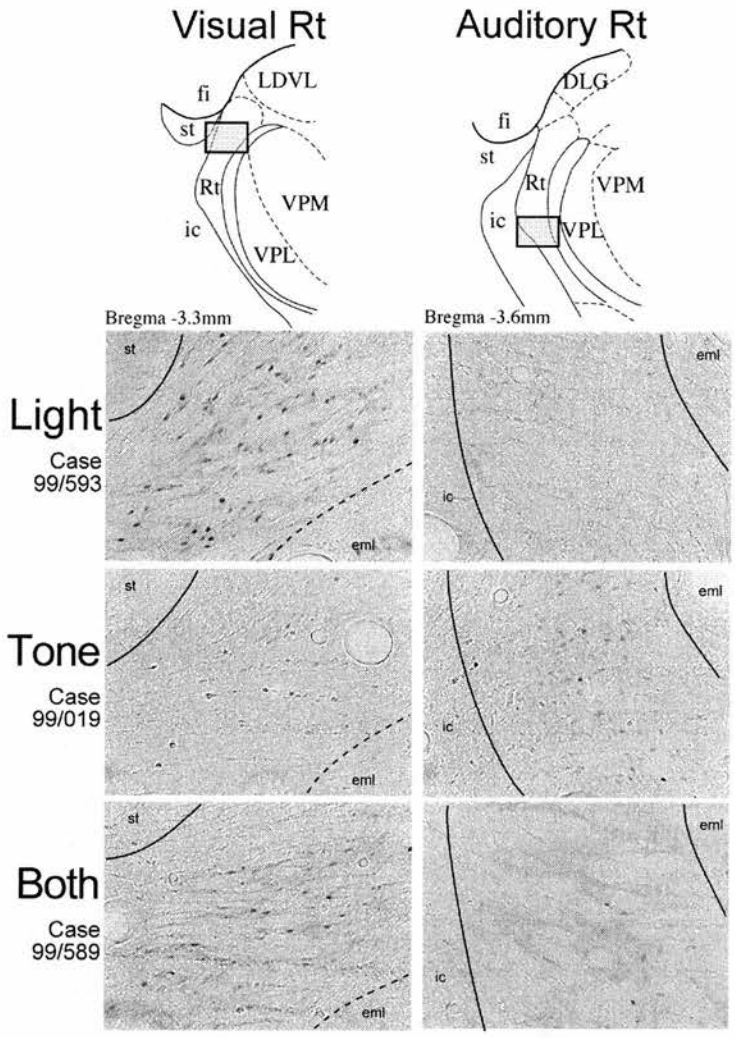


Figure 3.3. Coronal sections, with schematics (adapted from Paxinos and Watson 1997), through the thalamic reticular nucleus of the same hemisphere of one rat from each group, showing Fos immunoreactive neurons in different sectors of the thalamic reticular nucleus according to the group. Fos labelling was seen in the dorsocaudal region (visual) of the thalamic reticular nucleus in the light-conditioned, but not in the dorsocaudal region (visual) in the tone-conditioned group. The converse pattern of activity was found in the ventrocaudal region (auditory) of the thalamic reticular nucleus. In the compound stimulus-conditioned rats, Fos immunoreactive neurons were found in both regions. On the test day, all rats received the same, compound, stimulus presentations. Abbreviations: DLG, dorsal lateral geniculate nucleus; eml, external medullary lamina; fi, fimbria; ic, internal capsule; LDVL, laterodorsal nucleus, ventrolateral part; Rt, reticular thalamic nucleus; VPL, ventral /posterolateral thalamic nucleus; VPM, ventral posteromedial thalamic nucleus; ZID, zona incerta, dorsal part; ZIV, zona incerta, ventral part; (all abbreviations taken from Paxinos and Watson 1997).

Across rats in all groups, there was a positive correlation between magnitude of conditioned responding to the light and the number of Fos immunoreactive neurons in visual Rt (Fig. 4; partial correlation, removing variability due to auditory conditioned responding, $r = 0.45$, $df = 12$, $p = 0.053$, one-tailed). There was a stronger positive correlation between the magnitude of auditory conditioned responses and the number of Fos immunoreactive neurons in auditory Rt (partial correlation, removing variability due to visual conditioned responding, $r = 0.62$, $df = 12$, $p < 0.01$, one-tailed). This is shown in Figure 3.4. Thus, in addition to the mean differences between groups, the number of Fos immunoreactive neurons in each sensory sector predicts the magnitude of behavioural effects in individual animals.

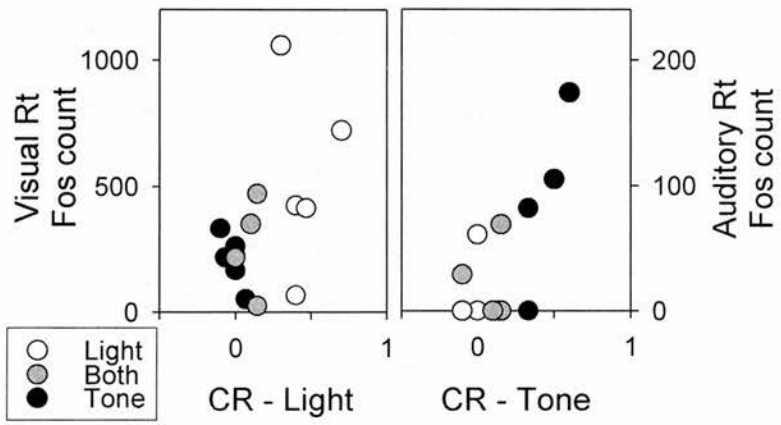


Figure 3.4. Plots showing the residual conditioned response against the residual Fos count. The visual responses (visual conditioned response and the mean Fos count in the visual portion of the thalamic reticular nucleus) were predicted from the auditory responses and vice versa. The observed responses were then subtracted from predicted responses, to obtain the residual. Thus variability in the residuals of the visual (or auditory) responses is independent of variability in the auditory (or visual) response. 0-1 indicates the degree of correlation between conditioning to light, tone or compound stimuli and Fos labelling in visual and auditory Rt.

Discussion

Activation of Rt was observed in rats attending to conditioned stimuli in a blocking procedure. The activation was specific to the sector of Rt associated with the sensory modality of the conditioned stimulus. There was less activation of the sector of Rt associated with the sensory modality of a blocked stimulus, which did not elicit a conditioned response. The degree of Fos labelling in Rt correlated with the magnitude of the conditioned response to the conditioned stimulus. This result not only supports the view that Rt regulates sensory transmission (Schlag and Waszak, 1971; Frigyesi and Schwartz, 1972; Steriade and Wysinski, 1972; Sumitomo et al. 1976; Yingling and Skinner 1976; Hale et al. 1982; French et al. 1985; Salt, 1989; Simons and Carvell, 1989; Lee et al. 1994 a, b; Warren and Jones, 1994; Hartings and Simons, 2000; Hartings et al., 2000) but also demonstrates that this regulation is modulated by attention.

The three groups of rats – Light, Tone and Both (light and tone) – differed only according to the stimuli to which they had received prior conditioning. On the test day, all rats were treated the same and received the same repeated presentations of the compound light-tone stimulus. The possibility that the rats, in attending and responding to the conditioned stimulus, might fail to orient to the second stimulus was considered. To avoid this objection, the light stimulus was presented within the food hopper, to which all the rats oriented and approached when the compound stimulus was presented. The tone was presented from an omni-directional speaker, located above the rat in the chamber. Thus, the stimuli impinging on the various sensory surfaces of the rats would not differ as a function of the rats orienting behavior, so that

neural differences can reasonably be expected to reflect differences in attention and not sensation. It should also be noted that no attempt was made to restrict other stimuli within the chamber during the test session: a ventilation fan was on throughout and neither the dispenser nor the hopper panel was silent. These results suggest reticular cells receive additional information related to stimulus relevancy (e.g., information on the rewarding properties or emotional significance of stimuli) that independently influence attentional processing. Thus, Rt may represent the neurological substrate of Broadbent's (1958) selective filter model of attention in which the filter of attention is adjusted according to the ability of cues to predict important consequences. These results suggest Rt represents an attentional 'gate' or 'filter' that samples and regulates the flow of information between thalamus and cortex according to its motivational/behavioural relevance. The selective processing of behaviourally relevant information by Rt may guide efficient, adaptive, goal directed behaviour in awake, behaving animals.

Although we interpret these results as evidence of a role for Rt in selective attention i.e. the enhanced processing of contextually significant, unblocked, stimuli at the expense of currently insignificant, blocked, stimuli, it has been suggested that the present results may also be open to an associative interpretation. Jones and Gonzalez-lima (2001) have argued that these results can be explained not by a blocking effect, but by differential excitatory effects of the CSs. They argue that because compound stimuli appear to be just as effective as blocked stimuli in inducing Fos within Rt these results can be explained not by a blocking effect, but by differential excitatory effects of the CSs, thus supporting an associative interpretation. However, upon closer inspection these criticisms may prove unjustified. The case being made for an

associative interpretation is based on the following findings. First of all, Fos counts in dorsal (visual) and ventral (auditory) sectors in the compound group are similar to the effects in the corresponding sectors produced by the blocked stimuli in tone and light groups (see Figure 3.2). For example, the dorsal (visual) sector effect in the 'both' group is similar to the dorsal effect seen in the tone group. Thus the 'compound' light effect is comparable to the 'blocked' light effect. Thus, the stimuli trained as compound were as effective as the blocked stimuli in inducing Fos in the corresponding sectors. It has been suggested that these results can be parsimoniously explained as a greater (neural) response to the stimulus that received the greater number of excitatory conditioning (due to the first phase of training) in each of the tone and light groups. That is, the light is paired more times than the tone in the 'light' group, and thus may be expected to show a greater neural effect than the tone. So the differential excitatory effects of the CSs are consistent with the differential Fos activation within the light and tone groups.

However, the finding that a blocked stimulus is just as effective as a compound stimulus in inducing Fos within Rt could still be explained without recourse to an associative interpretation. Preconditioning to a single stimulus predisposes animals to respond to a single stimulus, light or tone, selectively. By contrast, during compound conditioning trials, animals are required to detect and respond to a compound stimulus that is comprised of two, spatially incontiguous, stimulus dimensions (light and tone). Therefore, one could argue that while light- and tone-conditioned rats are attending selectively to a single stimulus (selective attention involves the enhanced processing of contextually significant stimuli at the expense of currently irrelevant stimuli) the attentional resources of compound conditioned rats are distributed or divided across

the spatial realm. Thus, during compound conditioning the level of attention devoted to the constituent components of the compound stimulus is potentially half that devoted to a single stimulus (as in light- and tone-conditioned groups. Furthermore, given that blocking merely 'attenuates' attention to irrelevant stimuli, one might expect the level of Fos induced in a given sector of Rt by a compound stimulus to be comparable to that induced by a 'blocked' stimulus. Finally, if these results are purely a result of differential excitatory effects, why isn't the level of Fos labelling within dorsal and ventral sectors of compound-conditioned rats comparable to the same sectors in light- and tone-conditioned animals? These rats have received the exact same number of light and tone presentations as light- and tone-conditioned rats, yet the magnitude of Fos labelling within their dorsal (visual) and ventral (auditory) sectors of Rt is considerably less.

It would appear that the present results are open to both associative and attentional interpretations. However, even if an associative account, based on competing associations of variant strengths explains the failure of a blocked stimulus to predict reward, it should be borne in mind that the purpose of this experiment was not to demonstrate whether the mechanisms underlying conditioned blocking are associative or attentional in origin but whether Rt regulates the relay of sensory stimuli according to their behavioural significance. The specificity of labelling within Rt, according to the modality of attended stimuli, supports the view that this nucleus acts as an attentional gate or filter, modulating the transmission of sensory stimuli according to their salience or significance. While these data do not demonstrate that the mechanism of blocking is attentional, they nonetheless provide support for the suggestion that

blocking has an attentional component, changing the transmission of stimuli according to the associative value of the stimulus.

The present results extend upon those of Montero (1997), who demonstrated selective *c-fos* activation in the visual sector of Rt in normal rats exploring a complex novel environment and in the somatosensory sector of Rt in blind rats, dependent upon tactile cues to explore. Montero (1997) suggested that sectors of Rt actively compete for limited attentional resources. However, the notion of inter-sector communication within Rt remains to be determined both physiologically and anatomically. The idea that Rt sectors might communicate with each other was originally proposed by Crick (1984) and was based on early anatomical studies of Rt in neonatal rodents (Scheibel and Scheibel, 1966). While these early investigations showed extensive dendritic profiles and axon collaterals that ramified throughout Rt often crossing the boundaries of modality specific sectors, subsequent contemporary studies have demonstrated that these processes are often lost (axon collaterals) or truncated (dendrites) during development (Pinault et al., 1997). Montero (2000) has recently demonstrated that Fos labelling in Rt, but not the dorsal lateral geniculate nucleus, is dependent on primary visual cortical input emphasising a role for cortico-reticulo-thalamic circuitry in feed-forward inhibitory mechanisms and selective attentional function (see conclusions in chapter 2 and general discussion). However, interactions between thalamus and Rt may also initiate 'feedback' inhibitory mechanisms that could complement feed-forward mechanisms in awake, behaving animals (see chapters 1 and 2). Rhythmic burst firing in the thalamus, including Rt, is prevalent during periods of neural synchronisation, such as during slow wave sleep, deep anaesthesia, or absence seizures (Steriade et al., 1985; Steriade et al., 1987; Steriade et al., 1991; Marks and

Roffwarg, 1993; for review see Steriade et al., 1993) and is thought to represent a functional detachment of relay cells from their sensory inputs (i.e. burst firing disrupts the transmission of the sensory signal). In periods of cortical desynchrony, Rt fires in a tonic mode (Marks and Roffwarg, 1993; for review see Steriade et al., 1993). Guido and Weyand (1995) demonstrated that arrhythmic burst firing in the thalamic relay cells in awake animals may support better signal detection by elevating the signal-to-noise ratio and suggested that the functional implications of burst versus tonic firing in the awake animal may relate to orienting versus focal attention. This suggestion is given further support by the recent works of Ramcharan et al., (2000) and Fanselow et al., (2001) who suggest that burst firing in the thalamic relay of awake animals provides an attentional 'wake-up call' to cortex that signals the presence of novel stimuli (see chapters 1 and 2). Indeed it has recently been demonstrated that burst firing represents a powerful way of getting sensory information into cortex (Swadlow and Gusev, 2001, Fanselow et al., 2001). The fos labelling seen here might represent neuronal excitation within Rt, which 'feeds-back' onto thalamic relay cells. This feedback inhibition, which probably operates through activation of $GABA_B$ receptors, may serve to hyperpolarize thalamic relay cells, consequently priming them for the initiation of burst firing (see Crunelli and Leresche, 1991). Rt's contribution to these mechanisms however, remains to be determined experimentally in awake, behaving animals.

Preliminary, although inconclusive, behavioural evidence was presented in Chapter 1 implicating caudal Rt in sensory-attentional mechanisms (Weese et al., 1999; Montero, 1997, 1999, 2000). Using more sophisticated reliable behavioural paradigms, Chapters 2 and 3 have strengthened these originally tentative findings,

supporting a role for caudal Rt in selective and potentially sustained attentional processes. While caudal Rt has been implicated in sensory-attentional processes (see especially Weese et al., 1999, but also see Montero, 1997, 1999, 2000), preliminary evidence points to a role for rostral Rt in the abstraction of behaviourally relevant information (Vann et al., 2000, Wilton et al., 2001)[3]. This function may be the cognitive equivalent of the filtering mechanism that characterises the modality specific regions of more caudal Rt. This is examined in Chapter 4 using an attentional set-shifting paradigm (Birrell and Brown, 2000). The term 'attentional set' refers to an acquired predisposition to direct attentional resources towards contextually significant aspects of the perceptual environment. The formation of an 'attentional set' requires that subjects filter sensory input from complex multi-dimensional stimuli allowing the abstraction and processing of behaviourally relevant features (i.e. those contingent with reward) at the expense of irrelevant features. Chapter 4 examines the acquisition, maintenance and shifting of attentional set in rats with neurotoxic lesions centred on the rostral pole of Rt.

[3] Caudal Rt can be distinguished from rostral Rt according to distinct organisational principles. Neurons within caudal sectors of Rt display a distinct 'slab-like' organization (Coleman and Mitrofanis, 1996; Conley and Diamond, 1990; Conley et al., 1991; Crabtree, 1992a, 1992b, 1996, 1998; Crabtree and Killackey, 1989; Montero et al., 1977; Symonds and Kaas, 1978; for a review see Guillery et al., 1998 or Crabtree, 1999) that is absent in the anterior 'limbic' and 'motor' regions of Rt (Lozsadi 1994, 1995; Cicirata et al., 1990). These 'slabs', which run parallel to the borders of the nucleus are identified using anterograde and retrograde tracing techniques and are defined by the terminal zones of input (imparted through collaterals) from discrete regions of modality specific cortices and their respective thalamic nuclei (see especially Crabtree and Killackey 1989, Crabtree 1992ab, 19996, 1998, 1999). These anatomical differences across rostral and caudal sectors of Rt may underlie the distinct psychological processes thought to be mediated by rostral and caudal sectors of Rt – discussed above.

Chapter 4: The role of the rostral portion of the thalamic reticular nucleus in the acquisition, maintenance and shifting of attentional set

Abstract

This study examined the acquisition, maintenance and shifting of attentional set in rats with neurotoxic lesions centred on the rostral portion of the thalamic reticular nucleus (Rt). Rats were trained to perform an attentional set-shifting task that is formally the same as a task used in monkeys and humans. Damage to this region did not disrupt the ability to acquire, maintain or shift attentional set, provided the mediodorsal and midline-intralaminar thalamic nuclei remained intact. Nor did such lesions impair performance on discrimination reversals. Only extensive thalamic lesions (in three animals) that extended to include the mediodorsal and midline-intralaminar nuclei selectively impaired extradimensional set-shifting. Thus, despite anatomical connectivity with regions mediating the acquisition and shifting of attentional set, rostral Rt does not appear to represent a functional component of this network. These results are consistent with negligible behavioural effects on tasks of spatial processing following rostral Rt lesions. However, detrimental effects of such lesions have been reported in the literature. Whilst these differences may be due to methodological factors, they might also be indicative of a fundamental misinterpretation of rostral Rt function in awake, behaving animals. This possibility is discussed in terms of the possible role played by this region in mechanisms of cortical arousal.

Introduction

Evidence was presented in Chapters 2 and 3 supporting a role for Rt in the orienting of limited attentional resources to behaviourally relevant stimuli. These results support the notion that Rt operates as an attentional gate that filters sensory input, facilitating the transmission of behaviourally relevant information at the expense of irrelevant information. By virtue of its connectivity with the anterior thalamic nuclei (Gonzalez-Ruiz et al., 1997, Lozsádi 1994, 1995), which are crucial to spatial learning and memory (Aggleton et al., 1995; Aggleton et al., 1996; Byatt et al., 1996; Vann et al., 2000; Warburton et al., 1997), the rostral sector of Rt has also been implicated in spatial processing (M'Harzi et al., 1991; Collery et al., 1993; Vann et al., 2000; Wilton et al., 2001). However, unlike the evidence obtained from studies of caudal sectors of Rt (i.e. visual and somatosensory) for a role in attention (see especially chapters 2 and 3 and Weese et al., 1999) investigations of the functional significance of rostral Rt have been less convincing (M'Harzi et al., 1991; Collery et al., 1993; Vann et al., 2000; see especially Wilton et al., 2001).

M'Harzi et al., (1991) and Collery et al., (1993) showed that rats with Rt lesions had a spatial working, but not reference memory impairment. However, neither M'Harzi et al., (1991) or Collery et al., (1993) effected a lesion of rostral Rt without causing considerable damage to the adjacent anteroventral thalamic nucleus. Wilton et al., (2001) demonstrated that rats with neurotoxic lesions of rostral Rt were not impaired on a series of spatial learning and memory tests, provided damage to the adjacent anteroventral thalamic nuclei was minimal. There was evidence of a transient impairment during acquisition of a forced choice alternation in a T-maze, which

rapidly disappeared. In an attempt to explain these discrepant behavioural results, Wilton et al., (2001) argue these data support a role for rostral Rt, not in spatial processing per se, but in the attention to or abstraction of behaviourally relevant information. This function may represent the cognitive equivalent of the filtering mechanism that characterises the modality specific regions of more caudal Rt.

As rostral Rt receives overlapping projections from the medial prefrontal cortex and the lateral division of mediodorsal thalamus (Cornwall and Phillipson, 1988, 1988a; Cornwall et al., 1990), a role for this sector in attention to contextually significant events is at least anatomically plausible. Specifically, lesions to medial prefrontal cortex in rats impair the shifting of 'attentional set' between perceptual features of complex stimuli (Birrell and Brown, 2000). The concept of 'attentional set' assumes that subjects analyse stimuli in terms of dimensions, that they can only process a limited number of dimensions at any one time, and that reinforcement will strengthen attention and responses to particular dimensions. The formation of an 'attentional-set' requires that subjects filter sensory input from complex multi-dimensional stimuli, allowing the abstraction and processing of behaviourally relevant features (i.e. those contingent with reward) at the expense of irrelevant features. Preliminary behavioural findings already suggest rostral Rt might be involved in the abstraction or attention to such features (Wilton et al., 2001). Indeed, whilst attentional set shifting capacities are vulnerable to lesions of dorsolateral prefrontal cortex in primates (Dias et al., 1996a, b; 1997) and medial prefrontal cortex in rodents (Birrell and Brown, 2000), overall this capacity remains intact, as animals ultimately shift attention towards the newly rewarded perceptual dimension. Collectively, these results imply that the neuropsychological mechanisms underlying the ability to shift attentional set are not

the property of a single brain area, such as the prefrontal cortex, but represent the concerted effort of a network of functionally and anatomically related brain regions, of which rostral Rt may represent an important component.

Attentional set-shifting paradigms (Humans: Owen et a., 1991, Primates: Dias et al., 1996 a, b, 1997 and Rodents: Birrell and Brown, 2000) typically also include a series of 'discrimination reversals', where subjects not only have to maintain an attentional set, but also learn and unlearn specific stimulus-reward associations within a perceptual dimension. Converging evidence suggests interconnections between the orbitofrontal cortex, mediodorsal thalamic nucleus and basolateral amygdala are critical for encoding and using associative information about the motivational significance of stimuli (Porrino et al., 1981; Amaral et al., 1992; Carmichael and Price, 1995; Baxter et al., 2000). Lesions to the mediodorsal nucleus of thalamus (Hunt and Aggleton, 1998) and orbitofrontal cortex (Gallagher et al., 1999) in rats are associated with deficits in the ability to adjust behaviour in response to altered stimulus-reward contingencies. Rats with bilateral lesions of orbitofrontal cortex fail to alter their actions in response to changes in the value of a reinforcer (Gallagher et al., 1999), whilst lesions to mediodorsal thalamus are associated with a deficit in the shifting of response rules in a delayed matching to place task in rats (Hunt and Aggleton, 1998). As rostral Rt receives overlapping projections from both orbitofrontal cortex and the central division of mediodorsal thalamus (and projects back topographically to central mediodorsal thalamus - see Cornwall et al., 1990), this sector might also contribute to the psychological processes mediating the acquisition or reversal of stimulus-reward contingencies.

Here we investigate the effect of rostral Rt lesions on the ability to acquire, maintain and shift attentional set in rodents. This was achieved using an attentional set-shifting task that is formally the same as that used monkeys (Dias et al., 1996a,b; 1997), humans (Owen et al., 1991) and rodents (Birrell and Brown, 2000). The task was comprised of a series of compound perceptual discriminations that required subjects either to (a) maintain an attentional set and transfer behavioural responding from one pair of exemplars to another within the same perceptual dimension; an intradimensional shift, or (b) to shift an attentional set from one perceptual dimension (i.e. texture, odour or medium) to another; an extradimensional shift. The test also included a series of 'discrimination reversals', where subjects not only have to maintain an attentional set, but also learn and unlearn specific stimulus-reward associations within a perceptual dimension (rule reversal). The behavioural or inhibitory control required for attentional set-shifting is at the level of attentional selection, whereas the inhibitory control required for discrimination reversals is at the level of stimulus-reward and stimulus response associations (Dias et al., 1996a,b, 1997). Rt receives afferents from orbitofrontal cortex, medial prefrontal cortex and mediodorsal thalamus and sends efferents back to central, medial and lateral divisions of mediodorsal thalamus (Cornwall et al., 1990). Through these connections Rt might be involved in both aspects of behavioural control. The inclusion of 'intra/extradimensional shifts' and 'discrimination reversals' allows a direct assessment of Rt's contribution to these mechanisms in awake, behaving rodents.

Experimental Procedures

Subjects

Twelve Lister hooded rats (Charles River, Margate, Kent, UK) were housed individually in 25 x 45 x 15 cm plastic cages. Testing was conducted in the light phase of a 12 hr light/dark cycle (lights on at 7 a.m.). The rats were maintained on a restricted diet (15-20 gm of standard rat chow) with water freely available in the home cage. All procedures were carried out in accordance with the requirements of the United Kingdom Animals (Scientific Procedures) Act 1986.

Apparatus

Test apparatus was identical to that described by Birrell and Brown (2000). Briefly, ceramic pots with an internal diameter of 7cm and a depth of 4cm were used to hide food reward. Bowls were varied according to their odour, and the type of digging medium used to conceal food reward. Food reward consisted of one-half of a honey nut loop (Kellogg, Manchester, UK).

Testing apparatus consisted of an adapted home cage (dimensions 40 x 70 x 18 cm) – see Figure 4.1. Two-thirds of this cage, represented a 'holding area' where rats were confined to between trials, whilst a third of the cage was comprised of a 'discrimination area'. Removable Plexiglas panels separated these areas. Prior to trial initiation, digging bowls were placed in the two compartments of the discrimination area. Rats were given access to the digging bowls by lifting the plexiglass divider.

Figure 4.1. Test apparatus used in attentional set-shifting task.

Surgery

Anaesthesia was induced with an intraperitoneal injection of pentobarbitone sodium (1.0ml/kg, 65mg/ml). Rats were then placed in a stereotaxic frame with atraumatic ear bars (Kopf, Tujunga, CA) with the nose bar set at –3.30mm (an approximate measure of level skull). Level skull was confirmed by measuring dorsoventral co-ordinates at both bregma and lambda. Provided these two figures corresponded, level skull was achieved. Any deviation (between measurements) was corrected by adjusting the nose bar until both measures corresponded. A midline incision was made along the scalp, and the skin and fascia were retracted to expose the skull. A small burr hole was drilled bilaterally to allow the vertical stereotaxic insertion of a 30 gauge injection needle. Bilateral lesions of Rostral Rt were made by injection of ibotenic acid 1.40mm posterior to Bregma, 2.0mm lateral to the midline and 6.6 mm below skull surface using a cone-tipped 1.0µl Hamilton syringe. Ibotenic acid (0.06M) was administered using a sterile phosphate buffered saline as a vehicle (pH = 7.4) in a volume of 0.15µl/site. Each injection was made over a period of 5 minutes using a microdrive, and the needle was left in position for a further 5 minutes before being retracted. Unlesioned, control rats received equivalent injections of vehicle only at the same co-ordinates. Skin incisions were cleaned and closed using sterile metal clips. A heated pad was kept under the rats at all times during surgery to maintain normal body temperature. Seven days were allowed for post-operative recovery before habituation and testing began.

Habituation

The training and testing protocols were essentially the same as that described by Birrell and Brown (2000). The day before testing rats were presented with 2 sawdust

filled bowls. Bowls were rebaited every 5 minutes, for 60 minutes. When rats were reliably digging to retrieve food rewards, they were presented with three simple discriminations. These included a texture (rubber versus masking tape), odour (vanilla versus blackcurrant scented tea) and medium discrimination (confetti versus polystyrene balls). During habituation, only one exemplar of each discrimination was positively associated with reward; Table 4.1. All rats were habituated using the same discriminations, in the same order. These exemplars were not used again.

Discrimination	Positive Exemplar (always contingent with reward)	Negative Exemplar
1) Odour	Vanilla	Blackcurrant
2) Medium	Confetti	Polystyrene balls
3)Texture	Rubber	Masking tape

Table 4.1. Outline of simple discriminations presented during habituation (Based on Birrell and Brown, 2000).

Testing

Testing consisted of a series of perceptual discriminations that required subjects either to maintain an attentional set and transfer behavioural responding from one pair of exemplars to another within the same perceptual dimension or to shift attentional set from one perceptual dimension to another: Odour-Medium versus Medium to Odour. Subjects were randomly assigned to a particular shift type prior to testing. Each

discrimination required subjects to learn which of two exemplars from any given dimension was positively correlated with reinforcement. Table 4.2 lists the stimuli used during testing. Exemplars were always presented in pairs. For example cinnamon and cumin were always presented together in either a leaf or ground tea medium, thyme and paprika were always presented in either a stone chip or wooden bead medium and cloves and nutmeg were always presented in a sawdust or wooden shaving medium. The order in which pairs of exemplars were presented was pseudorandomized *a-priori*, such that no two rats in the same group received the same combination of exemplars.

	Pair 1		Pair 2		Pair 3	
Exemplars: Dimensions:	1	2	3	4	5	6
Odour	Cinnamon	Cumin	Thyme	Paprika	Cloves	Nutmeg
Medium	Leaf tea	Ground tea	Stone chips	Wood beads	Wood shavings	Sawdust

Table 4.2. Exemplars used during testing (Based on Birrell and Brown, 2000).

The initial stage of testing was a simple discrimination (SD), where subjects were required 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 subject 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 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 pseudorandomized across animals. The shifts performed by each lesioned rat were matched by those of a control rat. Table 4.3 summarises the experimental procedure.

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

Table 4.3. The order of the discriminations and examples of the positive (shown in bold) and negative exemplars (Based on Birrell and Brown, 2000).

Immunohistochemistry

Following experimentation, rats were deeply anaesthetised with sodium pentobarbital (1ml/kg, 65mg/kg) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1M phosphate buffered saline (PBS). Brains were removed and cyroprotected for 24 hours in 20% sucrose solution in PBS. Brains were blocked and cut coronally in 50μm sections using a freezing microtome. Three sets of sections were collected for each subject. Two sets were incubated separately for 48 hours in primary antibodies directed against Parvalbumin and healthy neuronal nuclei (NeuN) and processed as follows: thirty minute wash in 20% sucrose solution (in PBS); two rinses in PBS followed by a 60 minute wash in blocking solution (100ml PBS, 20ml goat serum, 1ml triton); two PBS rinses; 48 hours incubation in primary *c-fos* antibody (Ab-5; 1:20000. Oncogene Research Products, Cambridge, MA); five PBS rinses; 45 minute incubation period on a shaker in Vector IgG solution (5μl per ml. Vectastain rabbit ABC Kit, Vector Laboratories, Peterborough, England); five PBS rinses; 45 minute incubation in ABC complex (antibody diluting solution, substrate A and substrate B, each 20μl per ml) on a shaker; five PBS rinses; incubated for 2 – 10 min in diaminobenzidine peroxidase substrate (Sigma Fast 3-3'- diaminobenzidine tetrahydrochloride with metal enhancer tablet sets; Sigma-Aldrich, Poole, England). For each animal, a third set of sections was mounted on slides and stained with cresyl violet.

Data analysis

Trials to criterion were recorded for each rat for each discrimination. 2 repeated measures ANOVA were employed, the first with three factors, one within subjects (shift: SD, CD, ID, ED) and two between subjects (group: lesion and control and

dimension: odour-medium and medium-odour) and the second again with three factors, one within subjects (reversal: Rev1, Rev2, Rev3) and two between subjects (group: lesion and control and dimension: odour-medium and medium-odour).

Results

Histological Analysis

Six rats sustained bilateral damage to anterior thalamus. Figure 4.2 represents a series of schematics (adapted from Paxinos and Watson, 1997) illustrating the maximal and minimal extent of lesions within anterior thalamus. In all cases, damage extended beyond the rostral portion of Rt to include the anterior thalamic nuclei. In three extreme cases, significant cell loss extended to the mediodorsal and midline-intralaminar nuclei. In these cases, the only sparing of midline/intralaminar thalamus was restricted to the dorsal tip of the midline paraventricular nucleus. This is illustrated in Figure 4.3, a series of photomicrographs with schematics, showing the cases with the largest and smallest lesions. In every case, there was limited cortical damage restricted to the injection tract. Sham rats showed equivalent levels of mechanical damage through cortex and thalamus.

Behavioural Analysis

Discrimination Learning

Figure 4.4 shows the trials to criterion for each of the discriminations. Analysis of the stages before the ED shift confirmed that there was no effect of the lesion on these initial stages (main effect of group, $F_{(1, 8)} = 0.54$, NS; stage by group interaction $F_{(2, 16)} = 0.28$, NS). Also, performance did not significantly differ within the two perceptual dimensions (main effect of initial dimension, $F_{(1, 8)} = 1.50$, NS).

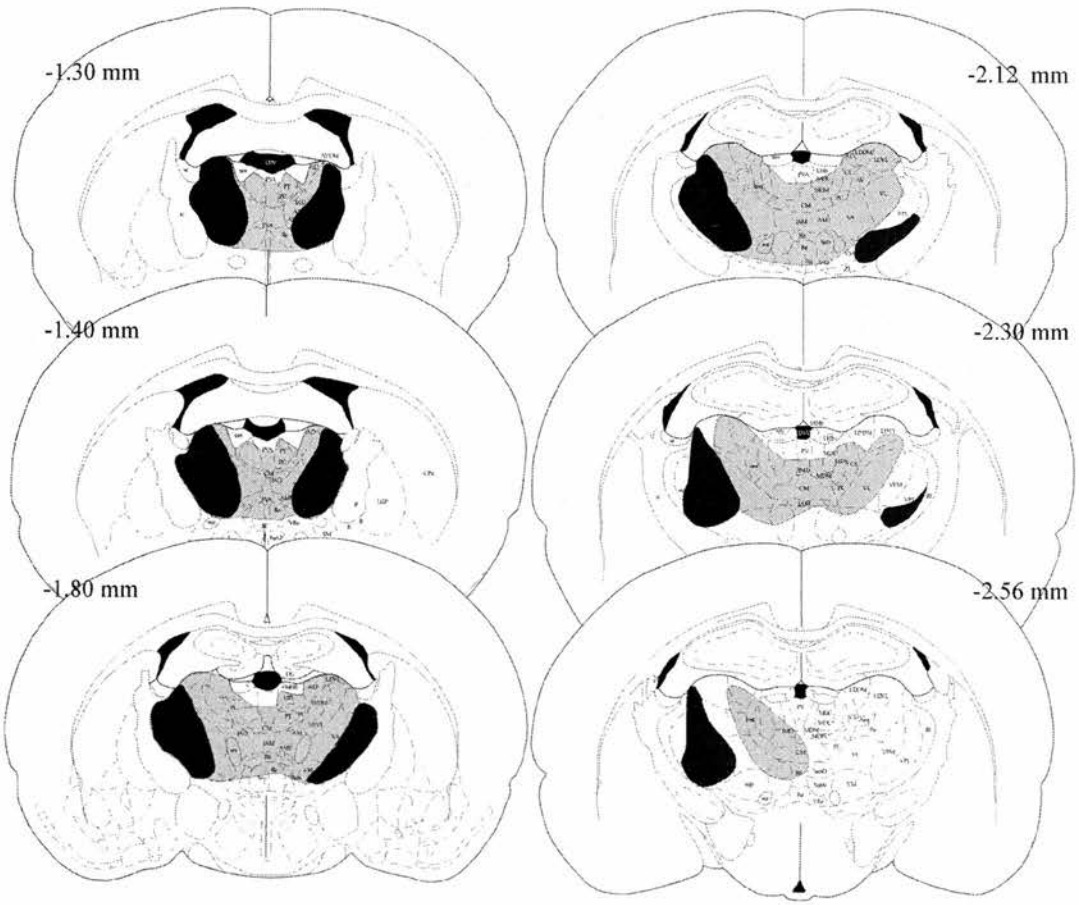


Figure 4.2. Diagrammatic reconstructions showing the cases with the largest (grey) and smallest (black) lesions plotted onto standard coronal sections (adapted from Paxinos and Watson, 1997). The numbers correspond to the distance from bregma. Abbreviations: AD, anterodorsal thalamic nucleus; AM, anteromedial thalamic nucleus; AMV, anteromedial nucleus, ventral; AVDM, anteroventral nucleus, dorsomedial; AVVL, anteroventral nucleus, ventrolateral; B, basal nucleus (Meynert); CL, centrolateral thalamic nucleus; CM, central medial thalamic nucleus; Cpu, caudate putamen; IAD, interanterodorsal thalamic nucleus; ic, internal capsule; LDDM, laterodorsal thalamic nucleus, dorsomedial; LDVL, laterodorsal thalamic nucleus, ventrolateral; IAM, interanteromedial thalamic nucleus; IMD, intermediodorsal thalamic nucleus; MD, mediodorsal thalamic nucleus; MDC, mediodorsal thalamic nucleus, central; MDL, mediodorsal thalamic nucleus, lateral; MDM, mediodorsal thalamic nucleus, medial; MDPL, mediodorsal paralaminar thalamic nucleus; MHb, medial habenular nucleus; PC, paracentral thalamic nucleus; PT, paratenial thalamic nucleus; PC, paracentral thalamic nucleus; Po, posterior thalamic nuclear group; PVA, paraventricular thalamic nucleus, anterior; Rt, thalamic reticular nucleus; LGP, lateral globus pallidus; Re, reuniens thalamic nucleus; Rh, rhomboid thalamic nucleus; st, stria terminalis; sm, stria medullaris; Sub, submedius thalamic nucleus; VA, ventral anterior thalamic nucleus; VL, ventrolateral thalamic nucleus; VM, ventromedial thalamic nucleus; VPL, ventral posterolateral thalamic nucleus; VPM, ventral posteriomediodorsal thalamic nucleus.

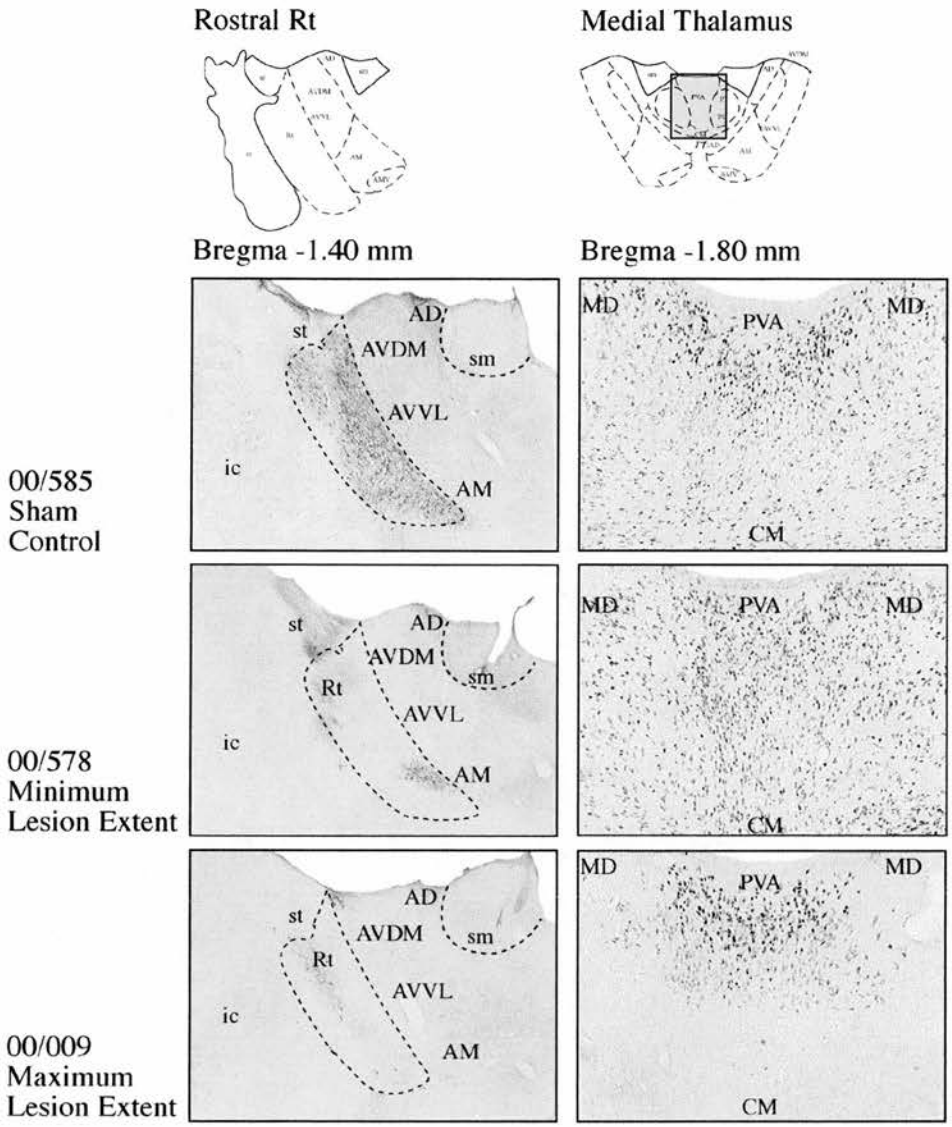


Figure 4.3. A series of photomicrographs (adapted from Paxinos and Watson, 1997), showing the cases with the largest (Case: 00/009) and smallest lesion (Case: 00/578), compared with a surgical control (00/585). All lesioned animals sustained bilateral lesions centred upon the rostral component of Rt (illustrated in left hand panels: sections are stained with an antibody against parvalbumin, which is found in the GABAergic cells of Rt and their terminals). In extensive cases, neurotoxic damage extended to include medial thalamus, encroaching upon the midline, mediodorsal and paraventricular nuclei (illustrated in right hand panels: sections are stained with an antibody against healthy neuronal nuclei – NeuN). Abbreviations, as in Figure 4.2.

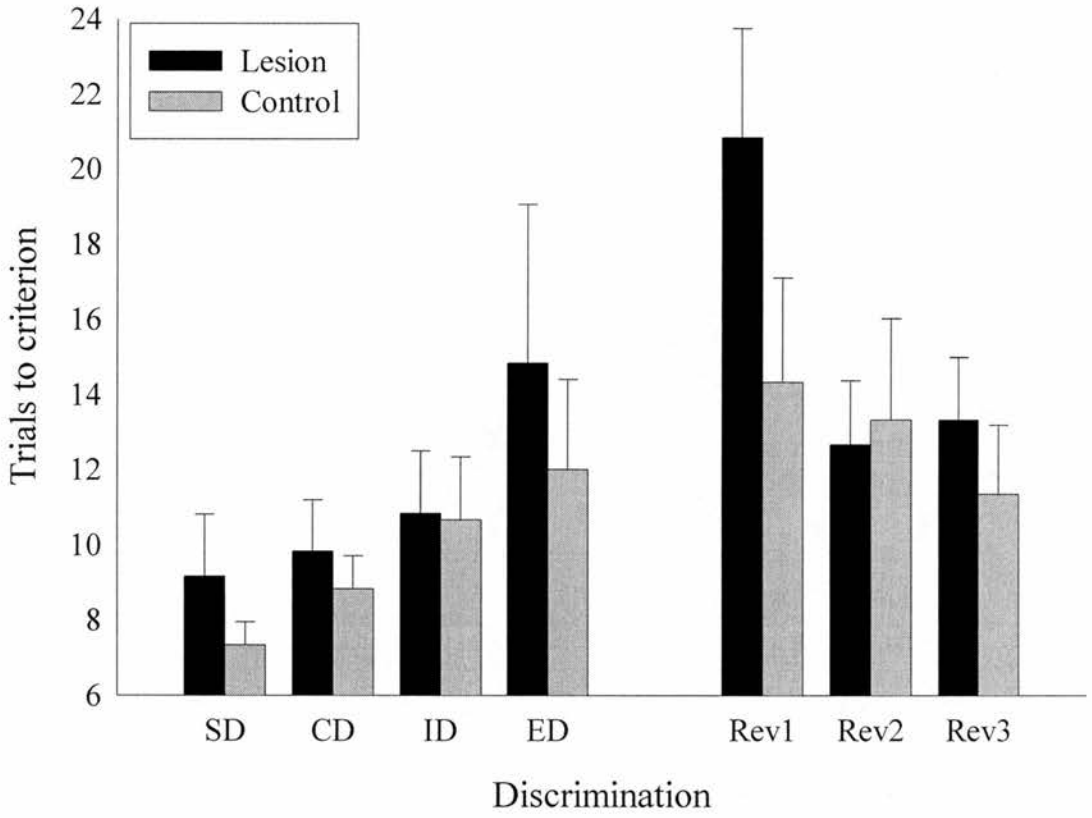


Figure 4.4. Mean (+/- SE) trials to acquire a response at each of the stages of testing, for both sham and thalamic lesioned rats.

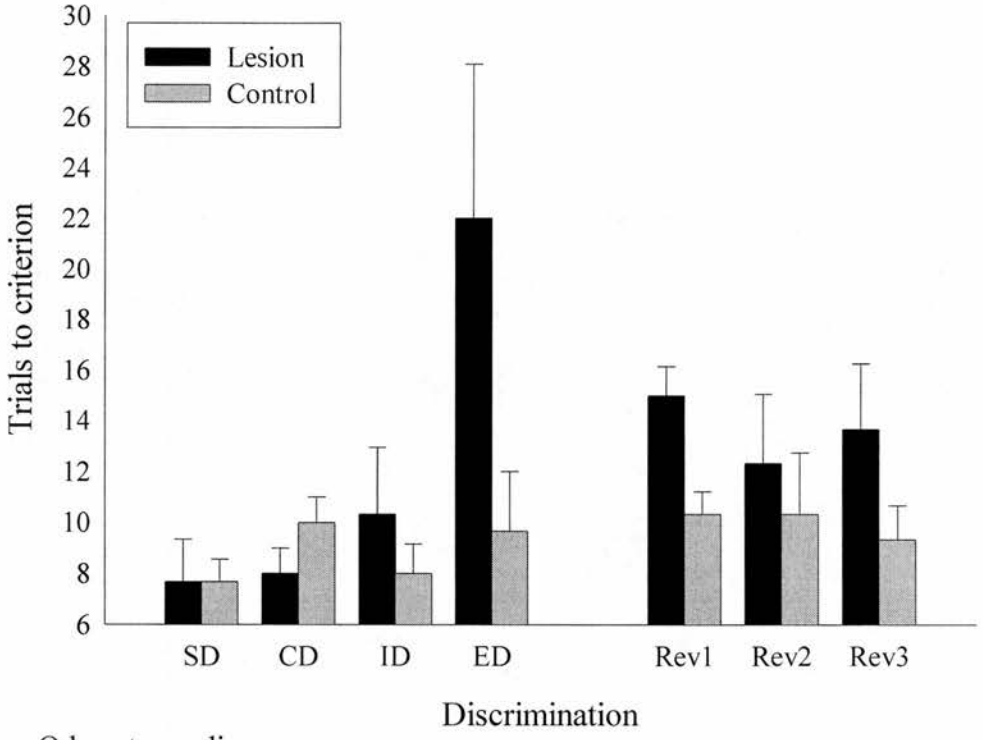
Reversal Learning

Following CD, ID and ED stages, the correct and incorrect exemplars were reversed. Figure 4.4 shows the trials to criterion for each of the reversals (Rev1, Rev2 and Rev3). In spite of an apparent tendency for the lesioned group to show a greater number of errors at the first reversal, this was not statistically reliable. The two groups of rats did not differ in their rate of acquisition following the reversal of reward contingencies (main effect of group $F_{(1, 8)} = 2.44$, NS; reversal by group interaction, $F_{(2, 16)} = 1.58$, NS).

ID versus ED shifts

On average both lesioned and control rats performed ID shifts more rapidly than ED shifts (Figure 4.4), however this difference was not significant (planned contrast, ID versus ED, after main effect of shift, $F_{(1, 8)} = 2.84$, NS). There was however, a significant interaction between group, shift and dimension ($F_{(1, 8)} = 5.37$, $p<0.05$), implying that the ability to shift attentional set towards a specific dimension, odour, was selectively impaired in lesioned animals. Figure 4.5 shows the trials to criterion for both discriminations for each shift type: (a) medium to odour and (b) odour to medium. On medium to odour shifts, lesioned animals took twice as many trials to perform an ED (a discrimination which required a shift in attentional resources towards a newly rewarded dimension) than an ID shift (a discrimination in which the exemplars changed, but the attended dimension remained the same). This implied a modality specific impairment in extradimensional set-shifting following rostral Rt lesions. However, closer inspection of these cases revealed consistent, extensive damage to various thalamic nuclei, not observed in lesioned animals performing odour to medium shifts; Figure 4.3.

a) Medium to odour



b) Odour to medium

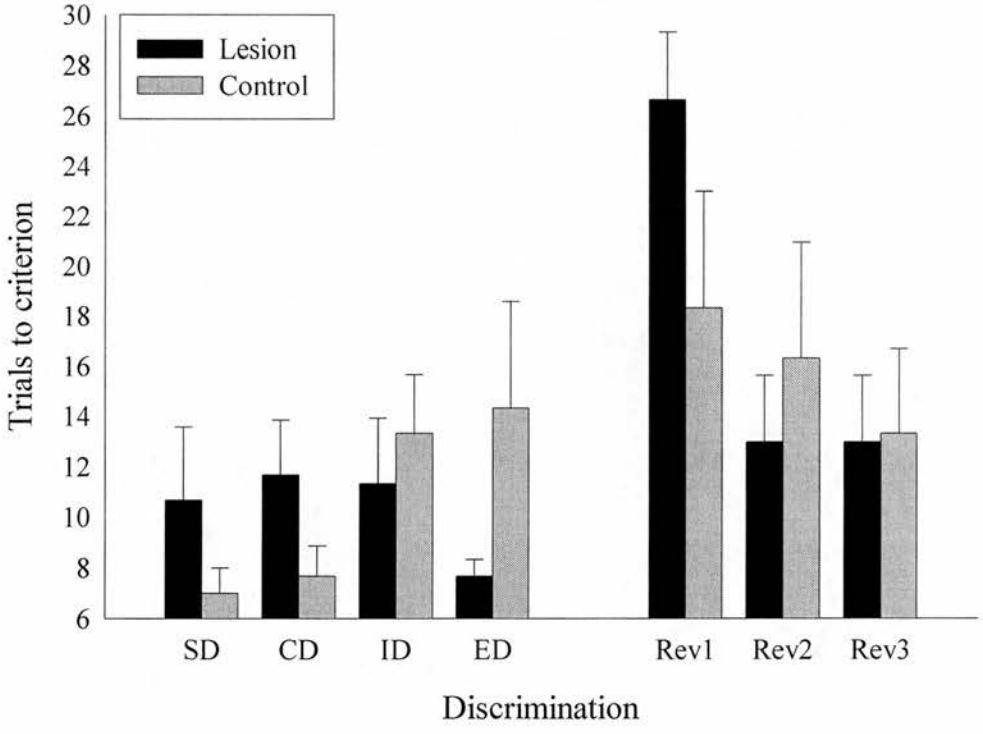


Figure 4.5. Mean (+/- SE) trials to acquire a response at each stage of testing for both control and thalamic lesioned rats, according to shift type.

Discussion

Rats with sizeable lesions of rostral Rt and the anterior thalamic nuclei (anterodorsal, ventroanterior and anteromedial) showed no impairment in attentional set shifting capacities. Neither were these animals impaired in their acquisition or reversal of stimulus-reward contingencies. These results imply that neither rostral Rt nor the anterior thalamic nuclei contribute to the psychological processes underlying the ability to form, maintain or shift attentional set. This is in spite of Rt's afferent connectivity with regions involved in this process. Only extensive thalamic lesions that extended beyond rostral Rt and the anterior thalamic nuclei, to include the mediodorsal, intralaminar (central medial, paracentral, central lateral, parafascicular), and midline (paratenial, paraventricular, intermediodorsal, rhomboid) thalamic nuclei impaired extradimensional set-shifting. Following substantial thalamic lesions which included the mediodorsal nucleus and the midline-intralaminar complex, the ability to shift attentional set towards a newly rewarded dimension (an ED shift) was selectively impaired; preliminary evidence of a role for these nuclei in the processes underlying extradimensional attentional set shifting in rats. The relative absence of any learning, memory or rule-reversal impairments, in spite of (often extensive) damage to regions implicated in these cognitive processes, is surprising (see especially Hunt and Aggleton, 1998 but also Aggleton et al., 1995; Aggleton et al., 1996; Beracochea et al., 1989 and Markowitsch, 1982). Given the level of damage observed in extensive cases, one might have expected a global, non-specific impairment across all components of this task rather than a highly specific impairment in the shifting of attentional set. Given the magnitude of the lesions reported interpretation of these results is

impossible. This notwithstanding, a speculative account of the possible thalamic nuclei mediating this precise behavioural deficit is presented.

The thalamus and reversal learning

Previous work has reported deficits in reversal learning following lesions to orbitofrontal cortex (Gallagher et al., 1999) and mediodorsal thalamic nucleus (Hunt and Aggleton, 1998) in rats. Lesions to both areas are associated with deficits in the ability to adjust behaviour in response to altered stimulus-reward contingencies. Despite receiving dense, overlapping projections from both regions and projecting back upon mediodorsal thalamus with exclusively GABAergic, inhibitory axons, rostral Rt does not appear to contribute to the behavioural control mechanisms mediating the formation or reversal of stimulus-reward contingencies in rodents. The lack of a reversal deficit was surprising in light of the damage sustained by the three most extensive cases. In all three cases neurotoxic damage extended to include the mediodorsal thalamus which has been implicated in rule-reversal learning (Hunt and Aggleton, 1998). In spite of this no reversal deficit was present in these animals

The thalamus and set-shifting

Deficits in extradimensional set-shifting have been reported in rats following lesions to the medial frontal cortex (Birrell and Brown, 2000). Rats with lesions of medial frontal cortex are selectively impaired on compound perceptual discriminations that require a transfer of attentional resources towards a newly rewarded, previously incorrect, perceptual dimension. Converging evidence suggests interconnections between the prefrontal cortex and mediodorsal nucleus are critical for the development of a 'central strategy' or 'attentional set' in rodents (Mishkin, 1964;

Kolb, 1984; Hunt and Aggleton, 1998; Birrell and Brown, 2000). Despite receiving overlapping projections from both regions (Cornwall et al., 1990), rostral Rt does not appear to contribute to extradimensional set-shifting, as lesioning this region, albeit in addition to adjacent anterior thalamic nuclei, does not appear to impair the ability to shift attentional set.

The inhibitory control required for attentional set-shifting is at the level of attentional selection, whereas the inhibitory control required for discrimination reversals is at the level of stimulus-reward and stimulus response associations (Dias et al., 1996a,b, 1997). Despite *anatomical* connectivity with regions implicated in both aspects of behavioural control, rostral Rt does not appear to comprise a *functional* component of these networks. Although these results are somewhat confounded by the size of the lesion, it should be borne in mind that in 50% of the cases, damage was reasonably contained to the anterior thalamic nuclei and rostral Rt. Despite extensive damage to both regions, no behavioural deficit was apparent, suggesting an insignificant role for either of these regions in the acquisition, maintenance or shifting of attentional set. Although confounded, these results are consistent with negligible behavioural effects on tasks of spatial processing following lesions to rostral Rt (Wilton et al., 2001). Wilton et al., (2001) demonstrated that rats with lesions of rostral Rt were not impaired on a series of spatial learning and memory tests, despite interconnectivity with regions involved in spatial processing (Aggleton et al., 1995; Aggleton et al., 1996; Byatt et al., 1996; Vann et al., 2000; Warburton et al., 1997). It should be borne in mind that Wilton et al., (2001) also failed to make precise, circumscribed lesions of rostral Rt. Their lesions invariably extended to include the anteroventral nuclei, the laterodorsal nuclei, the ventral anterior complex and the ventrobasal complex *in*

addition to adjacent sectors of Rt (motor and somatosensory). Despite significant encroachment of numerous adjacent thalamic nuclei, as in 50% of cases in the present study, Wilton et al., (2001) failed to demonstrate a robust behavioural deficit. However detrimental effects of rostral Rt lesions have been reported in the literature. M'Harzi et al., (1991) and Collery et al., (1993) both reported that excitotoxic lesions of rostral Rt result in significant impairments on the performance of spatial memory tasks. These findings are not however, conclusive as in both studies lesions spread to the adjacent anterior thalamic nuclei. This is important as even partial lesions of the anterior thalamic nuclei can impair spatial memory (Aggleton et al., 1995; Aggleton et al., 1996).

The present (albeit confounded) findings and those of Wilton et al., (2001) appear to be in direct contrast to those of M'Harzi et al., (1991) and Collery et al., (1993). This disparity is most apparent when comparing studies in which the same behavioural tasks were used to assess Rt function. It is not clear why ibotenate lesions of rostral Rt in rodents impair spatial learning and memory (M'Harzi et al., 1991; Collery et al., 1993) whereas NMDA lesions do not (Wilton et al., 2001). It is parsimonious to assume that unintentional damage to surrounding regions was less when neurotoxic lesions were achieved using NMDA. Indeed it is noteworthy that such incidental damage was responsible for the discrepancy in memory deficits following ibotenic acid and AMPA injections into the basal forebrain of rats (Connor et al., 1991; Dunnet et al., 1987; Muir et al., 1994). In conclusion it appears Rt damage alone is neither necessary nor sufficient to produce impairments in tasks of spatial processing or attentional set-shifting. In the present experiment, only substantial thalamic lesions that extended to include the mediodorsal and midline-intralaminar nuclei impaired

extradimensional set-shifting. Likewise, in the Wilton et al., (2001) study only larger Rt lesions that include anterior thalamus produce transient impairments of delayed spatial alternation.

The apparent failure of rostral Rt lesions to produce a consistent pattern of behavioural deficits might be indicative of a fundamental misinterpretation of the role played by this region in awake behaving animals. Rostral Rt receives extensive input from the basal forebrain cholinergic system (see especially Hallanger et al., 1987). The basal forebrain cholinergic system is implicated in mechanisms of cortical arousal (for a review see Semba, 1999). Via its afferent connectivity with this region, rostral Rt might comprise part of the network mediating such cortical arousal. Vann et al., (2000) showed that not all spatial memory tasks, but only 'spatially demanding' tasks, were associated with elevated levels of Fos immunoreactivity in rostral Rt. Thus, these authors concluded that their data suggest a role for rostral Rt in mechanisms of spatial processing. However given that this region of Rt corresponds to sector that receives input from the basal forebrain cholinergic system, Fos immunoreactivity within this region might be suggestive not of Rt's involvement in spatial learning per se, but perhaps in mechanisms of cortical arousal associated with the acquisition and performance of cognitively demanding tasks. The results of Wilton et al., (2001) indirectly support this hypothesis, as lesions to rostral Rt have no demonstrable effect on tasks of spatial processing.

Is there a role for Mediodorsal thalamus in attentional set-shifting?

The present results demonstrate that rats with substantial thalamic lesions extending to include the midline/intralaminar thalamic complex and the mediodorsal thalamic nuclei are significantly impaired in their ability to shift attention to a previously irrelevant dimension (ED shift) but not to in their ability to shift behavioural responding to novel exemplars of a previously relevant dimension (ID shift). Nor did such lesions impair performance on discrimination reversals. The result reported here is similar in nature and magnitude to the set-shifting deficit reported in rats after lesions of medial frontal cortex (Birrell and Brown, 2000) and that in marmosets following lesions of lateral prefrontal cortex (Dias, et al., 1996 a, b, 1997). Like humans, damage specific to the frontal lobes is sufficient, although not necessary to produce a selective deficit in extradimensional set-shifting ability (Owen et al., 1991). Collectively, these results imply that the neuropsychological mechanisms underlying the ability to shift attentional set are not the property of a single brain area, such as the prefrontal cortex, but represent the concerted effort of a network of functionally and anatomically related brain regions, of which thalamus represents an important component.

Further investigations are required to determine precisely which thalamic nuclei contribute to the extradimensional set-shifting impairment seen here in rodents. The contributions of the anterior thalamic nuclei and rostral Rt can be disregarded as extensive damage to these regions affects neither attentional set-shifting or discrimination reversals. In 50% of the cases, lesions were far more substantial including the mediodorsal nuclei and the midline intralaminar complex. Given the confounded nature of the results, the precise nuclei mediating this behavioural effect are

not known. However, by virtue of its connectivity, a potential candidate is the mediodorsal nucleus. The mediodorsal nucleus forms dense reciprocal interconnectivity with the prefrontal cortex. It is of great interest that damage to the prefrontal cortex produces perseverative deficits which are comparable in nature and magnitude to those observed in the present study (Mishkin, 1964; Kolb, 1984; Birrell and Brown, 2000). The perseverative deficits following both prefrontal and rats with lesions extending to mediodorsal thalamus might reflect dysfunction in the same system. These results, although tentative imply the mediodorsal thalamic nucleus and prefrontal cortex represent crucial components of a common system subserving extradimensional set-shifting in rats. However, the central segment of mediodorsal thalamus also receives a significant input from the olfactory cortex (Powell et al., 1965). Rats with large electrolytic lesions of the central (olfactory) component of mediodorsal thalamus are severely impaired in their acquisition of an olfactory-based learning set (Lu and Slotnick, 1990; Slotnick and Kaneko, 1991). Thus it has been suggested that mediodorsal thalamus forms part of the thalamocortical circuitry mediating complex olfactory learning. However, in the absence of adequate controls – namely comparable tactile, medium and visual perceptual disciminations - it remains to be determined whether these deficits seen by Lu and Slotnick (1990) and Slotnick and Kaneko (1991) are specific to complex odour discriminations or whether they represent a more general disruption to cognitive flexibility; such as that seen by mediodorsal lesioned rats performing tasks of spatial processing which have no olfactory component (Hunt and Aggleton, 1998). The failure of mediodorsal lesioned rats to solve complex olfactory discriminations might be indicative of a problem not with olfactory discrimination per se, but with the adoption of a central set or strategy. From the present, albeit confounded, results it is impossible to determine whether the observed behavioural

deficit represents a form of cognitive inflexibility that transcends perceptual dimensions or whether it merely represents an inability to acquire complex olfactory discriminations. Studies using other sensory stimuli and/or task paradigms are essential to understanding the precise functions of this nucleus.

Chapter 5: The cholinergic system and selective attention

Abstract

The midbrain cholinergic system, in conjunction with Rt, is known to modulate the transmission of sensory information in anaesthetised animals. The precise role played by acetylcholine in sensory-attentional mechanisms in awake, behaving animals remains to be determined. Specifically, it remains to be determined whether acetylcholine contributes to specific cognitive processes such as attention, or whether it contributes to general non-cognitive processes such as alertness or arousal – particularly during attentional orienting. The present experiment was designed to address this issue and extend upon previous investigations in anaesthetised animals. This was achieved by examining attentional performance, in a task of attentional orienting, following manipulation of cholinergic neurotransmission. One group of rats carried out a visual reaction time task, which allowed the measurement of attentional orienting separately from alertness following systemic administration of nicotine. Nicotine systematically and reliably shortened reaction times to both valid and invalid cues. Conversely, nicotine had variable and inconsistent effects on reaction times to bilateral and non-cued trials. A second group of rats carried out a visual reaction time task to measure covert orienting of attention following systemic administration of scopolamine. Scopolamine disproportionately lengthened reaction times to invalidly cued targets, increasing the validity effect. Taken together, these data indicate that cholinergic transmission represents an important neurochemical substrate of visuospatial attention, influencing specifically spatial aspects of attentional orienting. These results are discussed in terms of the conjoint roles played by Rt and the cholinergic systems (midbrain and basal forebrain) in sensory-attentional

mechanisms. Although somewhat tangential to the central theme of this thesis - Rt's contribution to subcortical attentional processes - this chapter represents the first of many ongoing experiments attempting to determine the relative roles played by Rt and acetylcholine in sensory-attentional mechanisms in awake, behaving animals.

Introduction

The midbrain cholinergic system (which includes the PPTg and LDTg) operates in conjunction with Rt (see especially Ben Ari et al., 1976 and Dingledine and Kelly, 1977) collectively regulating thalamocortical transmission. This is achieved by way of PPTg's inhibitory projections (Ben Ari et al., 1976; Godfraind, 1978; Sillito et al., 1983; McCormick and Prince, 1986; Marks and Roffwarg, 1991) to modality specific regions of Rt and disinhibitory projections (Eysel et al., 1986; Phillis, 1971; Sillito et al., 1983; McCormick and Prince 1987; McCormick, 1991) to functionally related dorsal thalamic nuclei (Shiromani et al., 1990; Uhlrich et al., 1988). Although the cholinergic system, in conjunction with Rt, is known to modulate the transmission of sensory information in anaesthetised animals (Ben-Ari et al., 1976; Dingledine and Kelly, 1977) the precise role played by this neurotransmitter, with respect to attention (especially covert orienting), remains to be determined in awake, behaving animals. Specifically, the extent to which the cholinergic system contributes to 'cognitive' factors such as attention rather than 'non-cognitive' factors such as arousal or alertness in attentional orienting remains to be determined. The present experiment attempted to shed light on this, and other issues (detailed below) by examining attentional performance, in a task of attentional orienting, following manipulation of cholinergic neurotransmission. The attentional orienting task allows specific cognitive processes, such as attention, to be studied separately from general non-cognitive processes, such as alertness and arousal. Although somewhat tangential to the central theme of the thesis - Rt's contribution to subcortical attentional processes - this experiment represents the first of many ongoing experiments attempting to determine the relative roles played by acetylcholine and Rt in sensory-attentional phenomena (Tait et al., 2001).

As noted earlier, the behavioural evidence bearing on a role for the cholinergic system in selective attention, particularly covert attentional orienting, is largely inconclusive. Attention can be oriented in space without necessarily being accompanied by an overt orienting response of the head and eyes. This has been referred to as 'covert orienting' and such shifts in attention have been quantitatively analysed in humans using a cued reaction time paradigm designed by Posner and colleagues (Posner, 1980; Posner et al., 1987; Rafal and Posner, 1987). Reaction time to a visual target is speeded when attention has been drawn to the location of the target by a preceding cue in that location (valid cue) and conversely, reaction times are slowed when the cue misdirects attention away from the subsequent target (invalid cue). This reaction time difference is commonly referred to as the "validity effect" and is thought to represent the cost of disengaging attention and shifting it to a new spatial location. In addition to being spatially informative, cues also provide subjects with a general warning, that increases the general readiness to respond and reduces the temporal uncertainty of the target appearance (Posner, 1980; Witte et al., 1996, 1997; Fernandez-Duque and Posner, 1997). Classically, the contribution of alerting to the target reaction times are assessed by comparing the effect of a so-called "neutral" cue that lacks spatial information with the effects of cues that provide spatial information (i.e. the cost benefit analysis) (Posner, 1980). However because of the difficulties in achieving stimulus "neutrality" (Jonides and Mack, 1984), contemporary studies have typically compared double (neutral) cued trials with a non-cued trial to arrive at a metric for the alerting effect (Witte et al., 1997). This reaction time difference represents the benefit of being alerted to the presence of a forthcoming target. The spatial and alerting provisions of cues may collectively determine the efficacy with which subjects respond to target stimuli.

Evidence from several lines of research has suggested that these mechanisms, orienting and alerting, are mediated by distinct neurotransmitter systems. For example, studies in primates examining the effect of pharmacological manipulations of the nicotinic cholinergic system have revealed consistent, facilitatory effects of nicotine (an agonist) on the orienting of attention (Witte et al., 1997). Conversely, nicotine appears to have no discernible effect on alertness. Both primate and human studies (Witte et al., 1997; Murphy and Klein, 1998) have demonstrated that nicotine, either by subcutaneous injection or inhalation of tobacco smoke, reduces specifically the validity effect by disproportionately reducing reaction times to invalidly cued targets. This would appear to be due to enhanced disengagement of attention as reorientation is more rapid after a distracting invalid cue (Murphy and Klein, 1998). Recently, these results were partially extended to rodents. Using a rodent analogue of Posner's paradigm Brown et al., (1998) demonstrated that nicotine facilitates the reorientation of attention to uncued visual locations. This was manifest as a disproportionate reduction in reaction times to invalidly cued targets, which effectively abolished the validity effect. As of yet no attempt has been made to assess the effect of nicotine on non-spatial aspects of attentional orienting in rats.

Conversely, the blockade of cholinergic activity has been associated with impaired attentional capacities (Callaway, 1984; Sahakian 1988; Warburton and Rusted, 1993; Rusted, 1994). Using the Posner paradigm, Parasuraman et al., (1992) have shown that patients with Alzheimer's disease have significantly larger validity effects, a consequence of increased costs following invalidly cued targets. Parasuraman et al., (1992) concluded that these individuals are specifically impaired in their ability to disengage attention from invalidly cued locations. Excitotoxic lesions of the basal

forebrain in primates reveal an analogous selective deficit in attentional orienting (Voytko et al., 1994). Pharmacological manipulations of the muscarinic cholinergic system have proved less consistent. A recent study carried out by Davidson et al., (1999) found a reduction in the validity effect in rhesus monkeys following scopolamine administration (a muscarinic cholinergic antagonist). Scopolamine produced a dose dependent increase in all reaction times and a decrease in accuracy, which was most prominent for valid cues. This result is somewhat anomalous, as cholinergic blockade is typically associated with an increase in reaction times to invalidly cued targets (Parasuraman et al., 1992; Voytko et al., 1994). Davidson et al., (1999) suggest their results might still be due to an impairment in disengaging attention, but from the fixation point rather from an invalidly-cued target. Of course, it is somewhat unsatisfactory if either an increase or a decrease in the validity effect is used to support the same conclusion. Furthermore, scopolamine was reported to have no demonstrable effect on alertness (Davidson et al., 1999). This results contrast with the findings of Witte et al., (1997), who report that atropine (another muscarinic antagonist) reduces overall reaction times overall and does not influence the validity effect. It is unclear why manipulations of the primate muscarinic cholinergic system have proved inconsistent and invariable. A parsimonious explanation of these disparate findings may lie in the unacceptably low doses of scopolamine administered to primates, 1000 times lower than that typically given to primates (Lynch et al., 1999) and 100 times lower than that administered to rodents (Brown et al., 1998).

The present study was designed to clarify the effects of both nicotine and scopolamine on covert orienting in the rat. Cholinergic agonists (nicotine) and antagonists (scopolamine) were administered to rats trained to perform a cued target detection task

which is formally the same to that used in humans (Posner, 1980) and primates (Bowman et al., 1993; Witte et al., 1996; Witte et al., 1997; Davidson et al., 1999). Rats were required to fixate a central visual stimulus and respond to the onset of visual targets presented randomly in two visual field locations. The location of the target's appearance was preceded by a cue that was either valid (cue and target at same location), invalid (cue and target at opposite locations), spatially uninformative (bilateral cues) or omitted altogether. It was predicted that nicotine would selectively facilitate the orienting of attention and have no effect on alerting to cued targets. This would provide evidence that the neural mechanisms mediating orienting and alertness are subserved by distinct neurotransmitter systems in rodents. The present experiment also intends to contribute to the debate over the effect of muscarinic cholinergic blockade, which has been less consistent in primates. It was predicted that scopolamine would increase in the validity effect, manifest as a disproportionate lengthening of reaction times to invalidly cued targets.

Experimental Procedures

Subjects

21 experimentally naïve male Lister Hooded rats (Charles River, UK), approximately 2 months old at the start of experimentation served as subjects (weight range 210-290gm). Prior to experimentation, subjects were placed on a controlled food schedule, comprised of standard laboratory chow, earned sucrose pellets and freely available water. Subjects were on a 12:12 hour dark/light cycle, experimental manipulations taking place during the light phase. All of the aforementioned practices were performed in accordance with the UK Animals (Scientific Procedures) Act 1986.

Apparatus

Training was conducted in 4 identical nine hole operant boxes enclosed in sound attenuating chambers (CeNes Ltd., Cambridge, U.K). Chambers were ventilated by quiet operation fans, which provided a steady stream of background noise. The operant box was made entirely of aluminium with the exception of the front wall, which had an outward opening Plexiglas division. The floor of the chamber was comprised of a metal grid. A magazine tray with an inward opening Plexiglas panel was recessed into the front wall of the camber. Nine capped holes (with recessed light bulbs) were situated at the back of the chamber, 1cm above the grid floor. For the purposes of this experiment, the middle three holes were uncapped. Photocell light source assemblies across the front of each hole detect entries and withdrawals into each nose-poke hole. When trials were completed successfully (i.e. the rat responded in the target stimulus hole successfully) a sucrose pellet (45mg, Bioserv Incl., New

Jersey) was delivered to a food hopper positioned on the wall opposite the response holes.

Behavioural training

The training and testing protocols are essentially the same as that described by Ward and Brown (1996). After the rats were habituated to handling, they received several weeks of training, shaping them to maintain a nose-poke in the central of three holes and then to respond to visual targets in the peripheral holes. First rats were habituated to the chambers for 1 hr with food pellets placed in the hopper. Secondly, rats were trained to collect food from the hopper by making food delivery and light onset in the hopper contingent on a panel press. Third, a trial was initiated by a panel press, which illuminated the bulb in the central hole. Nose pokes into the central hole were rewarded by the onset of the panel light and delivery of a food reward into the hopper. The required duration of the nose poke was systematically increased over a period of days, until rats could make, competent sustained nose pokes (up to 0.5 sec), upon which the house and hopper lights were turned on, food reward was delivered and the rat could initiate another trial with a panel press). Failure to do so (i.e. premature withdrawal or failure to initiate a nose poke within the central hole) resulted in deactivation of the house light for a 1 sec 'time out' period and no food reward.

Behavioural testing

The covert orienting paradigm has already been extensively described (Brown and Robbins, 1989; Ward and Brown, 1996). The trial events are depicted in Figure 5.1. Briefly, following illumination of the central hole subjects were required to initiate a sustained nose poke, maintaining fixation for variable foreperiod (0.2, 0.3, 0.4 and 0.5

secs) during which a bright target stimulus (150 msec duration) was presented in either the left or right nose hole. To measure attentional 'orienting' two types of trials were presented. In a valid trial, the cues and target are located on the same side of fixation (Fig. 5.1a). In an invalid trial, cue and target are on opposite sides of fixation (Fig. 5.1b). To measure 'alerting' separately from 'orienting', nicotine administered rats received two additional trial types: bilateral trials, where both cues are illuminated and the target appears either on the right or left (Fig 5.1c), and non-cued trials (Fig. 5.1d), where cues are omitted, but the timing of target appearance is the same as for the other trials. For each trial type, targets appeared equally as often on the right and left sides of fixation. A single sucrose pellet was dispensed to the magazine tray following successful trial completion (i.e. rat responds in target stimulus hole). Anticipatory errors (premature withdrawal from the central hole < or equal to100msecs), failure to respond (late errors >2 sec) or incorrect responses resulted in deactivation of the chamber lights for a 1 sec 'time out' period and no food reward. Each testing session lasted until successful completion of 120 trials or until 60 minutes had elapsed. It should be emphasised that while no attempt was made to measure eye movements, exploratory eye movements are thought to be minor and infrequent in rats (Sefton and Dreher, 1995). Rather, orienting head movements are made towards stimuli of interest. Therefore, for confirmation of covert orienting in the rats, it is important that during fixation the head of the rat is maintained centrally and still (Weese et al., 1999). The configuration and sensitivity of sensors of the nose poke holes is such that during fixation the head of the rat is oriented forward and level. For this reason, we feel that this study measures covert, rather than overt (Bushnell, 1995) attentional shifts.

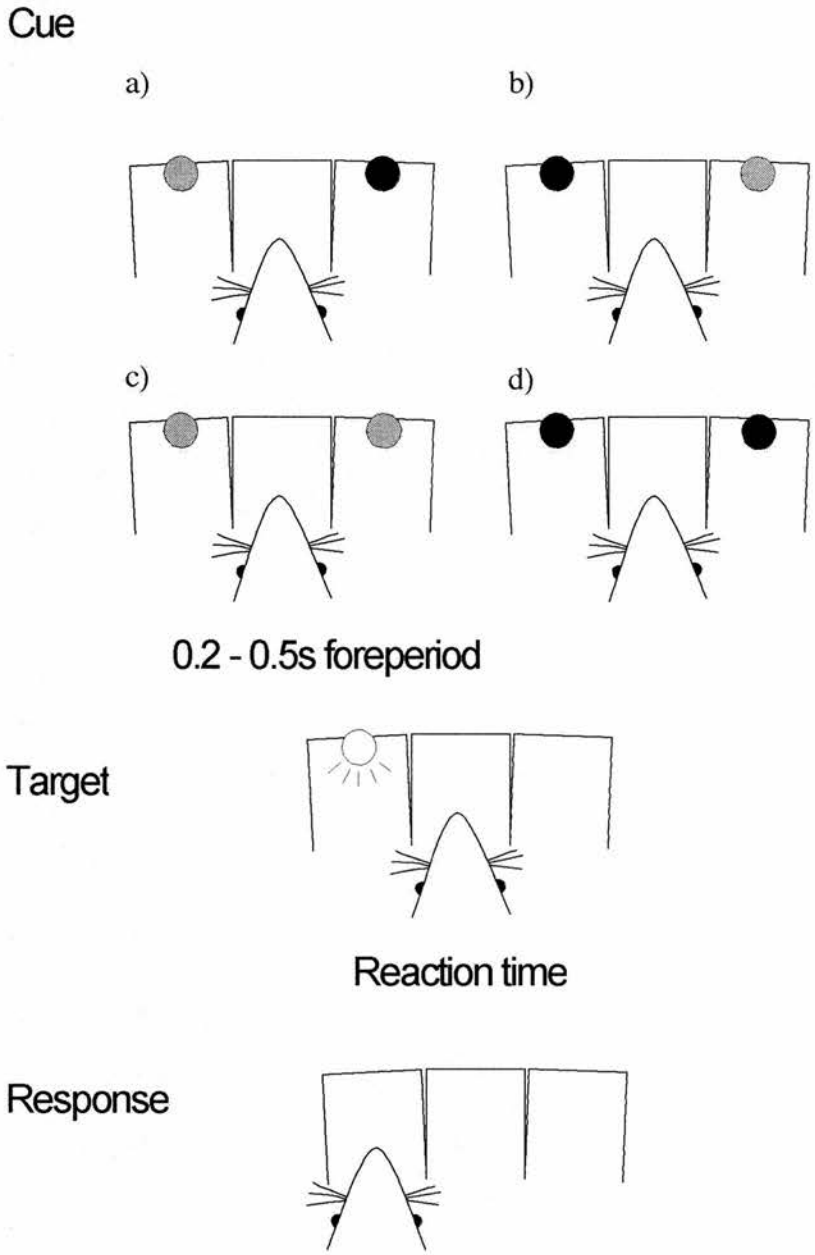


Figure 5.1. Schematic representation of trial events. Rats were trained to make a sustained nose-poke and wait (0.2 – 0.5 secs) for the target. At the start of the variable foreperiod, a brief, dim, cue was presented at the beginning of the foreperiod. The side of the cue did not predict the side of the target. This cue could be either a) on the same side of the target (validly cued) b) the opposite side (invalidly cued), c) bilateral or d) omitted altogether. Rats reported the location of the target by making a nose poke in the side hole.

Chemicals

Nicotine tartrate (Sigma) was dissolved in 0.9 saline and titrated to pH7 using sodium hydroxide. Scopolamine hydrobromide (sigma) was dissolved in 0.9% saline and administered with equimolar solutions of neostigmine methylsulphate. All doses were calculated from the weight of the salt. All drugs were kept at room temperature and light protected.

Drug administration and procedure

Nicotine administration

Nicotine was administered subcutaneously 15 minutes prior to testing. Prior to nicotine testing, subjects received 2 priming doses of nicotine (0.8 mg/kg). It has been suggested that prior to sensitisation, the effects of nicotine may be variable and opposite to those expected (see Balfour, 1998). Following sensitisation each subject was injected with nicotine (0.0, 0.2, 0.4 and 0.8 mg/kg) subcutaneously 15 minutes prior to experimentation. Nicotine testing was carried out over four test days with each rat receiving one of four doses on each testing day. Dose order was randomised and counterbalanced across testing chambers.

Scopolamine administration

Scopolamine hydrobromide was administered intraperitoneally (0.0, 0.03, 0.06 mg/kg) with equimolar solutions of neostigmine methylsulphate 30 minutes prior to experimentation. Neostigmine, which does not cross the blood-brain barrier, is used to control for any non-central effects of muscarinic blockade. Scopolamine testing was carried out every second day with each rat (n=10) receiving each of three doses in

three separate blocks of sessions. Dose order was randomised and counterbalanced across testing chambers.

Data analysis

Reaction times to invalid, valid, bilateral or non-cued trials were defined as the time taken from target onset to withdrawal from the central hole.

Nicotine Testing

Mean reaction times were calculated for each rat and analysed using 2 repeated measures ANOVAS, one with dose (0.0, 0.2, 0.4, 0.8 mg/kg), cue condition (valid and invalid), and foreperiod (0.2, 0.3, 0.4, 0.5 sec) as within subject variables and another with dose (0.0, 0.2, 0.4, 0.8 mg/kg), cue condition (bilateral and no-cues) and foreperiod (0.2, 0.3, 0.4, 0.5 sec) as within subject variables. Two additional data sets were constructed to represent the validity and alerting effects. Validity effects were calculated by subtracting mean reaction times for valid cues from that of invalid cues, for each foreperiod and analysed separately using a repeated measures ANOVA with dose (0.0, 0.2, 0.4, 0.8 mg/kg) and foreperiod (0.2, 0.3, 0.4, 0.5 sec) as within subject variables. Alerting effects were calculated by subtracting mean reaction times for bilateral cue conditions from that of no cue conditions for each foreperiod and were analysed using a repeated measures ANOVA with dose (0.0, 0.2, 0.4, 0.8 mg/kg) and foreperiod (0.2, 0.3, 0.4, 0.5 sec) as within subject variables.

Scopolamine testing

Mean reaction times were calculated for each rat and analysed using a repeated measures ANOVA, with dose (0.0, 0.3, 0.6 mg/kg), cue condition (valid and invalid), and foreperiod (0.2, 0.3 0.4, 0.5 secs) as within subject variables. Validity effects were calculated by subtracting mean reaction times for valid cues from that of invalid cues for each foreperiod and analysed using a repeated measures ANOVA with dose (0.0, 0.3, 0.6 mg/kg), and foreperiod (0.2, 0.3, 0.4, 0.5 sec) as within subject variables.

Nicotine sensitisation: effects on spatial provisions of cues

Nicotine reduced reaction times overall as a function of sensitisation (main effect of dose: $F_{(2,20)} = 5.7$ $p<0.01$) – see Figure 5.2. This decrease was equal for both valid and invalidly cued targets (no interaction between dose and validity: $F_{(2,20)} = .160$ $p>0.05$). A restricted analysis – following a main effect of dose – revealed that overall reaction times (to either cue type) were not significantly different from baseline following priming dose 1 ($F_{(1,10)} = .183$ $p>0.05$), highlighting the general ineffectiveness of initial priming doses.

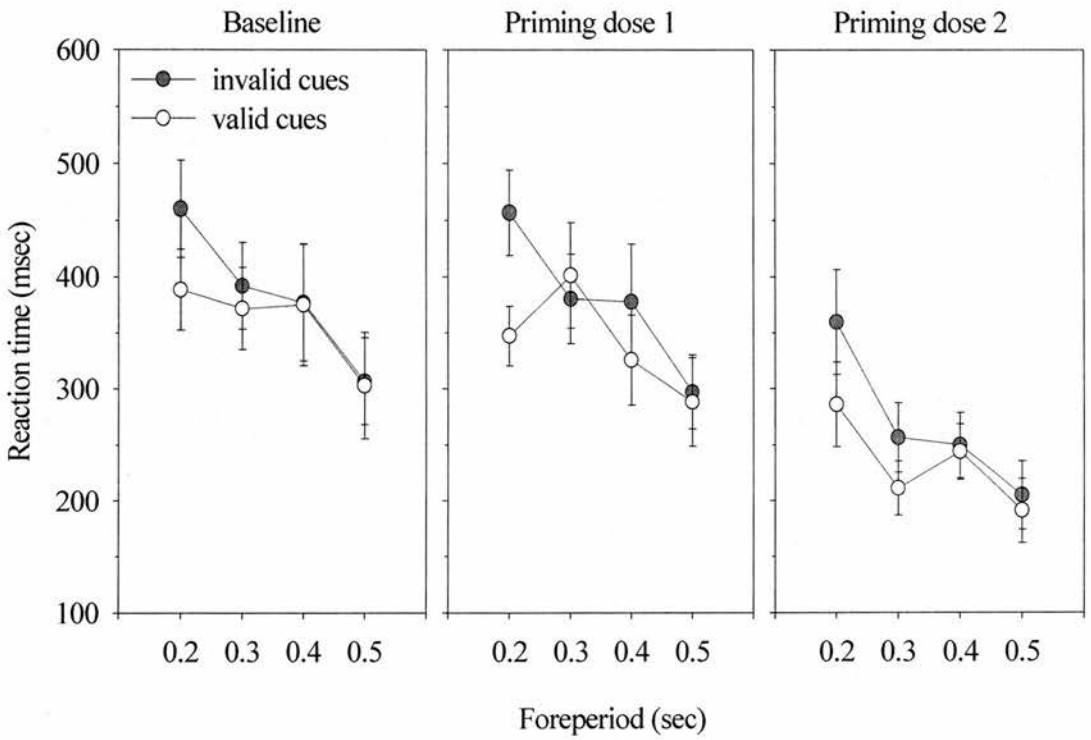


Figure 5.2. Effects of nicotine sensitisation on reaction times (mean ± SEM) for different cue types (invalid versus valid) at each foreperiod.

Nicotine sensitisation: effects on non-spatial provisions of cues

Figure 5.3 shows the effect of nicotine sensitisation on mean reaction times to bilateral and non-cued targets at each foreperiod. Mean reaction times reduced overall as a function of sensitisation (main effect of dose: $F_{(2,20)} = 5.17$ $p<0.05$; no interaction between dose and cue-type: $F_{(2,20)} = .510$ $p>0.05$). A restricted analysis – following a main effect of dose – revealed that overall mean reaction times to either cue type were not significantly different from baseline following priming dose 1 ($F_{(1,10)} = .723$ $p>0.05$).

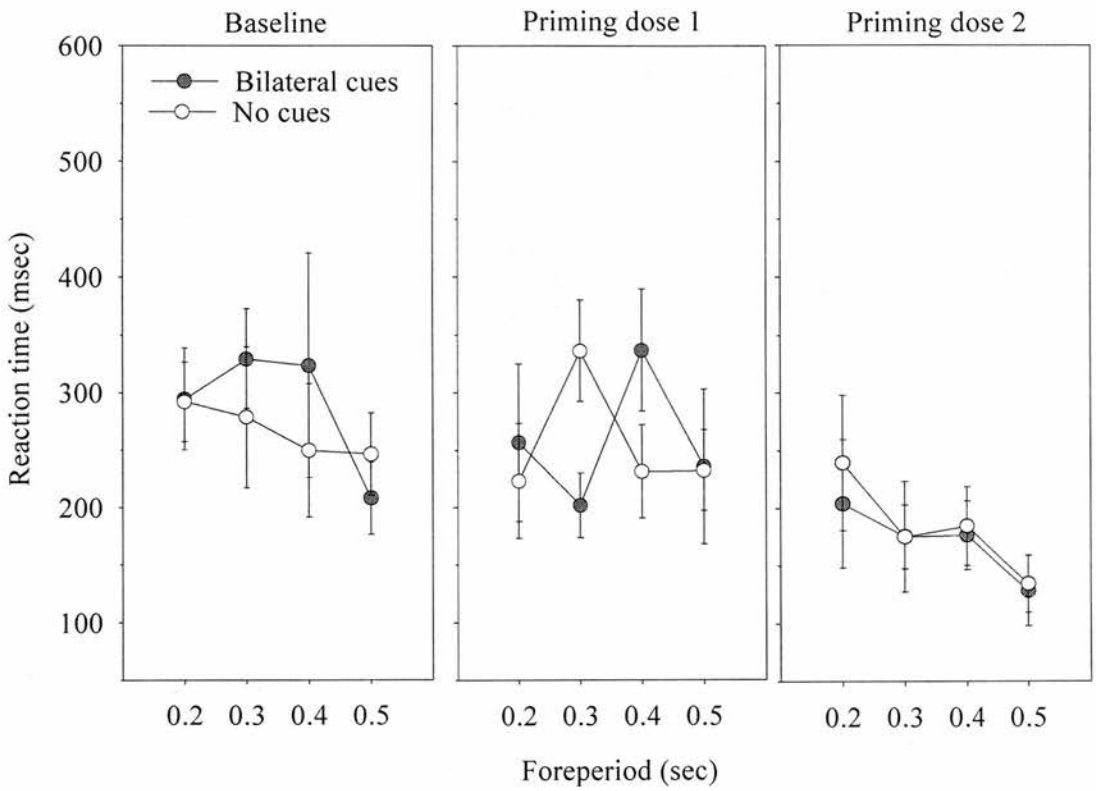


Figure 5.3. Effects of nicotine sensitisation (0.8 mg/kg) on reaction times (mean ± SEM) for bilateral and non-cued trial types.

Nicotine dosing: effects on spatial provisions of cues

Figure 5.4 shows the effect of nicotine on mean reaction times for invalid and validly cued targets at each foreperiod. Nicotine (0.0, 0.2, 0.4, 0.8 mg/kg) reduced overall reaction times in a dose-dependent manner which approached significance (main effect of dose: $F_{(3,30)} = 2.54$ $p=0.08$). As this reduction was comparable for both cue-types (no interaction between dose and validity: $F_{(3,30)} = .874$ $p>0.05$), validity effects (mean reaction times to invalid cues minus valid cues) were not significantly different across doses (no main effect of dose: $F_{(3,30)} = .874$ $p>0.05$).

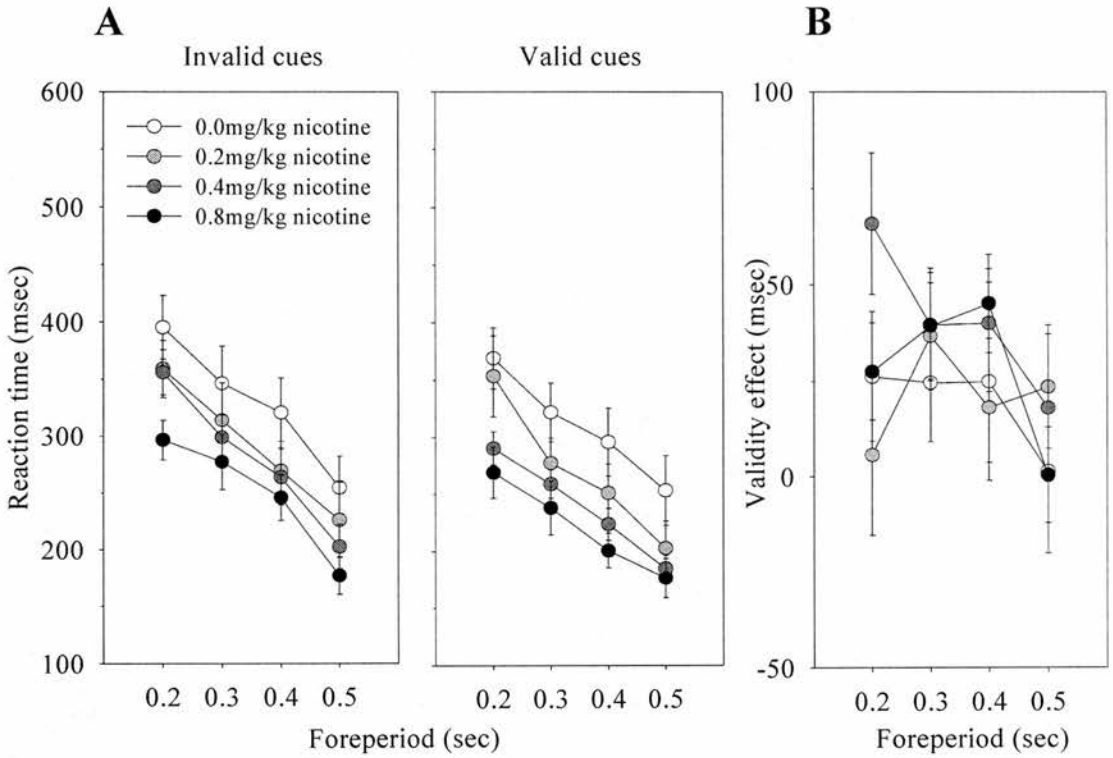


Figure 5.4. ***A*** *Effects of nicotine (0.0, 0.2, 0.4, 0.8 mg/kg) on reaction times (mean ± SEM) for different cue types (invalid versus valid) at each foreperiod.* ***B*** *Effects of nicotine (0.0, 0.2, 0.4, 0.8 mg/kg) on validity scores (mean ± SEM) are shown for each foreperiod.*

Nicotine dosing: effects on non-spatial provisions of cues

Figure 5.5 shows the effect of nicotine on reaction times to bilateral and non-cued targets. Overall, nicotine (0.0, 0.2, 0.4, 0.8 mg/kg) had no effect on mean reaction times to bilateral or non-cued targets (main effect of dose: $F_{(3,30)} = .738$, $p>0.05$). As there was no interaction between dose and cue-type ($F_{(3,30)} = .325$, $p>0.05$) alerting effects remained unchanged (no main effect of dose: $F_{(3,30)} = .325$, $p>0.05$) across doses.

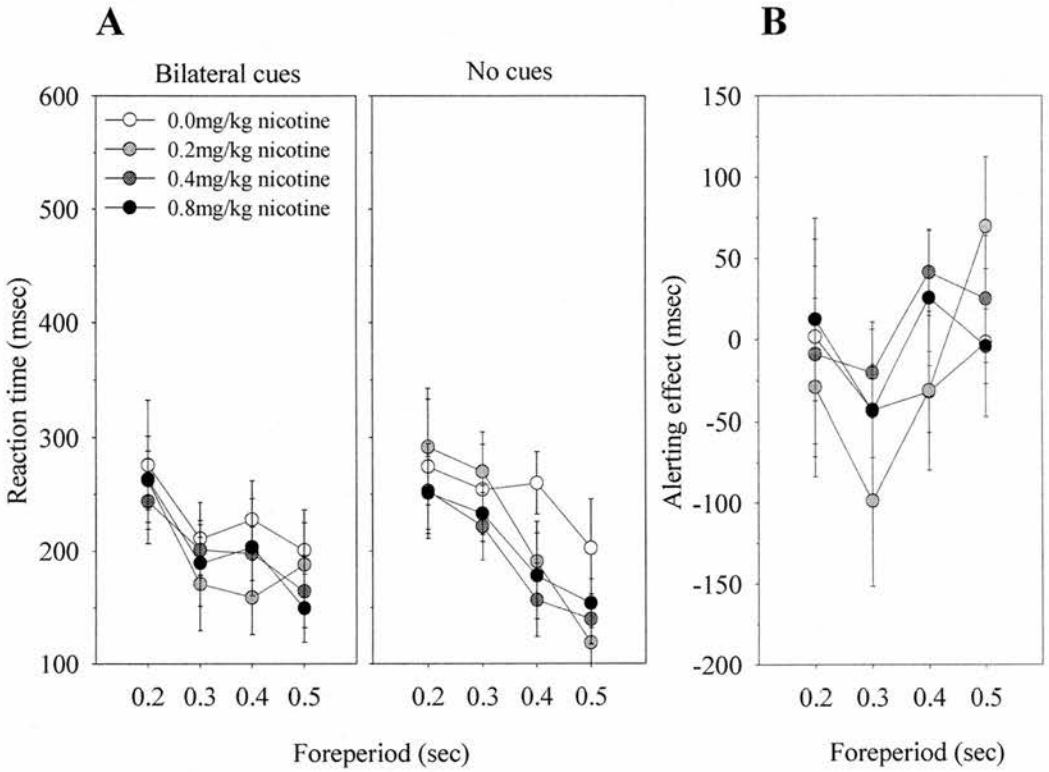


*Figure 5.5. **A** Effects of nicotine (0.0, 0.2, 0.4, 0.8 mg/kg) on reaction times (mean ± SEM) for different cue types (bilateral and non-cued) at each foreperiod. **B** Effects of nicotine (0.0, 0.2, 0.4, 0.8 mg/kg) on alerting scores (mean ± SEM) are shown for each foreperiod.*

Scopolamine dosing: effects on spatial provisions of cues

Mean reaction times increased as a function of increasing dose of scopolamine (main effect of dose: $F_{(2,18)} = 4.71$, $p<0.05$) at each cue target interval (no interaction between dose and delay: $F_{(2,18)} = .76$, $p>0.05$) – see Figure 5.6. This increase was disproportionate for invalidly cued targets (interaction between dose and validity: $F_{(2,18)} = 4.44$ $p<0.05$). Validity effects were significantly increased across scopolamine doses (main effect of dose: $F_{(2,18)} = 4.94$, $p<0.05$) at each cue-target interval (no interaction between dose and delay: $F_{(6,54)} = .520$, $p>0.05$).

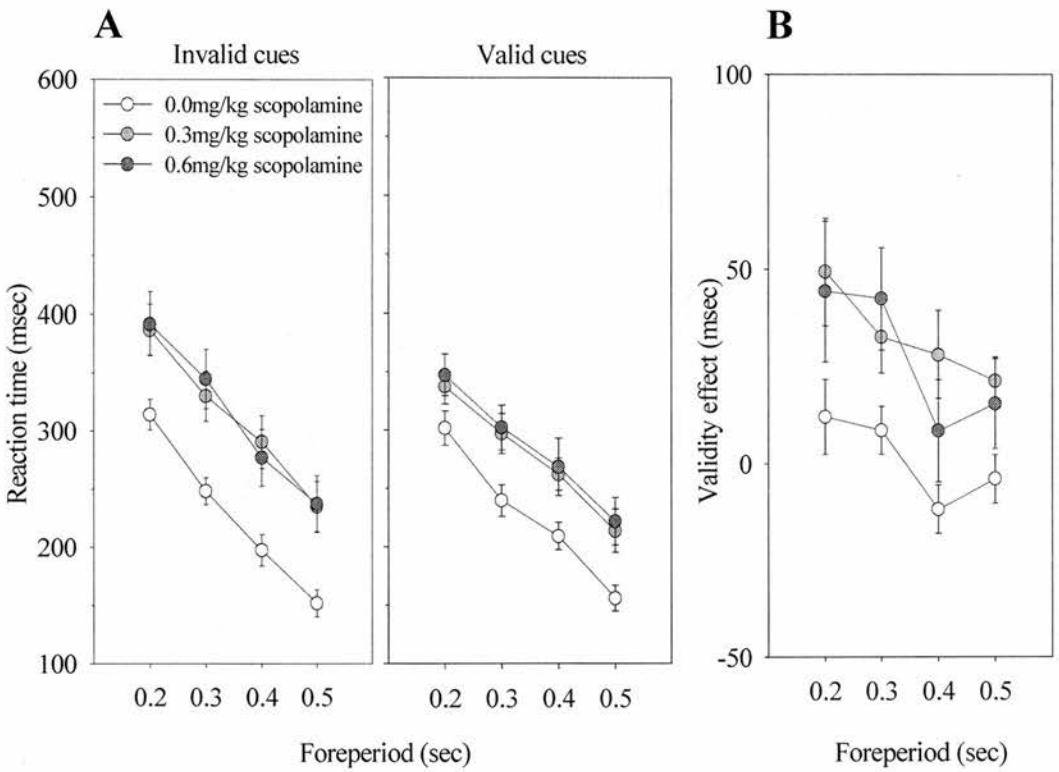


Figure 5.6. ***A*** *Effects of scopolamine (0.0, 0.3, 0.6 mg/kg) on reaction times (mean ± SEM) for different cue types (invalid versus valid) at each foreperiod.* ***B*** *Effects of scopolamine (0.0, 0.3, 0.6 mg/kg) on validity scores (mean ± SEM) are shown for each foreperiod.*

Discussion

The midbrain cholinergic sytem, in conjunction with Rt, has been demonstrated to regulate the transmission of sensory information in anaesthetised animals (see especially Ben Ari et al., 1976 and Dingledine and Kelly, 1977). The present experiment sought to examine the role of acetylcholine in sensory-attentional mechanisms in awake, behaving animals. This was achieved by examining attentional performance, in a task of attentional orienting, following manipulation of cholinergic neurotransmission using the cholinergic agonist, nicotine and the cholinergic antagonist, scopolamine. The primary objective of the present experiment was to determine the extent to which acetylcholine contributes to specific cognitive processes such as attention rather than general non-cognitive processes such as alertness or arousal. This experiment also sought to elucidate the relative roles played by the nicotinic and muscarinic cholinergic systems. Although somewhat tangential to the central theme of this thesis, this experiment represents one of the first attempts to elucidate the relative importance and neurochemical substrate of the pathways involved in sensory-attention modulation.

Nicotine: effects on spatial and non-spatial provisions of cues

Nicotine, a nicotinic receptor agonist, systematically reduced reaction times to invalid and validly cued targets overall. Conversely nicotine had inconsistent, transitory effects on alerting to visual targets. Reaction times to invalid and validly cued targets decreased with increasing doses of nicotine (an effect which approached significance). This dose-dependent effect contrasts with the essentially random effects of nicotine on reaction times to bilateral and non-cued trials; see Figure 5.5. These results are

consistent with a substantial body of evidence to suggest a cholinergic involvement in attention in general (e.g., Callaway, 1984; Sahakian, 1988; Warburton and Rusted, 1993; Rusted, 1996; Muir et al., 1995; Turchi and Sarter, 1997; Mancuso et al., 1999) and covert orienting in particular (Parasuraman et al., 1992; Voytko et al., 1994; Witte et al., 1997; Murphy and Klein, 1998; Davidson et al., 1999). In humans and primates orienting is independent of the level of alertness in Posner's paradigm (Fernandez-Duque and Posner, 1997; Murphy and Klein, 1997; Witte et al., 1997). The present results partially extend this to rodents, as overall nicotine had a transient, modest effect on alerting to visual cued targets. This can be seen in Figure 5.3, where a second 'priming' dose of nicotine significantly reduces reaction times to bilateral and non-cued trials. These results imply that the mechanisms mediating alertness and orienting are not completely separate in rodents and interact to some degree. It isn't immediately obvious why subsequent doses of nicotine failed to reduce reaction times to bilateral and non-cued trials. It may be parsimonious to assume that mechanisms other than nicotine administration come into play during these initial injections. The failure of initial priming doses of nicotine to reduce reaction times to valid, invalid, double or non-cued trials (See Figures 5.2 and 5.3) cued targets is consistent with the suggestion that nicotine may have little or no effect in the absence of a 'sensitising' procedure. (Balfour et al., 1998). This finding may represent an important caveat to be borne in mind when interpreting so called 'negative' results in the absence of a sensitising procedure.

Scopolamine: effects on covert orienting of attention

Conversely, scopolamine, a muscarinic receptor antagonist, resulted in an overall slowing of reaction time and an increase in the validity effect, due to a

disproportionate lengthening of reaction times to invalidly cued targets. While consistent with effects reported in humans (Parasuraman et al., 1992) and non-human primates (Voytko et al., 1994), these present results contrast with those reported in non-human primates by Davidson et al., (1999), that scopolamine reduced the validity effect by increasing reaction times to valid cues more than to invalid cues. They also contrast with the results of Witte et al., (1997), who report that atropine (also a muscarinic antagonist) reduced overall reaction time and did not influence the validity effect. It is not obvious why two antagonists of the same receptor would have such contrasting effects in the non-human primate. It is also not obvious why neither of the primate studies are consistent with the data from the rats. Davidson et al., (1999) conclude that scopolamine results in a deficit of disengagement from the fixation point to orient towards the cues. However, this account may be regarded as inconsistent, given that there is no problem in disengagement from an invalid cue (the manifestation of which would have been a greater increase in invalid reaction times). As for the inconsistency of the effect of scopolamine and atropine in the non-human primate, Davidson et al., (1999) suggest that this might be due to unpredicted differential effects of blockade of pre and post-synaptic muscarinic receptors. Given that their highest dose of scopolamine was only 0.0005 mg/kg (more than 1000 times lower than other authors have used in non-human primates (e.g. Lynch et al., 1999) and almost 100 times lower than the dose of 0.06mg/kg we used in the rat) this would seem to be the most parsimonious explanation. We do agree with Davidson et al., (1999) that blockade of muscarinic cholinergic receptors results in a deficit in the disengagement or movement of attention. We base this conclusion on our finding that once attention is directed to a cue, reaction time to a subsequent target in a different location is lengthened. The deficit reported here is comparable in nature and

magnitude to the attentional deficits displayed by Alzheimer's patients performing a human analogue of this task (Parasuraman et al., 1992). The scopolamine-administered rodent provides a relatively inexpensive model of the attentional orienting deficits manifest in early stages of this neurodegenerative disorder (Parasuraman et al., 1992). Such cross-species generalisation is valuable as it provides a comparative model, which can be used to investigate the neural basis of both normal and abnormal attentional function. Further progress in understanding the neurobiology of attentional dysfunction in human diseases may depend on the use of such accessible experimental animals (see Bushnell, 1998).

The neural and neurochemical basis of selective attention

These data strongly suggest that normal cholinergic activity is necessary for attentional orienting. The present results support a role for acetylcholine in specific cognitive processes, such as selective attention, rather than non-cognitive processes, such as general alertness or arousal. These data extend upon previous investigations in anaesthetised animals (see especially Ben Ari et al., 1976 and Dingledine and Kelly, 1977) demonstrating a role for acetylcholine in the regulation of sensory transmission. The present results suggest this regulation may be modulated by attention. However, the effects of systemic drugs do not shed light either on the issue of which cholinergic system is involved in covert orienting or the neural basis of these systemic effects. An obvious candidate is the basal forebrain cholinergic projection to frontal and/or parietal cortex, based on lesion work in rats (Muir et al., 1995, Muir et al., 1996) and non-human primates (Voytko et al., 1994). However, because of previous works implicating Rt in attentional orienting (Weese et al., 1999), a more likely candidate is

the cholinergic innervation, from basal forebrain and the pedunculopontine tegmental nucleus, of the thalamus and Rt (Hallanger, 1987).

The midbrain cholinergic system and Rt

It has been suggested that the internal 'spotlight' which facilitates target detection in Posner's covert orienting paradigm is initiated (switched on) by Rt and that the functional basis of this searchlight is the arrhythmic, amplified 'burst' firing patterns in thalamic relay neurons (Crick, 1984). As noted earlier, thalamic relay neurons respond to sensory input in two modes, 'tonic' and 'burst'. It has been suggested that burst and tonic firing represent the neurophysiological basis of two distinct, but inter-related forms of visual attention referred to as orienting and focal attention (Posner and Peterson, 1990). Orienting attention is used to detect salient visual stimuli thus may benefit from the amplified response of relay cells during burst firing. In contrast focal attention, which requires the tonic firing of dorsal thalamic relay cells, is used to analyse visual stimuli in detail. The tonic discharge of Rt neurons during wakefulness may provide the priming actions (membrane hyperpolarization) required to induce the burst discharge thought to under lie attentional orienting in the awake, behaving animal. However, because this burst responding is unsuitable for sensory analysis, somehow the system has to be switched from burst to tonic firing allowing the faithful relay and analysis of sensory input (see Guido and Weyand, 1995). The midbrain cholinergic system (PPTg and LDTg), in conjunction with Rt, is known to modulate the relay of sensory information in anaesthetised animals (Ben-Ari et al., 1976; Dingledine and Kelly, 1977). This system may also modulate the excitability of the thalamocortical attentional network during wakefulness, so that once targets have been detected, the system can be appropriately modified (i.e. transferred to tonic

firing) allowing the analysis of sensory information, and the execution of an appropriate behavioural response. The present results, in conjunction with previous findings (see especially Ben Ari et al., 1976, Dingledine and Kelly, 1977, and Weese et al., 1999) support the notion that the Rt and the midbrain cholinergic system (PPTg and LDTg) may operate in unison, collectively determining orienting and focussed attention to task relevant cues during wakefulness.

The basal forebrain cholinergic system and Rt

Previous works have indicated that the basal forebrain cholinergic system also plays an important role in the orienting of attention to visual stimuli (Muir et al., 1995, 1996). In rodents, projections from the substantia innominata project to visual Rt (see Kolmac and Mitrofanis, 1999). Because lesions to either area in rodents impairs the attention to and subsequent detection of brief, unpredictable target stimuli (Weese et al., 1999; Muir et al., 1995, Muir et al., 1996), it may be parsimonious to assume that attentional deficits following lesions to either region reflects dysfunction of a common system serving attentional orienting. A series of ongoing, collaborative experiments are currently attempting to elucidate the relative importance and neurochemical substrates of the basal forebrain and midbrain projections to Rt (Tait et al., 2001).

Although this study clearly demonstrates a role for cholinergic transmission in attentional processes, specifically the covert orienting of attention, the present finding are limited in that they fail to address the neural basis of these systemic drug effects. A series of parallel experiments are currently being undertaken in an attempt to understand the relationships between attention, Rt and the midbrain and basal forebrain cholinergic systems. The fact that previous works (see Chapter 2 and especially Weese et al., 1999)

have implicated Rt in this task of attentional orienting make this structure and the ascending and descending cholinergic pathways suitable candidates for further investigation.

Chapter 6: General Discussion

Subcortical mechanisms of attentional selectivity and control

Preliminary behavioural evidence suggests that the Rt operates as an attentional gate or 'filter' at both sensory (see especially Weese et al., 1999, and Montero, 1997, 1999, 2000) and higher cognitive levels (Vann et al., 2000; Wilton et al., 2001). Caudal sectors of Rt, which form complex topographical connections with modality specific thalamic nuclei and their respective cerebral cortices (for a review see Guillery et al., 1998 or Crabtree, 1999), are thought to mediate sensory attentional processes (Weese et al., 1999; Montero 1997, 1999, 2000), while rostral Rt, which forms connectivity with the anterior, mediodorsal and midline/intralaminar thalamic nuclei, is thought to be involved in more complex 'higher cognitive' processes, such as spatial learning and memory (M'Harzi et al., 1991; Collery et al., 1993; Vann et al., 2000; Wilton et al., 2001). However, there is considerable doubt first, as to whether Rt mediates selective attention as opposed to sensory or motor functions (see especially Montero 1997, 1999, 2000), and second, whether there is a dissociation of function across the rostrocaudal extent of this nucleus (see especially Wilton et al., 2001). Chapters 2-4 attempted to investigate these issues systematically. Chapter 5 sought to examine the role played by acetylcholine in a task of attentional orienting. Although somewhat tangential to the overall theme of the thesis, the cholinergic system was chosen for investigation as this system, in conjunction with Rt, is known to modulate thalamocortical transmission (see especially Ben-Ari et al., 1976; Dingledine and Kelly, 1977). This thesis suggests that the Rt (in conjunction with the midbrain and basal forebrain cholinergic systems) contributes to sub-cortical stages of attentional

processing where currently significant sensory information is selected for further processing at the expense of currently irrelevant input.

Chapters 1-3: Rt and selective attention

Selective attention involves the filtering of aspects of the environment to allow processing of any given stimulus, dependent on its intrinsic nature or its position in space (Treisman, 1969). Preliminary behavioural evidence suggests Rt operates as an attentional gate or filter (see Chapter 1) which facilitates the transmission of task-relevant cues. Montero (1997) demonstrated selective Fos activation in the modality specific sector of Rt as a function of the cues (visual or somatosensory) with which subjects (normal or blind) explored a novel environment. However, the methodological limitations of this task (see Chapter 1) make it impossible to determine whether Fos activity following exploration of a novel complex environment represents attentional rather than sensory or motor task requirements. Using more sophisticated attentional paradigms, Chapters 2 and 3 sought to elucidate the mechanisms underlying Fos labelling within Rt. In Chapter 2, Fos expression was examined in rats trained to perform a visual reaction time task. The target stimuli to which the rat was required to respond were preceded by cues, which served to direct attention towards the subsequent target. The visual cues and targets were presented to one side of the rat's head only. Different rats were trained to respond towards or away from the side of the visual target. Uniform Fos expression, across hemispheres, was observed in the first-order visual thalamic relay nucleus, the dorsal lateral geniculate. Of most interest, there was non-uniform Fos protein in the visual sector of the Rt contralateral to the visual stimuli. This was regardless of the direction of the response. These data support the suggestion that the visual sector of Rt is selectively activated in a task involving

the allocation of visual attention. However, it could be argued that non-uniform labelling of visual Rt contralateral to attended visual stimuli merely represents disproportionate visual input and not necessarily the disproportionate allocation of attentional resources. In order to demonstrate that Fos immunoreactivity within visual Rt represents the attentional and not stimulatory properties of stimuli, Fos expression was examined following a behavioural task - Kamin's (1969) conditioned blocking task - in which attention is directed to one stimulus and not another. In this procedure, a stimulus that reliably predicts reward evokes a conditioned response. A second stimulus, introduced after conditioning but presented simultaneously with the first, is redundant and therefore results in no conditioned response. The second stimulus is referred to as the 'blocked stimulus'. Despite the simultaneous presentation of visual and auditory stimuli to all rats, during a final test session, Fos-immunoreactive neurons were more numerous in the sector of Rt associated with the attended (conditioned) stimulus than in the sector associated with the unattended (blocked) stimulus (see Figure 3.3). These demonstrations of selective activation in the sector of Rt associated with attended stimuli support the view that this structure is more than a component of the sensory relay, but rather acts as an attentional gate or filter, modulating the flow of information between thalamus and cortex.

Collectively, Chapters 1 and 2 suggest that Rt mediates the shifting of attention to brief sensory stimuli that predict biologically relevant events such as the presentation of a food reward. Furthermore, in Chapter 3 the degree of Fos labelling within Rt correlated with the magnitude of the conditioned response to the conditioned stimulus. This result not only supports the view that the Rt is involved in the processing of sensory information (Schlag and Waszak, 1971; Frigyesi and Schwartz, 1972; Steriade

and Wysinski, 1972; Sumitomo et al., 1976; Yingling and Skinner 1976; Hale et al., 1982; French et al., 1985; Salt, 1989; Simons and Carvell, 1989; Lee et al., 1994 a, b; Warren and Jones, 1994; Hartings and Simons, 2000; Hartings et al., 2000) but demonstrates that it is also involved in attention. These results imply reticular cells receive additional information, possibly from the orbitofrontal cortex, related to stimulus relevancy, e.g., information on emotional state, rewarding properties of stimuli, which independently influence the orienting aspects of attentional processing. Thus, reticular cells appear to modulate the transmission of sensory information through thalamus according to their behavioural relevance. These results support and extend those of Montero (1997, 1999, 2000), by demonstrating that Fos immunoreactivity within Rt is contingent upon attentional and not on uncontrolled sensory or motor task requirements. Together, these results support a role for Rt in the transmission of essential, behaviourally relevant cues in order to guide appropriate, efficient behaviour (Montero 1997, 1999, 2000). There are two alternative, although not mutually exclusive, mechanisms by which Rt might contribute to selective attention in rodents.

The neurophysiological basis of selective attention

Internuclear communication: the relationship between Rt and thalamus

It is parsimonious to assume that the Fos labelling observed in Chapters 2-3 reflects neuronal excitation in the sector of Rt associated with the attended modality. At first glance, it may seem reasonable to explain these results based on Rt cells inhibiting local thalamic GABAergic interneurons, thereby facilitating the relay of behaviourally relevant 'attended' sensory information from the thalamus to the cortex. However, with

the exception of the dorsal lateral geniculate nucleus[4], there is a relative paucity of local interneurons within the nuclei of rodent thalamus, (Arcelli et al., 1997). Rt must therefore primarily hyperpolarize thalamic relay cells. Indeed numerous physiological investigations have confirmed the powerful inhibitory effect Rt exerts on thalamic relay neurons (Schlag and Waszak, 1971; Frigyesi and Schwartz 1972; Steriade and Wysinski 1972; Sumitomo et al., 1976; Yingling and Skinner 1976; Hale et al., 1982; French et al., 1985; see especially von Krosigk et al., 1993; Lo and Sherman, 1994; Kim and McCormick, 1998). Crick (1984) has suggested that Rt acts as an 'attentional spotlight', which amplifies or intensifies the relay of active thalamic inputs (usually sensory) to cortex. The functional basis of this 'amplification' is the arhythmic 'burst' firing expressed by hyperpolarized thalamic relay cells (Crick suggested this was mediated by Rt's inhibitory influence). Thalamic relay cells operate in two different response modes, 'burst' and 'tonic'. Whichever mode is in operation will determine how information is relayed to cortex. Whether a relay cell is in burst or tonic mode depends on the inactivation state of their voltage-gated, T-type Calcium channels. Hyperpolarization leads to de-inactivation of the of these T channels, whereupon a suitably large depolarising input or an excitatory post-synaptic potential (EPSP) produces a large low threshold spike which is followed by a high frequency 'burst' of conventional action potentials (up to ten at a time). Depolarization of relay cells leads to inactivation of the T channels, so a depolarising input or EPSP, will produce a sustained steady stream of action potentials – known as tonic firing (See Figure 1.11). Rhythmic burst firing is thought to disrupt the relay of the thalamocortical signal, hence its presence during stages of functional sensory detachment (i.e. slow wave sleep, deep anaesthetic or absence seizures). Tonic firing, with its steady stream of action

[4] In rodents, the dorsal lateral geniculate nucleus, the first-order visual thalamic relay, has approximately 25% local interneurons (for a comprehensive review see Arcelli et al., 1997).

potentials, is thought to provide a faithful relay of sensory information. However, arhythmic burst firing has been recorded from the visual thalamic relay neurons of both lightly anaesthetised and awake, behaving animals (McCarley et al., 1983; Guido et al., 1992; 1995; Guido and Weyand 1995; Albrecht et al., 1998; Mukherjee and Kaplan, 1995; Ramcharan et al., 2000). These bursts were found to correlate with enhanced detectability of near threshold visual stimuli. Bursting typically comprises the initial cellular response to visual stimuli. The presence of burst firing during wakefulness suggests this firing mode plays an important role in signal transmission. Specifically, it is thought that burst firing may serve as a "wake up call" alerting cortex to behaviourally relevant stimuli (Sherman, 1996). The tonic, possibly even burst discharge of Rt neurons during wakefulness (Steriade et al., 1985; Steriade et al., 1987; Steriade et al., 1991; Marks and Roffwarg, 1993; Hartings et al., 2000; for review see Steriade et al., 1993) might provide the priming effects (membrane hyperpolarization) necessary for the induction of arhythmic burst firing in the thalamic relay neurons of awake behaving animals. De-inactivation of the low threshold spike is both voltage and time dependent. Specifically, a sustained inhibitory post-synaptic potential lasting ≥100 msec is required to de-inactivate the low threshold spike. As noted earlier, the electrophysiological characteristics of $GABA_B$ mediated inhibitory post-synaptic potentials, particularly their lengthy temporal characteristics, suggest $GABA_B$ mediated inhibition plays an important role in the controlled de-inactivation of the low threshold spike. Unlike the $GABA_B$ mediated response, which produces lengthy, stereotyped changes in thalamic neuron excitability lasting ≥ 100 msec, the $GABA_A$ mediated response to a single input is typically over in 10-20msec (see Crunelli and Leresche, 1991). If Rt contributes to event-related burst firing during wakefulness, this effect must be mediated through activation of $GABA_B$ and not $GABA_A$ receptors. The idea that Rt

contributes to $GABA_B$ mediated induction of the low threshold conductance is supported by the elimination of $GABA_B$ inhibitory post-synaptic potentials in thalamocortical relay cells following Rt lesions (Steriade et al., 1985). While $GABA_B$ inhibition is thought to be principally involved in controlled de-inactivation of the low threshold spike (which consequently determines the firing mode of relay cells) it should be borne in mind that reticular mediated $GABA_A$ inhibition can also produce low threshold spiking in relay cells. However, such $GABA_A$ mediated activation of the low threshold spike is restricted to conditions where large assemblies of Rt cells burst in synchrony, such as during slow wave sleep (see Sherman et al., 2001).

Several important caveats should be borne in mind before allocating this function to Rt. Sherman's hypothesis has recently come under attack (See Weyand et al., 2001 and Steriade, 2001). However these criticisms may prove premature and unjustified. Weyand et al., (2001) recently demonstrated that during wakefulness *"less than 1% of action potentials in the visual relay of cats were associated with burst firing"* and *"increased vigilance was negatively associated with bursting probability"*. Based on these findings Weyand et al., (2001) have argued that *"although attractive (the wake up call to cortex hypothesis), our present results indicate that this idea is too limited...Further as appealing and intuitive as the idea that bursting is more potent than single spike activity in activating cortical circuits, empirical evidence in a thalamocortical system is lacking"*. For several reason these criticisms may prove unjustified. First of all, it seems highly unlikely that repeated presentations of stimuli over protracted periods of time would capture an animal's attention indefinitely. Such in-attentiveness may manifest itself as a low incidence of burst firing across protracted recording sessions. Indeed it is interesting that burst-firing typically occurs

during trials where novel, potentially interesting stimuli are presented for the first time (Weyand et al., 2001). Secondly, given that burst firing is thought to constitute a *wake up* call to cortex, one might expect such activity to precipitate (i.e. be negatively correlated) rather than be contingent with attentive, vigilant behaviours. Finally, and perhaps most importantly, Swadlow and Gusev (2001) have provided compelling new evidence that bursting thalamic impulses potently activate cortical circuits in awake alert rabbits. Specifically, initial impulses of each burst train were shown to have a greatly enhanced ability to elicit cortical action potentials. Swadlow and Gusev argue that the powerful activation of cortex by thalamocortical bursts is fully consistent with an involvement of these thalamic impulses in sensory/attentional processes (Crick, 1984; Sherman, 1996). The recent findings of Fanselow et al., (2001) have provided confirmatory evidence that burst activity provides a 'wake up call' to cortex. In the somatosensory thalamic relay of rodents, burst activity both precipitates and occurs throughout 'whisking' behaviours. Such whisking typically occurs during periods of alert immobility when animals are anticipating potential incoming, particularly novel or subthreshold, stimuli. This contrasts with the quiet immobile state, in which animals are awake, but less alert and not anticipating an impending stimulus. Crucially, burst firing is substantially reduced during periods of quiet immobility, supporting the idea that burst firing represents a event related 'wake-up-call' which alerts cortex to potentially interesting or dangerous stimuli (Sherman, 1996). It should be borne in mind that while there is no direct evidence supporting a role for Rt in the initiation of thalamic burst discharge, the fact that both Rt synapses and T-channels are disproportionately represented on the dendrites of thalamic relay neurons provides preliminary anatomical support of Rt's involvement (see Wang et al., 2001).

The neurophysiological findings of Warren and Jones (1994) provide more direct evidence of Rt's involvement in the gating of thalamocortical activity. Warren and Jones (1994) investigated the effects of glutamate activation of Rt neurons on the responses of single ventroposterior lateral (VPL) neurons to controlled mechanical stimulation of their receptive fields in anaesthetised cats. These investigators found that tonic activation of Rt neurons had little or no effect on the responses to peripheral stimulation of the VPL neurons, but exerted a strong suppressive influence on background activity, thus increasing the signal-to-noise ratio. The enhancement of the responses of VPL neurons relative to background activity implies that the tonic discharge of Rt neurons heightens the contrast between response and background noise. Consequently, it has been suggested that the role of Rt during wakefulness is to modulate the responsiveness of thalamic relay neurons to specific inputs, by reducing the level of background noise. Hartings and Simons, (2000) and Hartings et al., (2000) have since demonstrated that the tonic activity of Rt neurons also contributes to cortical responsiveness. Collectively these findings are fully consistent with a role for Rt in selective attentional mechanisms.

Intranuclear activation: the relationship between Rt neurons (cell-cell communication) and potentially sectors

Fos immunoreactivity within Rt might also represent intranuclear (cell-cell) inhibition within Rt, as opposed to internuclear inhibition between Rt neurons and dorsal thalamic neurons. Physiological investigations have demonstrated that neurons within visual Rt actively communicate with each other (Bal et al., 1995; Huguenard and Prince, 1992) presumably via extensive inhibitory collaterals (Cox et al., 1996). Via such intranuclear communication, a locus of excitation within Rt could effectively

eliminate activity in adjacent regions, allowing the relay of behaviourally relevant information at the expense of irrelevant information. It has been suggested that the extensive dendritic and axonal spread of Rt neurons across the rostrocaudal extent of this nucleus (Scheibel and Scheibel, 1966) might promote active communication between sectors (see Crick, 1984 and Montero, 1997). Such lateral inhibition within Rt could improve the relay of relevant information whilst attenuating the relay of competing stimuli, *between* modalities. However, this remains to be determined both anatomically and functionally. The anatomical findings of Scheibel and Scheibel (1966) have to be interpreted cautiously as they were performed on the brains of neonatal rats. Contemporary investigations have since demonstrated that the lengthy dendritic profiles and axon collaterals found in the Rt of neonatal rats (a potential neural substrate of inter sector communication) are subsequently lost or truncated during development (see especially Pinault et al., 1997). As far as current anatomical and electrophysiological evidence indicates, lateral inhibition within Rt can only improve the relay of relevant information, at the expense of competing stimuli, *within* the same modality.

Chapter 4: Rt and attentional/executive control

Rt can be divided into several distinct sectors, each related to a particular functional group of thalamocortical or corticothalamic pathways (Jones, 1975; for a review see Guillery et al., 1998 and Crabtree, 1999). It has been suggested that a dissociation of function may exist across the rostrocaudal extent of Rt. It has been proposed that caudal sectors of Rt mediate selective attention (through their connectivity with modality specific first- and higher-order thalamic nuclei) (Weese et al., 1999; Montero 1997, 1999, 2000) while rostral Rt mediates higher cognitive processes, such

as spatial learning and memory (M'Harzi et al., 1991; Collery et al., 1993; Vann et al., 2000; Wilton et al., 2001). Unlike the evidence obtained in studies of the sensory modality specific regions of Rt for a role in attention, investigations of rostral Rt have been less convincing. By virtue of its connectivity with the anterior thalamic nuclei (Gonzalez-Ruiz et al., 1997, Lozsádi, 1994, 1995) rostral Rt was originally implicated in spatial processing (see Chapter 1, page 19). M'Harzi et al., (1991) and Collery et al., (1993) showed that rats with rostral Rt lesions had a spatial working memory (but not reference memory) impairment. However, it is difficult to make a lesion of rostral Rt without causing damage to the adjacent anterior nucleus of the thalamus. Wilton et al., (2001) subsequently demonstrated that rats with lesions of rostral Rt were not impaired on a series of spatial learning and memory tests, provided the anterior thalamic nuclei were reasonably spared. There was a transient impairment during acquisition, which is suggestive not of an involvement in spatial learning per se but perhaps in attention to the spatial stimuli required for acquisition. Vann et al., (2000) showed that only 'spatially demanding' tasks, were associated with elevated levels of Fos immunoreactivity in rostral Rt. Thus, these authors concluded that their data suggest a role for rostral Rt in the attention to or abstraction of behaviourally relevant information rather than in spatial processing per se. This function may be the cognitive equivalent of the filtering mechanism that characterises the caudal sectors of Rt. The hypothesis that rostral Rt is involved in the abstraction of contextually significant events was examined in Chapter 4 using an attentional set-shifting task. The acquisition, maintenance and shifting of attentional set was examined in rats with large to extremely large regions of both dorsal and ventral thalamus. Despite anatomical connectivity (see Cornwall and Phillipson 1998, 1988abc; Cornwall et al., 1990), with regions known to mediate attentional set-shifting (Birrell and Brown,

2000) and the shifting of response rules in rodents (Hunt and Aggleton, 1998) Rt does not appear to contribute to these processes in rodents. These results are consistent with those of Wilton et al., (2001) who failed to demonstrate a role for Rt in spatial learning and memory, despite connectivity with regions mediating these processes. The present results, in conjunction with those of Wilton et al., (2001) do not support either a role for Rt in higher cognitive functions such as spatial learning, memory or attentional control or a dissociation of function across the rostrocaudal extent of this nucleus. However this does not preclude the possibility of rostral Rt mediating functions not required by these particular paradigms.

In Chapter 4, only extensive lesions that extended to include the midline/intralaminar and mediodorsal thalamic nuclei impaired the ability to shift attentional set, further support for subcortical mechanisms of attentional selectivity and control in rodents. Thus in rodents (as in humans – see Rodgers et al., 2000) the neuropsychological mechanisms underlying the ability to shift attentional set are not the property of a single brain area, such as the prefrontal cortex (Birrell and Brown, 2000), but represent the concerted effort of a network of functionally and anatomically related brain regions of which midline/intralaminar thalamus represents an important, if not crucial, component. Because of the confounded nature of these results, further investigations are required to determine precisely which thalamic nuclei contribute to the highly specific extradimensional set-shifting impairment seen in these rodents. Because lesions were extensive in rats displaying this deficit, the precise nuclei mediating this behavioural effect are not known. A potential candidate is the mediodorsal nucleus, which not only forms dense reciprocal interconnectivity with the prefrontal cortex (Cornwall and Phillipson, 1988), but also, when lesioned, produces

perseverative deficits similar to those reported in Chapter 4 (Hunt and Aggleton, 1998).

However, the central segment of the mediodorsal thalamic nucleus also receives a significant input from the olfactory cortex (Powell et al., 1965). Rats with large electrolytic lesions of the central (olfactory) component of the mediodorsal thalamic nucleus are severely impaired in their acquisition of an olfactory-based learning set (Lu and Slotnick, 1990; Slotnick and Kaneko, 1991). Consequently, it has been suggested that mediodorsal thalamus forms part of the thalamocortical circuitry mediating complex olfactory learning. Unfortunately, in the present study, rats with damage extending to midline/intralaminar and mediodorsal thalamus only performed medium to odour shifts. Therefore it is impossible to determine whether the observed behavioural deficit in these rodents represents a form of cognitive inflexibility which transcends perceptual dimensions or whether it merely represents an inability to acquire complex olfactory discriminations. However, such an interpretation seems unlikely. Rats with midline/intralaminar thalamic lesions in the present paradigm were specifically impaired on discriminations which required a redirection of attentional resources towards a newly rewarded perceptual dimension: an extradimensional shift. The same rats showed no impairment in olfactory reversal discriminations, which were equally challenging from an olfactory perspective. These results imply that the resultant deficit in extradimensional shifting following midline/intralaminar and mediodorsal thalamic lesions is probably not due entirely to a disruption of the olfactory sytem (of which the mediodorsal thalamic nucleus comprises a central component) but represents a more general disruption of cognitive flexibility, namely extradimensional set-shifting. Future investigations with more precise circumscribed

lesions of mediodorsal thalamus, using other sensory stimuli and/or task paradigms are essential to understanding the precise functions of this nucleus.

Chapter 5: the cholinergic system and selective attention

Chapters 1-4 were concerned primarily with the neural basis of selective attention. Collectively, these chapters provide compelling support for subcortical mechanisms of attentional selectivity and control in rodents. However, the neurochemical bases of many of these fundamental psychological processes remains to be determined. Considerable confusion surrounds the role of cholinergic neurotransmission in attentional orienting – a task known to be vulnerable to cell body lesions of visual Rt (Weese et al., 1999). Specifically, it remains to be determined whether acetylcholine contributes to specific 'cognitive' processes, such as attention, or whether it contributes to general 'non-cognitive' processes, such as alertness or arousal in this paradigm. Chapter 5 examined whether the cholinergic agonist (nicotine) and/or antagonist (scopolamine) would influence specifically the covert orientation of attention in the rat. One group of rats carried out a visual reaction time task, which allowed the measurement of attentional orienting separately from alertness following systemic administration of nicotine. Nicotine systematically and reliably shortened reaction times to both valid and invalid cues. Conversely, nicotine had variable and inconsistent effects on reaction times to bilateral and non-cued trials. A second group of rats carried out a visual reaction time task to measure covert orienting of attention following systemic administration of scopolamine. Scopolamine disproportionately lengthened reaction times to invalidly cued targets, increasing the validity effect. Taken together, these data indicate that cholinergic transmission represents an important neurochemical substrate

of visuospatial attention, disproportionately influencing spatial aspects of attentional orienting. These results are consistent with a substantial body of evidence to suggest a cholinergic involvement in attention in general (e.g., Callaway, 1984; Sahakian, 1988; Warburton and Rusted, 1993; Rusted, 1994; Muir et al., 1995; Turchi and Sarter, 1997; Mancuso et al., 1999) and covert orienting in particular (Parasuraman et al., 1992; Voytko et al., 1994; Witte et al., 1997; Murphy and Klein, 1998; Davidson et al., 1999).

The cholinergic system was chosen for investigation as the neurotransmitter acetylcholine, in conjunction with Rt, modulates thalamocortical transmission (see Ben-Ari et al., 1977 and Dingledine and Kelly, 1976) in anaesthetised animals. It has been suggested that by way of its inhibitory projections to dorsal thalamus, Rt might contribute to the burst firing recorded in the visual thalamic relay of awake behaving animals (see Chapters1-3). While burst activity heightens signal *detectability* (Guido et al., 1992) and perhaps even the *relay* of sensory information (Reinagel et al., 1999), this response mode is unsuitable for sensory *analysis*. Somehow the attentional system has to be switched from burst to tonic firing allowing the faithful relay and analysis of sensory input. It has been suggested that this transition from burst to tonic (and tonic to burst firing) in thalamic relay cells is mediated by the neurotransmitter acetylcholine (see Sherman, 1996). The cholinergic neurotransmitter system probably modulates the excitability of the thalamocortical attentional network so that once targets have been detected, the system can be appropriately modified (i.e. transferred to tonic firing) thus allowing the analysis of sensory information, and the execution of an appropriate behavioural response. A speculative account was presented of which cholinergic system might be involved in covert orienting. An obvious candidate is the basal forebrain cholinergic projection to frontal and/or parietal cortex, based on lesion work in rats

(Muir et al., 1995; Muir et al., 1996) and non-human primates (Voytko et al., 1994). However, a second candidate is the cholinergic innervation, from basal forebrain and the pedunculopontine tegmental nucleus, that innervates both Rt and thalamus (Hallanger et al., 1987). Because it is impossible to determine the neural basis of such systemic effects, the results of Chapter 5 were discussed in a purely speculative context. These results were discussed in terms of the conjoint roles played by Rt and the basal forebrain and midbrain cholinergic systems in sensory-attentional mechanisms. Although Chapter 5 seems tangential to the central theme of this thesis, the neural basis of attention, this experiment represents a preliminary attempt to determine the relative roles played by Rt and midbrain and basal forebrain cholinergic systems in attentional processes. Future investigations using central injections of cholinergic agonists/antagonists into discrete neural regions (basal forebrain versus midbrain cholinergic systems) would allow us to confirm the neural basis of sensory-attention modulation in rodents.

Conclusions

In an attempt to elucidate the functional significance of the Rt in rodents, this thesis has successfully applied a range of neuroscientific techniques in conjunction with sophisticated attentional paradigms. These include lesion studies, functional immunohistochemistry (Fos protein immunoreactivity) and a preliminary psychopharmacology experiment. Using a multi-methodological approach in conjunction with robust, reliable behavioural paradigms these studies have contributed significantly to our understanding of Rt function in awake behaving rodents. Despite forming part of the circuitry involved in sensory transmission, it is now clear that Rt function is more than the mere regulation of sensory information. Several lines of evidence have converged supporting Rt's role in *attention guided* modulation of

sensory information (Weese et al., 1999; Montero 1997, 1999, 2000). The exact mechanism by which this is achieved is still a subject for speculation, but recent insights into the dynamic nature of the thalamic relay of awake behaving animals have provided a clue (McCarley et al., 1983; Guido et al., 1992; 1995; Guido and Weyand, 1995; Albrecht et al., 1998; Mukherjee and Kaplan 1995; Ramcharan 2000; also see Warren and Jones 1994). It appears that in addition to heightening both thalamic and cortical responsiveness to sensory input, the tonic discharge of Rt neurons during wakefulness might support the episodic burst firing found in awake behaving animals (although this remains to be determined). The transition from burst to tonic firing in thalamic relay cells, which precipitates sensory analysis, is thought to be modulated by cholinergic afferents from the mesopontine region (Sherman, 1996). In Chapter 5 incontrovertible evidence was presented supporting a role for acetylcholine in attentional orienting – a phenomenon known to be vulnerable to cell body lesions of Rt (Weese et al., 1999). The midbrain cholinergic system has been shown to modulate the relay of sensory information in *anaesthetised* animals (see especially Ben-Ari et al., 1976 and Dingledine and Kelly, 1977). This system probably modulates the excitability of the thalamocortical attentional network during *wakefulness*, so that once targets have been oriented towards and detected, the system can be appropriately modified (i.e. transferred to tonic firing) thus allowing the analysis of sensory information, and the execution of an appropriate behavioural response.

While it has been suggested that rostral Rt might operate as a attentional gate or filter at more 'cognitive' levels (see especially Wilton et al., 2001) , there was no evidence to support a role for Rt in mechanisms of attentional selectivity or control. Extensive thalamic lesions that extended to include the mediodorsal thalamic nuclei were found

to selectively impair extradimensional set-shifting capacities in rodents. Although these results are somewhat tentative and confounded, the highly selective deficit observed, despite extensive thalamic damage warrants further discussion and investigation. In summary, this thesis has presented several compelling demonstrations of sub-cortical mechanisms of attentional selectivity and control. These results challenge the traditional notion that thalamus operates as a simple relay station, which mediates the transfer of sensory information to cortex. Rather, reciprocal interactions between dorsal and ventral divisions of the mammalian thalamus, probably in conjunction with cholinergic afferents from the brainstem and basal forebrain, determine the nature of the thalamic relay (i.e. whether it operates in burst or tonic mode) and ultimately the contents of our limited attentional resources. While many of these points are still speculative and indeed controversial, they nonetheless constitute the basis for future investigations into sub-cortical mechanisms of attentional selectivity and control.

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