Behavioural analysis of the role of caudal thalamic reticular nucleus in attention

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by

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i) I, Iraklis Petrof, hereby certify that this thesis, approximately 41,000 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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i. Contents

ii. Acknowledgments

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iii. Abstract

The thalamic reticular nucleus (TRN), and especially its caudal, sensory-related, half (cTRN), has been hypothesised for years to be at the very heart of thalamic sensory processing modulation, and attentional processes in particular. Very limited behavioural evidence is available, nonetheless, in support of such a functional attribution. In this thesis we carried out a series of investigations, combining immunocytochemical and lesion techniques with tests of behaviour, in order to examine the potential role of cTRN in attention and identify the attentional processes, if any, that it is more likely to contribute to.

In chapter *II*, we looked at the Fos activation levels within modality-specific sectors of cTRN following attentive behaviours to stimulation of different modalities. We observed a selective activation of the visual sector of cTRN in visually attentive animals but not in tactilely attentive, yet visually stimulated, animals, thus demonstrating an involvement of that area in processes of visual attention.

In chapter *III* we looked at the role of cTRN in cross-modal expressions of divided attention. We found that its removal, through neurotoxic lesioning, did not result in any behavioural costs with regard to the division of attention. Detriments in response accuracy, however, suggested that cTRN may be involved in stimulus processing enhancement operations, unrelated with the division of attention.

Finally, in chapters *IV* and *V*, we looked at the effects of lesions of the visual sector of cTRN (TRNvis) on the ability to orient attention covertly within visual space. We found that the removal of TRNvis did not affect visual covert orienting behaviour, both when this is triggered by exogenous and endogenous means. Overall our results suggest that even though cTRN appears to be involved in some aspects of attention, it does not represent a necessary structure for the generation and operation of certain other forms of attention.

Chapter I. General Introduction

1.1 *The Thalamus*

Seen for decades as a simple relay station of information between the periphery and cortex, the thalamus was not considered to contribute, in any significant way, to the elaboration of the information it relayed. Situated in a central anatomical location within the encephalon, thalamus is composed of several modality-specific nuclei that receive peripheral inputs (mainly, but not exclusively, from the sensory organs) before projecting them to cortex. Indeed, the term thalamus comes from the greek *θάλαμος:* (chamber) which implies little functional significance. Early functionally anatomical descriptions of the diencephalic structures focused heavily on the characterisation of the functionally distinct modality pathways they belonged to and the apparent linear relationship between their input and output, overlooking the possibility of a more profound functional attribution for these structures (e.g. von Monakow, 1895, Wallenberg, 1909, Gurdjian, 1927, as cited in Le Gros Clark, 1932; Brouwer and Zeemann, 1926; Overbosch, 1927, as cited in Le Gros Clark and Penman, 1934). It was soon realised however that a structure of the size and complexity of the thalamus is likely to play a more dynamic role than that of a simple relay (Walker, 1938, as cited in Sherman and Guillery, 2001; Sherman and Guillery, 1998; Guillery and Sherman, 2002; Sherman, 2001; 2005; 2006). Indeed the role of thalamus does not stop in the relay of peripheral information to cortex but it also extends in representing an indirect pathway for cortico-cortical communication (Guillery and Sherman, 2002). Furthermore, the fact that the vast majority of its input arrives not from the periphery but

from cortex and also from various subcortical areas (see below) suggests that the thalamus may act as a hub for various modulatory influences which could then actively and dynamically alter the relay of sensory information.

1.1.1.“Driver” and “modulator” thalamic input

The input to the thalamus can be segregated in two broad categories: *driver* and *modulator* input. Driver input determines the receptive fields of the post-synaptic thalamic cells and carries the main information to be relayed to cortex. Modulatory inputs, on the other hand, as their name indicates, carry information that modulates the input of drivers by affecting non-receptive field features of the relay cells, influencing properties such as baseline firing rates and/or firing mode (see *1.3.3.*). Driver and modulator inputs can be identified on the basis of a number of features that range from connectional (anatomic), to neurochemical, to electrophysiological (see Sherman and Guillery, 1998 for a detailed review). Briefly, driver inputs are exclusively excitatory (glutamatergic in particular) and they form few, but relatively large in size, contacts with post-synaptic cells, concentrated around their somata. Furthermore, driver inputs act mainly on, fast-activated, ionotropic glutamate receptors. As a consequence of the above, and despite being relatively few in number, driver inputs can exert strong post-synaptic effects on thalamic cells, thus ensuring the successful communication of their signal. Modulators are considerably more variable in nature compared to drivers, exerting both excitatory and inhibitory post-synaptic effects, through both ionotropic and metabotropic receptors of various neurotransmitters. Moreover, modulatory

inputs tend to form more, but smaller and more distant from the cell-soma, synapses with post-synaptic thalamic cells, compared to drivers. Modulator input can therefore exert post-synaptic effects that are strong enough to influence the profile of the relayed information, but too weak to allow the alteration of its identity. At the thalamic level, the nature of modulators' influence on sensory (driver) input is not, however, thought to be directly related to signal processing. Instead, as will be described in more detail later, it is thought to be concerned with the *preparation* of sensory signal for its subsequent cortical processing.

1.1.2 Thalamic Subdivisions

1.1.2.1. Dorsal Thalamus

When speaking of the thalamus, one usually refers to the *dorsal thalamus*, the largest and most prominent component of the diencephalon. The dorsal thalamus contains a number of distinct nuclei, each associated with a separate sensory, motor or limbic function. With regard to sensory nuclei, these can be segregated into two classes, namely *first order* and *higher order* nuclei. First order nuclei receive their driving input from the sensory organs and they subsequently transmit it to the functionally equivalent primary sensory cortical areas, from which, in turn, they receive modulatory feedback projections. These nuclei typically contain a map of the sensory modality they represent and form topographically precise connections with their associated cortical areas. Higher order nuclei, on the other hand, receive driving input from primary sensory cortices and driver and modulatory input from higher-order sensory cortices. In turn, higher order nuclei project to higher order

sensory cortical areas only (Guillery, 1995; Guillery and Sherman, 2002). The principal feature of sensory dorsal thalamic nuclei, therefore, is that they communicate information, either from periphery to cortex or between cortical areas (typically from primary cortices to higher order ones, but also between higher order cortices too).

1.1.2.2. Ventral Thalamus

The thalamus includes also another, less well-investigated area, known as the *ventral thalamus;* an area developmentally, anatomically, neurochemically and functionally different from the dorsal thalamus (see Sherman and Guillery, 2001). Unlike its dorsal counterpart, ventral thalamus does not project to cortex but instead targets the dorsal thalamus. The most prominent part of the ventral thalamus is the *thalamic reticular nucleus* (TRN), a relatively thin sheet of exclusively GABAergic cells wrapped around the dorsal thalamus (Houser, Vaughn, Barber and Roberts, 1980; DeBiasi, Frassoni and Spreafico, 1986, see Figure 1.1). TRN's main input arrives from dorsal thalamus and cortex in the form of collateral projections from the thalamocortical (corticopetal thalamic) and corticothalamic (thalamus-terminating corticofugal) fibres that traverse it (see *1.2.4*). Representing the major source of inhibition of the dorsal thalamus (Thomson, 1988), TRN appears to be at the very heart of the sensory signal modulatory processes taking place in the latter. TRN's modulatory influences can be very broad, recruiting the whole of dorsal thalamus, but can also be very specific, exerted over very small areas of a dorsal thalamic sensory surface. With regard to the former, TRN, by virtue of its intrinsic ability to generate rhythmic oscillatory activity, which can initially

propagate to, and then synchronise over, widespread thalamic and cortical circuits, it can generate various wave rhythms (e.g. spindle oscillations) associated with some stages of sleep and other forms of sensory disengagement (e.g. anaesthesia or epileptic seizures: see Steriade, McCormick and Sejnowski, 1993 for a review). In the awake state however, TRN can implement the topographical organisation of most of its sensory afferents and efferents, and the inhibitory nature of the latter, to generate a selective and sensotopically precise filtering of the flow of sensory information through the dorsal thalamus. Such a mechanism could form the basis for processes such as selective attention to take place (see *1.3.2*.).

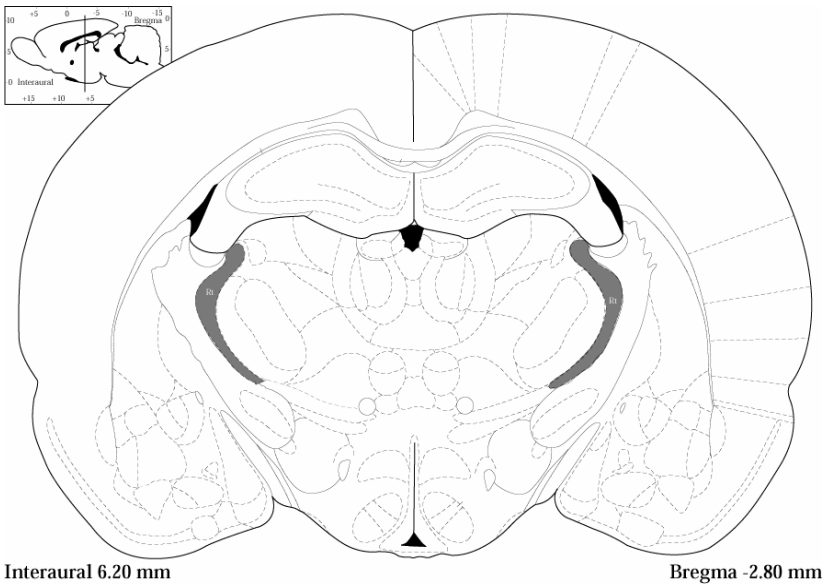

Figure 1.1. The position of TRN (seen in grey) within the rodent brain. Adapted from Paxinos and Watson, (1998).

1.1.3. Early speculation regarding TRN function

Suggestions for a potential involvement of the TRN in the modulation of thalamocortical transmission were formulated early on, upon the identification

of its efferent connections. Based on early anatomical observations that thalamic and cortical terminals converge on the TRN, that TRN cells' dendrites extended over widespread areas within the nucleus and finally that the axons of its cells terminate throughout the dorsal thalamus, Scheibel and Scheibel (1966), and later Jones (1975), suggested that TRN may act as an non-specific integrator of thalamocortical and corticothalamic activities of various modalities. It was reasoned that the modulatory output generated by the TRN on dorsal thalamus could reflect the combined effect of its variable, multi-modal, input, thus affecting overall levels of dorsal thalamic excitability. Later electrophysiological observations showed that dorsal thalamic sensory responses appear to decrease or abolish following the electrical activation of TRN (Yingling and Skinner, 1976). This suggested that TRN may be acting as a filter that could prevent sensory thalamocortical signals from being transmitted to cortex. However, electrical stimulation of certain TRN loci had an inhibitory effect only in adjacent (and not distant) dorsal thalamic areas, suggesting, therefore, that TRN's inhibitory output might not be unspecific and diffuse as previously proposed, but that it may instead be topographically organised to, at least, some degree. Indeed, subsequent, more advanced and thorough, anatomical investigations (see *1.2.4.* and *1.2.5*.) demonstrated that activity in TRN is far from non-specific and that it is instead modality-segregated and topographically organised. This allowed more specific speculations to be made regarding the nucleus' potential functions. For example, Yingling and Skinner's hypothesis of a thalamocortical filter was taken further by Crick (1984), who speculated that TRN might be the neural basis of an attentional "searchlight". Given its precise excitatory

thalamocortical and corticothalamic input and its equally precise inhibitory output on dorsal thalamus, the TRN, possibly under cortical instructions, could selectively suppress sensory signals that are weak or behaviourally irrelevant and allow salient or behaviourally significant signals to pass to cortex unaltered. Taking vision as an example, this could create a locus within the visual field where sensory signals are relayed unaltered to cortex and thus are attended, surrounded by a greater area of suppressed signals which would be not as effective in driving cortical cells and thus remain unattended. Despite the apparent plausibility of such a proposal, however, no available empirical evidence was available at the time to support it. Indeed, for more than a decade following Crick's "TRN-mediated attentional searchlight" idea, no known behavioural investigations of its plausibility were carried out. This is possibly due to the difficulties associated with TRN work; such as the difficulty in inflicting selective lesions and the complexity of in vivo electrophysiological recordings from its cells (see section *1.4*). As a consequence, most of TRN's investigations in the 80's and 90's focused only on the further delineation of the nucleus' precise anatomy, afferent and efferent connections, and the intrinsic electrophysiological properties of its cells, mainly *in vitro* and in *in vivo* anaesthesia. The additional information about TRN's characteristics offered by these investigations allowed more specific hypotheses to be made about the nucleus' functional capabilities, especially with regard to a role in the regulation of sensory activity and in particular in the generation of attentional processes. In turn, this led to the first behavioural investigations of TRN, with regard to these processes, in the late 90's (see *1.4.1; 1.4.2 and 1.4.3*). The

anatomical evidence presented in the following sections comes from the rodent, unless otherwise specified.

1.2. General TRN anatomy

1.2.1. Position and shape

As mentioned earlier, TRN is a narrow sheet of GABAergic neurons that curves around the rostral, lateral and, to some extent, the dorsal areas of the thalamus. It is separated from the dorsal thalamus by the external medullary lamina, and from cortex by the internal capsule (Jones, 1975). The rostral pole of the TRN is the thickest part of the nucleus, and also the least curved. The central and caudal parts of TRN, on the other hand, extend both more ventrally and more dorsally than the rostral pole, thus enveloping a greater area of the dorsal thalamus (Ohara and Lieberman, 1985, see Figure 1.2). With respect to the rostro-caudal axis, horizontal sections show that the rostral pole of the nucleus is more medially placed compared to the rest of the nucleus, in a position that allows it to abut the anterior thalamic nuclei. Caudal TRN extends more laterally adjoining at its posterior-most end the lateral geniculate nucleus (LGN) (Ohara and Lieberman, 1985). As a result of its shell-like shape and varying orientation, no section can offer a complete view of the TRN (Guillery, Feig and Lozsadi, 1998).

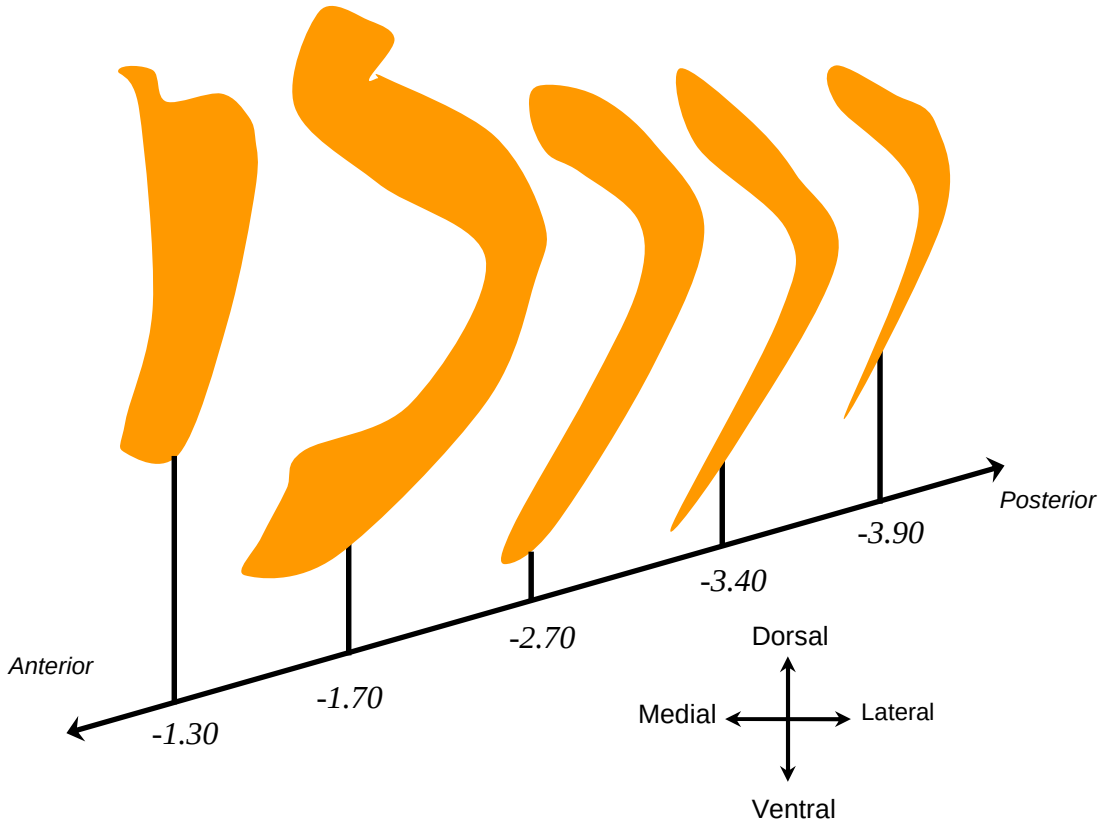

Figure 1.2: Sequential (rostral to caudal) coronal sections through the left TRN of the rat (developed according to Pinault, 2004). The numbers represent distance (in mm) from bregma.

1.2.2. Morphology of TRN neurons

There is considerable variation with regard to cell sizes and shapes within TRN. Cell sizes vary between 180 and 860 microns in diameter and can have round, ovoid or particularly elongated somata (Lübke, 1993; Ohara and Havton, 1996). Typically, dendrites emerge from the two poles of the cell soma, branching and extending for fairly long distances (up to 450 microns) within the nucleus (Ohara and Liebereman, 1985, Mulle, Madariaga, and Deschênes, 1986; Lübke, 1993). Neurons in caudal TRN have dendrites with a dorsoventrally elongated arbour, whereas in rostral TRN it is more common for neurons to exhibit multipolar dendritic arbours (Lübke, 1993). Despite the

variation in size, shape, and extent of dendritic arbour, there are no grounds for a classification of neurons according to anatomical criteria.

1.2.3. Intra-TRN (cell to cell) communication

Axons of TRN cells typically emerge from the cell body, even though there are also reports for axons emerging from proximal dendrites (Pinault, 2004). Before entering the dorsal thalamus axons often form collaterals that innervate somata and dendrites of neighbouring TRN cells (Liu, Warren and Jones, 1995). The most common form of intra-TRN communication however is by means of dendro-dendritic synapses (Scheibel and Scheibel, 1972, Deschênes, Madariaga-Domich and Steriade, 1985; Pinault, Smith and Deschênes, 1997, Williamson, Ohara, Ralstron, Milroy and Ralston, 1994; Deleuze and Huguenard, 2006). The existence of other types of intra-TRN communication, such as axo-axonic, is possible but has not yet been clearly demonstrated (see Pinault, 2004).

In addition to the inhibitory chemical interactions, TRN cells also communicate via electrical synapses (Landisman, Long, Beierlein, Deans, Paul and Connors, 2002; Long, Landisman and Connors, 2004; Deleuze and Huguenard, 2006). These electrical gap junctions are evident between proximal cells and enable the direct communication of excitatory effects between cells, without the involvement of neurotransmitters. Whereas chemical synapses are more prominent between adjacent, in the anteroposterior plane, TRN cells, electrical synapses are primarily formed between TRN cells in the dorsovental plane (Deleuze and Huguenard, 2006).

Electrical intra-reticular communication is thought to play an important role in the generation of firing synchronicity between TRN cells (Long *et al*., 2004), given that excitation in one TRN cell can, under the right circumstances, result in an almost instantaneous excitation of its electrically coupled TRN cells. It is possible therefore that electrical synapses play an important role in the generation of synchronous and rhythmic firing patterns within TRN (and by extension in dorsal thalamus and cortex) that underlie mechanisms of sleep and epileptogenesis (Deleuze and Huguenard, 2006)

1.2.4. TRN connections with dorsal thalamus and cortex

As a result of its anatomical position, all axons travelling from the dorsal thalamus to cortex and vice versa have to go through the TRN. Thalamocortical fibres give off collateral branches within TRN that terminate on local cells (Jones, 1975). The same is the case for corticothalamic fibres from layer VI, but it does not apply to fibres from other layers (e.g. layer V); these do not branch in TRN, but project directly and exclusively to dorsal thalamus (Jones, 1975; Bourassa and Deschênes, 1995). Both thalamocortical and corticothalamic collaterals supply the TRN with glutamatergic innervation (for a detailed description of the post-synaptic effects of glutamate on TRN cells see *1.2.9.1.*). In return, TRN provides dorsal thalamus with GABAergic projections (Jones, 1975). The inhibitory post-synaptic effects of TRN's projections are mediated by both $GABA_A$ (mainly) and $GABA_B$ receptors (Kim and McCormick, 1998, also see Lee, Friedberg and Ebner, 1994b).

1.2.4.1. Thalamocortical and corticothalamic projections to TRN

Corticothalamic axons traversing and innervating the TRN outnumber thalamocortical axons by as much as a magnitude of ten (cat: Guillery, 1967; rodent: Jones, 1985). Both thalamocortical and corticothalamic projections onto TRN tend to be topographically organised, especially those arising from sensory areas. That is, any anteroposterior, dorsoventral or mediolateral shift in a dorsal thalamic or cortical area is usually accompanied by a corresponding shift of its projection to the TRN (e.g. Crabtree and Killackey, 1989, see also Pinault, 2004).

Due to the considerably larger number of thalamocortical axons traversing the TRN compared to the cells present in the nucleus, it would be reasonable to expect a degree of convergence of thalamocortical projections onto TRN cells. This would also explain why the latter tend to have larger receptive fields than dorsal thalamic cells (see Sanderson, 1971; Pollin and Rokyta, 1982; Uhlrich, Cucchiaro, Hamphrey and Sherman, 1991, for evidence in felines and primates). Indeed, evidence suggests that thalamocortical projections converge on TRN cells (Harris, 1987). However, there is also evidence suggesting that thalamocortical axons collateralise (further) within the TRN, innervating more than one cell, thus contributing to a smaller-scale divergence pattern within a larger convergence one (Harris, 1987). A convergence pattern also appears to exist for the corticothalamic projections upon TRN. The degree of convergence for these projections is expected to be even greater as the number of corticothalamic terminals on TRN outnumber considerably those from thalamocortical cells (Bourassa and Deschênes,

1995). Surprisingly however, and despite the enormous potential functional significance of corticothalamic projections in TRN function (see Montero 2000, and also *1.3.5.1*), very little further anatomical evidence is available regarding their termination patterns in the nucleus.

1.2.4.2. Patterns of TRN projections onto thalamocortical relays

TRN's projections on dorsal thalamus were initially speculated to target local inhibitory interneurons, thereby promoting the disinhibition of thalamocortical relays (Steriade, Domich and Oakson, 1986). Subsequent anatomical evidence, however, revealed that TRN projections rarely target dorsal thalamic interneurons and that the frequency by which they do so varies between thalamic nuclei and species. In the somatosensory and visual thalamic nuclei of felines, for example, TRN terminals have been consistently reported to make extremely infrequent contacts with local interneurons (see Liu, Warren and Jones, 1995; Wang, Bickford, Van Horn, Erişir, Godwin and Sherman, 2001). This is also believed to be the case in the rodent, where with the exception of the LGN (Sumitomo, Nakamura and Iwama, 1976) the rest of the dorsal thalamus is virtually devoid of local interneurons (Jones, 1985; Arcelli, Frassoni, Regondi, De Biasi and Spreafico, 1997). In primates however it is somehow more common for TRN projections to target interneurons, especially in the non-sensory thalamic areas such as the anterior and mediodorsal nuclei (Kultas-Ilinsky, Yi and Ilinsky, 1995; Tai, Yi, Ilinsky and Kultas-Ilinsky, 1995). In primates too, however, the vast majority of TRN axons terminate on thalamocortical relay cells.

TRN's reciprocal connections with dorsal thalamus are organised in such a way so that a particular TRN area projects to the same dorsal thalamic nucleus from which it receives input (Jones, 1985, Ohara and Lieberman, 1985, Pinault, 2004). Furthermore, projections between TRN and dorsal thalamus tend to be topographically organised, even though the degree of topography varies considerably depending on which dorsal thalamic nuclei are involved (see *1.2.5.*). In the rodent brain, adjacent TRN cells usually send axons to the dorsal thalamus in a spatially segregated, yet parallel, way. Minor overlap in their dorsal thalamic terminals has been observed in some cases, usually when these TRN cells have overlapping dendritic arbours (Pinault and Deschênes, 1998). Projections with relatively loose topography are also present. For example, two overlapping TRN cells may send their axons to two different, but functionally related, dorsal thalamic nuclei. This however seems to be mainly a feature of TRN areas projecting to non-sensory or higher-order sensory thalamic nuclei. Even more complicated patterns of TRN-dorsal thalamic projections have been identified (see Pinault and Deschênes, 1998, Pinault, 2004, for reviews) but they are thought to be rare. Regardless of the pattern of synaptic connectivity between TRN and dorsal thalamus, however, TRN axons invariably terminate on distal dendrites of thalamocortical cells, where most corticothalamic axons also tend to terminate on these cells (Jones, 1975; Wang, Bickford, Van Horn, Erişir, Godwin and Sherman, 2001), thus suggesting a modulatory, rather than a driving, effect upon them (see *1.1.1.*).

Despite the connectional reciprocity between TRN and dorsal thalamus at the nucleus level, little such reciprocity seems to apply at the cellular level. FitzGibbon (1994) and later Pianault and Deschênes (1998) reported that, in the majority of cases, a thalamocortical cell is not innervated by the same TRN cell that it innervates. This suggested that there is only a limited cell-to-cell reciprocity between reticulo-thalamic and thalamo-reticular projections and that more *open-loop* than *closed-loop* projection circuits exist between dorsal thalamus and the TRN. Even though some more recent evidence suggests that the frequency of closed-loop thalamo-reticulal circuits has been underestimated (e.g. Desilets-Roy, Varga, Lavallee and Deschênes, 2002) it is generally accepted that cellular reciprocity in thalamo-reticular circuits is limited. Functionally, the existence of more open-loop than close-loop thalamo-reticular circuits suggests an increased likelihood for a TRN-mediated lateral inhibition mechanism within dorsal thalamus. Such a mechanism could be associated with the elaboration of receptive field properties of dorsal thalamic cells (Lee, Friedberg and Ebner, 1994a) and/or with processes associated with selective attention (McAlonan, Cavanaugh and Wurtz, 2006). The functional significance of a potential TRN-mediated dorsal thalamic lateral inhibition mechanism will be discussed in more detail later (see *1.3.2.1.*).

1.2.5. TRN sectors

On the basis of the dedicated connections between certain areas of the TRN, particular dorsal thalamic nuclei, and their associated cortical areas, several distinct sectors within the TRN can been identified (see Figure 1.3). Each one of these sectors is concerned with a particular function, equivalent to the

function of its input and output areas (Ohara and Lieberman, 1985). More specifically, caudal parts of TRN form connections with sensory thalamic nuclei and their functionally equivalent sensory cortical areas (Ohara and Lieberman, 1985; Crabtree and Killackey, 1989). As a consequence, four main sectors can be identified in caudal TRN, namely a visual, an auditory, a somatosensory and a gustatory one. No identifiable sector is devoted to olfaction (see Kay and Sherman, 2007 about the peculiarity of the olfactory pathways). On the other hand, rostral TRN areas contain sectors that are associated with thalamic nuclei and cortical areas that are implicated in motor, limbic and executive functions (Gonzalez and Sharp, 1985; Kolmac and Mitrofanis, 1997). Whereas the overlap between sectors is fairly modest in the caudal, sensory-related, TRN, it can be almost complete for some sectors of rostral TRN (see below). TRN inputs arriving from other (non-dorsal thalamic) subcortical areas (e.g. basal forebrain, brainstem) are scattered throughout TRN without forming identifiable sectors.

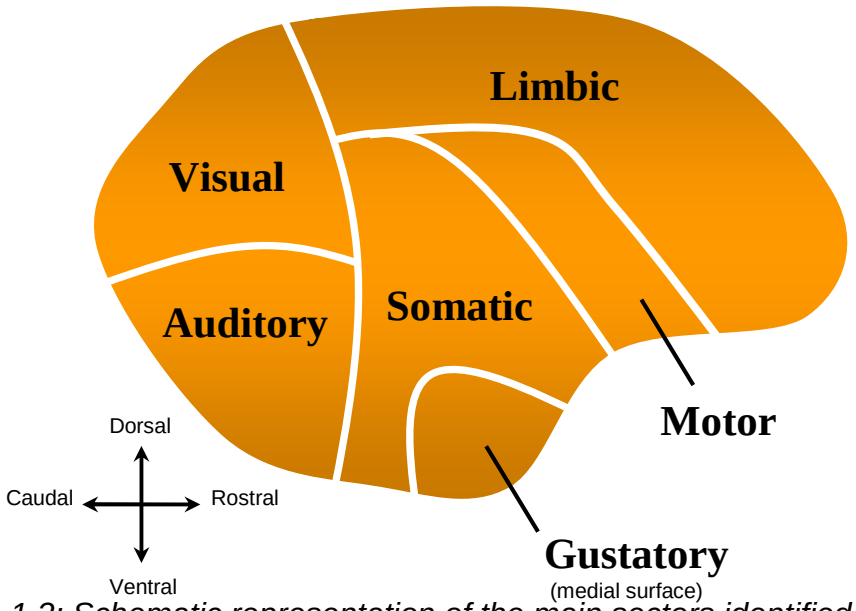

Figure 1.3: Schematic representation of the main sectors identified within TRN (sagittal view)

1.2.6 Caudal TRN: The sensory sectors

1.2.6.1 Visual TRN (TRNvis/PGN)

In every species that it has been studied so far, the visual sector of TRN (TRN*vis*) occupies the dorso-caudal part of the nucleus (*rodent*: Hale, Sefton, Baur and Cottee, 1982; Coleman and Mitrofanis, 1996; *bushbaby*: Conley and Diamond, 1990; Harting, Van Lieshout and Feig, 1991; *lagomorph*: Montero, Guillery and Woolsey, 1977). In felines and other carnivores, TRN*vis* is detached from the rest of the nucleus and it is known as the perigeniculate nucleus (PGN) (Uhlrich, Cuchiaro, Humphrey and Sherman, 1991). Even though the carnivore PGN is separate from the rest of the TRN, it is considered to be anatomically and functionally equivalent to TRN*vis* of rodents, lagomorphs and primates (Sherman and Guillery, 1996). TRN*vis*/PGN receives input from both primary and higher order visual cortices and forms reciprocal connections with the dorsal portion of the lateral geniculate nucleus (dLGN) and also with the lateral posterior nuclei (LP) in rodents (Coleman and Mitrofanis, 1996) and the pulvinar in carnivores and primates (Jones, 1975; Conley and Diamond, 1990). Two sub-sectors can be identified within TRN*vis* on the basis of the connections they form with either first- or higher-order visual areas. One sub-sector is associated with the *first-order* visual pathway (the pathway between dLGN and the primary visual cortex-V1) and the other with the *higher-order* visual pathways (the pathways between the Pulvinar/LP nuclei and their associated visual cortical areas) (Crabtree and Kilackey, 1989; Conley and Diamond, 1990; Lozsadi, Gonzalez-Soriano and Guillery, 1996).

1.2.6.1.1. First Order TRNvis

The first order visual sub-sector (foTRN*vis*) occupies the lateral 2/3^{rds} of the TRN*vis* and, similarly to its two main input sources (dLGN and V1), exhibits signs of retinotopic organization. That is, adjacent neurons or assemblies of neurons in foTRN*vis* represent adjacent areas of the visual field. Evidence for this arrives from tracer labelling studies in rats (Ohara and Lieberman 1985) and lagomorphs (Montero *et al.*, 1977; Crabtree and Killackey, 1989) which have shown that shifts in the injection sites within V1 or dLGN result in equivalent shifts in terminal labelling within foTRN*vis*. The way the retina is represented in foTRN*vis* differs from the retinotopic organization seen in either V1 or dLGN. In V1, areas of the visual field are represented by foci on the cortical surface, whereas in the dLGN by columns that run along the thickness of the nucleus (Kaas, Guillery and Allman, 1972). In foTRN*vis*, on the other hand, small areas of the visual field are represented by “slabs” of packed cells that run parallel to the medial and lateral borders of the nucleus (Crabtree and Killackey, 1989). A slab organization is evident not only for the projections foTRN*vis* sends to the dLGN, but also for the ones it receives from dLGN and V1 (Crabtree and Killackey, 1989; Coleman and Mitrofanis, 1996). The functional significance of this unusual form of sensotopic organization within foTRNvis remains unknown.

1.2.6.1.2. Higher Order TRNvis

The higher order sub-sector within the TRN*vis* (hoTRN*vis*) occupies the medial 1/3rd of the sector. This region forms reciprocal connections with the pulvinar in primates and felines, and with the LP nuclei in rodents (Crabtree

and Killackey, 1989; Conley and Diamond, 1990). In addition, hoTRN*vis* receives input from the higher-order visual cortical areas that the pulvinar and LP nuclei project (Lozsadi *et al.,*1996). Contrary to dLGN and V1, these visual areas do not possess clear retinotopic organization. As a consequence, the projections they send and/or receive from hoTRN*vis* also lack retinotopy. Furthermore, no slab organisation of visual input/output is evident in hoTRN*vis*. Tracer injections in its associated visual dorsal thalamic nuclei and cortical areas result in terminal labelling of large and often highly overlapping areas within hoTRN*vis* (Crabtree and Killackey, 1989; Lozsadi *et al.*,1996).

1.2.6.2. Auditory TRN (TRNaud)

TRN*aud* is located immediately ventrally to TRN*vis*, at the caudo-ventral end of TRN. This area has been found to anterogradely and/or retrogrately label after horseradish peroxidase injections in the auditory cortices and/or the medial geniculate nucleus (MGN) of rats (Ohara and Lieberman, 1985), cats (Rouiller and Colomb, 1985; Crabtree 1998) and primates (Conley, Kupersmith and Diamond, 1991). Additional evidence for the connectivity of this area with auditory centres was offered by Shosaku and Sumitomo (1983), who demonstrated that auditory stimulation, or the electrical stimulation of primary auditory cortex (A1), results in a fast and strong activation of cells in the ventro-caudal TRN.

1.2.6.2.1 TRNaud sub-sectors

Similarly to TRN*vis*, two sub-sectors can be found within the TRN*aud* of bushbabies (Conley *et al.*, 1991) and cats (Crabtree, 1992). The first sector occupies the central third of the sector's thickness and receives input from the ventral segment of the medial geniculate nucleus (vMGN), the first order auditory dorsal thalamic nucleus, and also from A1. In return, this sub-sector projects back to vMGN only. The second sub-sector occupies a *U*-shaped area within TRN*aud*, containing the medial and lateral thirds of the sector's thickness as well as its ventral-most end (see Figure 1.4). This sub-sector is associated with both the first-order and high-order auditory thalamic nuclei (vMGN and the magnocellular MGN (mcMGN) respectively) and their associated cortical areas (A1 and higher-order auditory cortices, respectively) (Conley *et al.,* 1991). No information is available for the existence of sub-sectors in rodent TRN*aud*.

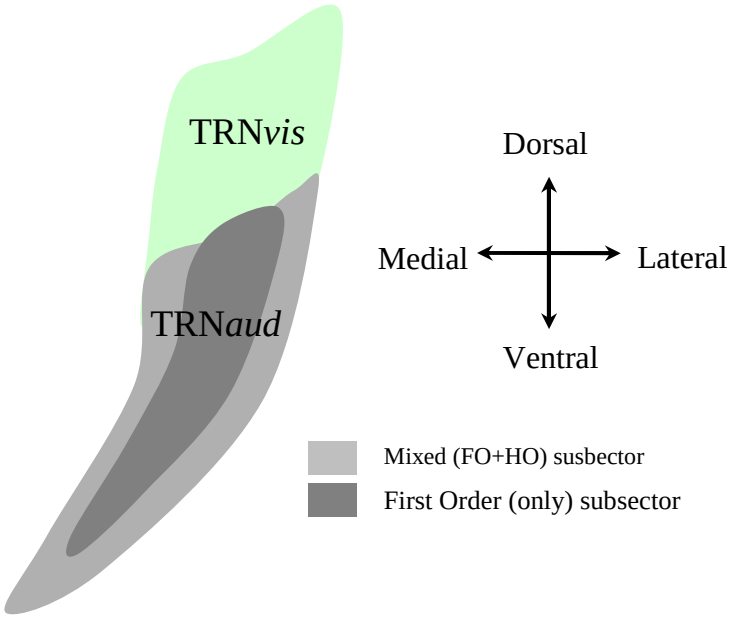

Figure 1.4. Schematic representation of a coronal section through the auditory TRN, illustrating the two subdivisions of the sector

Within TRN*aud*, a topographic/cochleotopic organization of projections exists only for those associated with the vMGN and A1. No such organisation exists for TRN*aud*'s connections with mcMGN and the higher-order auditory cortices (Conley *et al*., 1991; Crabtree, 1998). Similarly, a slab organisation of input/output is evident only for the first order TRN*aud* connections. More specifically, while small tracer injections in A1 and vMGN result in labelled slabs within TRN*aud*, equivalent injections in higher-order auditory cortices and mcMGN result in large labelled areas within the "U-shaped" TRN*aud* sub-sector (Crabtree, 1998).

1.2.6.3. Somatosensory TRN (TRNsom)

Sugitani (1979) was the first to identify the somatosensory area of the TRN (TRN*som*). He electrically stimulated small foci of the somatosensory cortex of anaesthetised rats and simultaneously recorded responses from various sites within TRN. Sugitani found that electric stimulation of the somatosensory cortex elicited discharge responses only in neurons of the centroventral TRN. Not surprisingly, this area of the TRN surrounds the ventrobasal nuclei (VB), the first order somatosensory thalamic nuclei. Analogous findings were later reported in cats and monkeys (Pollin and Rokyta, 1982). Ohara and Lieberman (1985) offered a more precise definition of TRN*som*'s anatomical position using axonal transport techniques. Injections of tracers in the primary somatosensory cortex (S1) and VB of rats gave rise to labelled cells in areas of TRN immediately rostral of TRN*vis* and TRN*aud*, excluding the most dorsal 1/3rd. Within TRNsom, distinct areas represent different body parts. The head, lips and vibrissae are over-represented in this map, occupying most of the

ventral and central extent of the sector, whereas the trunk and limbs occupy relatively small areas of the dorsal TRN*som* (Shosaku, Kayama and Sumitomo, 1984).

TRN*som* also forms reciprocal connections with higher-order somatosensory thalamic nuclei, namely the medial posterior thalamic nuclear group (mPo), and with the secondary somatosensory cortex (S2), in rodents (Pinault, Bourassa and Deschênes, 1995), felines (Yen, Conley, Hendry and Jones, 1985; Crabtree, 1992, 1996) and lagomorphs (Crabtree, 1992). In rodents, the areas of the TRN*som* associated with mPo and S2 are distinct from those associated with VB and S1. More specifically, similarly to the organizational pattern seen in TRN*vis*, the lateral 2/3^{rds} of TRN*som* connects with VB and S1 (first-order pathway) whereas the medial 1/3rd does so with mPo and S2 (higher-order pathway) (Shosaku, Kayama and Sumitomo, 1984). In felines and lagomorphs, however, the areas of TRN*som* associated with the first-order and higher-order pathways overlap completely and occupy the whole extent of the sector (Crabtree, 1992; 1996). Regardless of the presence of a spatial segregation between first-order and higher-order projections within TRN*som*, a “slab” somatotopic organization is evident only for the former and not for the latter (Shosaku *et al.,* 1984; Crabtree, 1992; 1996).

1.2.6.4 Gustatory TRN (TRN*gust*)

The gustatory area of rodent TRN (TRN*gust*) was identified by Hayama, Hashimoto and Ogawa (1994) following injections of antero- and retrograde tracers in the parvicellular part of the thalamic posteromedial ventral nucleus

(VPMpc) (the gustatory relay nucleus of the dorsal thalamus) and the gustatory cortex. TRNgust was identified as the ventromedial portion of the TRN, in an area immediately rostral to the VPMpc. Interestingly, injections of anterograde and retrograde tracers in that area of TRN resulted in a large number of labelled axon terminals but only a limited number of labelled cell bodies in VPMpc. This suggested that the projections of TRN*gust* to the gustatory dorsal thalamus are more numerous than the projections from the gustatory thalamus to the TRN*gust*, a somehow uncharacteristic example of thalamo-reticular synaptic relationship.

1.2.7 Rostral TRN

1.2.7.1.Motor TRN (TRNmot)

Using metabolic mapping techniques in rats, Gonzalez and Sharp (1985) observed that some areas of the rostral tip of TRN were activated during trained forelimb movements. They concluded therefore that this area represented the motor sector of TRN (TRN*mot*). A more precise localisation of TRN*mot* was offered by Cicirata, Angaut, Serapide and Panto (1990), who investigated the connectivity between TRN, motor cortical areas, and the motor thalamic nuclei (ventral lateral (VL) and ventral medial (VM) nuclei) using axonal transport techniques. They reported that there are two distinct projection systems, one linking directly the motor cortical areas with VL/VM and one indirect projection system involving the TRN. The area of TRN associated with the latter projection was reported to lie immediately rostrally of the TRN*som*. Furthermore, Cicirata *et al.* reported that tracer injections in motor cortical areas corresponding to the forelimb, hind limb and head

resulted in terminal labelling of adjacent but distinct areas in TRN*mot*. Head- and vibrissae-representing areas were found in the ventral TRN*mot*, whereas forelimbs and hindlimbs were represented by loci at dorsal TRN*mot*. Therefore, the rostro-caudal body surface appears to be represented by a ventro-dorsal organization in the TRN*mot*, similar to the body representation seen in TRN*som* (Shosaku, Kayama and Sumitomo, 1984; see *1.2.6.3*). Finally, more recent evidence suggests that TRNmot also receives direct projections from scattered areas of the cerebellum (Cavdar, Filiz, Yananli, Sehirli, Tulay, Saka, and Gurdal, 2002).

1.2.7.2.“Limbic”, “executive” and “non-specific” TRN

Lozsardi (1994) was the first to make reference to the existence of a “limbic” sector within rostral TRN. As its name suggests, this sector has connections with thalamic nuclei and cortical areas that are associated with limbic, but also executive, functions and it is confined to the dorsal and dorsolateral areas of the rostral pole of TRN. The most dorso-rostral area of the sector appears to be reciprocally connected to the anterior thalamic nuclear group (ATN) and also with the cingulate and retrosplenian cortices (Lozsadi, 1994; Gonzalo-Ruiz and Lieberman, 1995). Immediately caudally of that area, and dorsally to the TRN*som*, is the TRN area associated with the laterodorsal (LD) thalamic nucleus (Ohara and Lieberman, 1985). The topography of signal within these TRN areas is considerably loose, as different loci within ANT and LD project to considerably overlapping areas within dorso-rostral TRN (Gonzalo-Ruiz and Lieberman, 1995). Not surprisingly, no slab organisation of inputs/outputs is evident within the limbic sector of TRN. An exception is the connections with

the mediodorsal (MD) thalamic nucleus and the prefrontal cortex, which raise from, and terminate at, an area of TRN ventrally to the one associated with ATN (Cornwall and Phillipson, 1988; Cornwall *et al*., 1990). These connections posses a basic degree of topography, which however is not as acute or precise as the topographies found in the sensory areas of caudal TRN.

Apart from motor and limbic connections, the rostral TRN is also interconnected with a number of thalamic nuclei that have no single identifiable function. These thalamic nuclei send projections to widespread areas of the cortex and, for that reason, they are referred as “non-specific” (Ohara and Lieberman, 1985). The two main “non-specific” nuclear groups are the intralaminar and midline nuclei. Kolmac and Mitrofanis (1997) reported that these nuclear groups project to the rostral pole of the TRN, in a region that overlapped considerably with the areas that had been previously found to receive projections from the MD nucleus (Cornwall and Phillipson, 1988; Cornwall *et al*., 1990). The intralaminar projections tended to lie at more lateral areas within this region, whereas midline projections were primarily found to terminate at medial areas. Projections from individual intralaminar and midline nuclei were intermixed within these two separate layers illustrating no signs of topography, despite the fact that these same TRN areas illustrated topographical organization for their projections to and from the MD nucleus (Cornwall and Phillipson, 1988).

1.2.8. Other subcortical/non-glutamatergic, innervations

In addition to thalamic and cortical input, TRN also receives various inputs from numerous subcortical areas, including the basal forebrain, midbrain and brainstem. Unlike thalamic and cortical projections to TRN, which are exclusively glutamatergic, these projections vary in their neurotransmission (see below).

1.2.8.1. Basal forebrain innervation of TRN

Injections of retrograde tracers in TRN result in widespread labelling of various areas of the basal forebrain, including the nucleus basalis of Maynert, the vertical and horizontal limbs of the diagonal band of Broca, the substantia innominata and ventral pallidum (Hallanger, Levey Lee, Rye and Wainer, 1987; Levey, Hallanger and Wainer, 1987; Asanuma, 1989; Jourdain, Semba and Fibiger, 1989; Asanuma and Porter, 1990, Cornwall, Cooper and Phillipson, 1990). The majority of basal forebrain projections on TRN are cholinergic, although there are also GABAergic projections (Asanuma *et al.*, 1990). The bulk of GABAergic fibres stem from the caudal basal nucleus and appear to terminate principally in the caudal half of TRN. Cholinergic projections, on the other hand, tend to target mainly, but not exclusively, the rostral pole of TRN. The majority of these terminals rise from axon collaterals of basal forebrain corticopetal projections (Jourdain, Semba and Fibiger, 1989, also see Semba, 2000). This feature makes these collateral projections particularly interesting, as they could represent a functional branch of the cortical cholinergic input system that is involved in processes underlying

various forms of attention (see Sarter, Hasselmo, Bruno and Givens, 2005 for a review).

1.2.8.2. Midbrain and Brainstem

Besides basal forebrain, another major subcortical source of TRN innervation rises from various centres of brainstem and midbrain. More specifically, axon terminals within TRN have been identified to arise from the mesopontine tegmentum (Jourdain *et al.*, 1987, Spreafico, Amadeo, Angoscini, Panzica and Battaglia, 1993) and in particular the laterodorsal tegmental (LDTg) and pedunculopontine tegmental (PPTg) nuclei (Cornwall, Cooper, Phillipson, 1990). Choline acetyltransferase immunohistochemistry suggested that these projections are predominantly cholinergic (cat: Pare, Smith, Parent and Steriade, 1988; Raczkowski and Fitzpatrick, 1989). Unlike basal forebrain cholinergic projections, however, brainstem cholinergic terminals tend to be more dense in caudal TRN (cat: Uhlrich, Cucchiaro and Sherman, 1988; rat: Cornwall, Cooper and Phillipson, 1990).

In addition to cholinergic innervation, there is evidence for a diverse monoaminergic innervation of TRN, rising from midbrain and brainstem nuclei. For example, the raphe nuclei of the midbrain send widespread serotoninergic projections to the whole extent of the TRN (Cropper, Eisenman and Azmitia, 1984; Peschanski and Besson, 1984; Cornwall, Cooper and Phillipson, 1990). Furthermore, TRN receives scattered noradrenergic projections from the locus ceruleus (Kayama, Negi, Sugitani and Iwama, 1982; Asanuma, 1992). Finally, TRN also appears to receive dopaminergic innervation, as it is rich in

dopaminergic receptors of both the D1 and D2 types (rat: Huang, Zhou, Chase, Gusella, Aronin and DiFiglia, 1992; Khan, Gutierrez, Martin, Penafiel, Rivera, and De La Calle, 1998; primate: Mrzljak, Bergson, Pappy, Huff, Levenson, and Goldman-Rakic, 1996). One identified source of TRN dopaminergic innervation is the ventral tegmental area (VTA) and the nearby interfascicular nucleus (Cornwall, Cooper and Phillipson, 1990). Dopaminergic terminals from these areas seem to target primarily the medial surface of the TRN, both in the rostral and caudal halves of the nucleus. Another provider of dopaminergic innervation to the TRN is the substantia nigra pars compacta (SNpc) (Freeman, Ciliax, Bakay, Daley, Miller, Keating, Levey and Rye, 2001), which in addition provides the TRN with GABAergic input (cat: Pare, Hazrati, Parent and Steriade, 1990; rat: Gandia, de las Heras, Garcia and Gimenez-Amaya, 1993).

1.2.9. Neurotransmitter effects on TRN and relay cells

As is apparent from the above, TRN receives numerous different inputs involving a diverse range of neurotransmitters. The effects that these neurotransmitters exert on TRN cells, however, are not always the same as their effects on dorsal thalamic (thalamocortical) cells. The following sections detail the main differences and similarities in the way TRN and thalamocortical relay cells respond to six major neurotransmitters.

1.2.9.1.Glutamate

By and large, dorsal thalamic and TRN cells respond similarly to the application of glutamate. More specifically, both dorsal thalamic and TRN cells

respond to glutamate or glutamate agonists with membrane depolarisation and an overall reduction of input resistance, primarily through the activation of NMDA and AMPA receptors (e.g. de Curtis, Spreafico and Avanzini, 1989). Both NMDA and AMPA receptors are ionotropic, meaning that their activation (opening) is a direct consequence of the binding of glutamate (or glutamate agonists). NMDA receptor activation results in fast depolarisations caused by the influx of sodium and calcium ions whereas AMPA receptor activation leads to even faster depolarisations through the entry of sodium ions (see Dingledine, Borges, Bowie and Traynelis (1999) for a detailed review of glutamate receptors' function). In addition to ionotropic receptors, glutamate acts on thalamocortical and TRN cells through metabotropic glutamate receptors (mGluRs), the post-synaptic effects of which are somewhat more diverse. Unlike ionotropic receptors, metabotropic receptors are not ion channels themselves; rather their activation leads to a sequence of events that allows the opening of secondary ion channels. Due to the indirect nature of their operation, the effects of these receptors' activation are slower and lengthier than those of ionotropic receptors. In the thalamus, mGluRs can be classified in 3 family groups (mGluR-I: *mGluR1, mGluR5*; mGluR-II: *mGluR2, mGluR3*; mGluR-III: *mGluR4, mGluR7, mGluR8*) on the basis of their subunit composition (Nakanishi, 1992; Conn and Pin, 1997; Alexander and Godwin, 2006). Activation of mGluR-I in both thalamocortical and TRN cells results in slow depolarisations favouring the generation of tonic firing (McCormick and von Krosigk, 1992; Turner and Salt, 2000). TRN cells have been found however to also demonstrate a unique ability also to become inhibited by glutamate. More specifically, activation of mGluR-II (and particularly mGluR3)

in these cells has been found to result in membrane hyperpolarisation (Cox and Sherman, 1999, Alexander and Godwin, 2006, also see Govindaiah and Cox, 2006). In dorsal thalamus the distribution of mGluR-II is considerably poorer compared to TRN, explaining the lack of glutamatergic hyperpolarisations in thalamocortical cells. More specifically, thalamocortical cells are completely devoid of mGluR3s (Lourenco Neto, Schadrack, Berthele, Zieglgansberger, Tolle and Castro-Lopez, 2000), which are responsible for the generation of the majority of the glutamatergic inhibitory effects. The effects of activation of mGluR-III, which can be found in relatively small quantities in the diencephalon, have been less intensely studied but they are generally known to resemble those of mGluR-II in reducing TRN's output to dorsal thalamus (i.e. implying hyperpolarizing effects) (Turner and Salt, 2003).

1.2.9.2.GABA

GABA exerts hyperpolarising effects upon both TRN and dorsal thalamic cells (McCormick and Prince 1986, see McCormick 1992 for a review). Both $GABA_A$ (rat: Gibbs, Schroder and Coulter 1996; ferret: Sanchez-Vives, Bal and McCormick, 1997) and $GABA_B$ receptors (Ulrich and Huguenard, 1996a; 1996b; Cox, Huguenard and Prince, 1997) are involved in mediating these hyperpolarising effects. Activation of the ionotropic $GABA_A$ receptors typically results in fast and brief hyperpolarisations that rarely exceed 20 ms in duration in both classes of cells. Due to the metabotropic nature of $GABA_B$ receptors' activation, on the other hand, their hyperpolarising effects last considerably longer, often exceeding 100ms in duration. In thalamocortical cells, activation of extrasynaptic $GABA_A$ receptors (which typically contain the δ, β2 and α4

subunits) can create equally long hyperpolarisations (von Krosigk, 1992; Jia, Pignataro, Schofield, Yue, Harrison and Goldstein, 2005). These receptors possess a high affinity for GABA and as a consequence they can provide the cell with continuous (tonic) inhibition triggered by small amounts of GABA often diffused in extrasynaptic space (Lindquist and Birnir, 2006). Extrasynaptic $GABA_A$ receptors are less common in TRN (its cells mainly express synaptic $GABA_A$ receptors that tend to contain the α3, β1,3 and γ2 subunits, see Pirker, Schwarzer, Wiesalthaler, Sieghart and Sperk, 2000) and as a consequence they do not appear to exhibit extrasynaptic tonic inhibition (Belelli, Peden, Rosahl, Wafford and Lambert, 2005; Jia *et al*., 2005). The long duration of $GABA_B$ and extrasynaptic $GABA_A$ receptors' postsynaptic inhibition carries a great potential functional significance regarding the switching of firing mode in thalamocortical cells from tonic to burst, a process which requires a prolonged hyperpolarisation of the cells' membrane (see *1.3.3.*).Indeed, the δ-$GABA_A$ receptor agonist THIP selectively activates the extrasynaptic receptors of thalamocortical neurons and consequently promotes burst firing (Belelli, *et al*. 2005, Cope, *et al.* 2005) As will be described in more detail later, the term "inhibition" used here to describe the effects of GABA in thalamocortical cells' activity can be misleading as it only refers to the "lowering" of the post-synaptic cell's membrane potential (to more hyperpolarized levels) and does not necessarily imply inhibition of the cell's firing output. In fact GABAergic inhibition of thalamocortical cells, under certain circumstances, can act as an "enhancer" of their transmission of information to cortex, through the generation of burst firing (see *1.3.3.2.* for more details).

1.2.9.3.Acetylcholine

The most striking difference between TRN and dorsal thalamic cells relates to the way they respond to acetylcholine. Whereas acetylcholine has depolarising effects on dorsal thalamic cells (e.g. Lo, Lu and Sherman, 1991; Lu, Guido and Sherman, 1993), it appears to primarily hyperpolarise TRN cells (rat: Marks and Roffwarg, 1991; cat: Godfraind, 1978; Sillito, Kemp and Berardi, 1983; Hu, Steriade and Deschênes, 1989; Funke and Eysel, 1993; guinea pig: McCormick and Prince, 1986). This differential effect of acetylcholine seems to be due to the distribution of various subtypes of muscarinic and nicotinic receptors in TRN and dorsal thalamus. Whereas dorsal thalamic cells seem to be rich in m1 and m3 types of muscarinic receptors (Plummer, Manning, Levey, Rees and Uhlrich, 1999), which are responsible for prolonged depolarising post-synaptic effects (Jones, 1993; Zhu and Uhlrich, 1998), TRN is rich in m2 muscarinic receptors, known for their hyperpolarizing effects (McCormick and Prince, 1986; McCormick 1992; Jones, 1993). Even though TRN also contains some m1 and m3 muscarinic receptors, (e.g. Clarke *et al.*, 1985, in McCormick, 1992), prolonged depolarising effects (indicative of these receptors' activation) have rarely been reported in TRN cells as a result of cholinergic neurotransmission (Clarke *et al*., 1985, in McCormick, 1992). Nonetheless, activation of the ionotropic nicotinic receptors results in fast depolarising effects in both TRN (Léna and Changeux, 1997) and thalamocortical cells (Zhu and Ulrich, 1997). In the former, nicotinic receptors tend to be pre-synaptic (i.e. at the axonal terminals of TRN cells) and their activation enhances the release of GABA onto thalamocortical cells (Léna and Changeux, 1997). The, by and large,

differential post-synaptic effect of acetylcholine on the reciprocally connected TRN and thalamocortical cells has very interesting potential functional implications, which will be discussed in more detail later (see *1.3.5.2)*.

1.2.9.4.Noradrenaline

Both dorsal thalamic and TRN cells respond with depolarisation to both iontophoretic application of noradrenaline and to the stimulation of locus coruleous in rats (Kayama, Negi, Sugitani and Iwama, 1982; Asanuma, 1992; Holdefer and Jacobs, 1994). In cat and guinea pig TRN, however, noradrenaline has been found to exert differential postsynaptic effects depending on the firing state of the cell. More specifically, when TRN neurons fire at high frequencies, application of noradrenaline has been found to inhibit their firing. When the same TRN cells, however, fire in low frequency bursts, noradrenaline appears to have a depolarising effect that suppresses burst firing and enhances single spike firing (McCormick and Wang, 1991; Funke and Eysel, 1993). Noradrenaline effects in dorsal thalamus and TRN are believed to be mediated mainly by α1 adrenoceptors, which are prominent in both areas (Jones *et al.*, 1985; Palacios *et al.*, 1987, as cited in McCormick, 1992). Nonetheless, the involvement of β and α2 adrenoceptors cannot be excluded (Palcios and Kuhar, 1982; Unnerstall *et al.*, 1984, as cited in McCormick, 1992).

1.2.9.5. Serotonin

Despite initial suggestions that dorsal raphe serotonin inhibits TRN cells (Yoshida, Sasa and Takaori, 1984), serotonin seems to exert strong and

prolonged excitatory effects on TRN cells of cats (McCormick and Wang, 1991; Funke and Eysel, 1993) and guinea pigs (McCormick *et al*., 1991), which typically result in high frequency (35-120Hz) tonic activity. Similarly, serotonin has been found to excite dorsal thalamic cells (Pape and McCormick, 1989; McCormick and Pape, 1990). The type of serotoninergic receptors involved in these depolarising effects are not known, but are suspected to be 5-HT_2 (McCormick and Wang, 1991) and 5-HT_{1A} (McCune, Voigt, and Hill, 1993).

1.2.9.6. Dopamine

The limited information regarding the post-synaptic effects of dopamine in the rodent thalamus suggests analogous responses by dorsal thalamic and TRN cells to dopaminergic receptor agonists. Application of the D2-receptor agonist quinpirole in MD, the dorsal thalamic nucleus that receives possibly the heaviest dopaminergic input (see Melchitzky and Lewis, 2001; Sanchez-Gonzalez, Garcia-Cabezas, Rico and Cavada, 2006, for evidence in primates), appears to hyperpolarize a large proportion of its cells; an effect that is reversed by the application of the D2-receptor antagonist haloperidol (Lavin and Grace, 1998). On the other hand, D1-receptor agonist SK38393 does not seem to affect MD neurons' polarity in any significant way (Lavin *et al*., 1998). Similarly, application of quinpirole in TRN slices has been found to inhibit their depolarisation-mediated release of GABA, implying hyperpolarizing effects. Moreover, similarly to MD cells, SK38393 application results in no observable membrane potential differences in TRN cells (Floran, Floran, Erlij and Aceves, 2004).

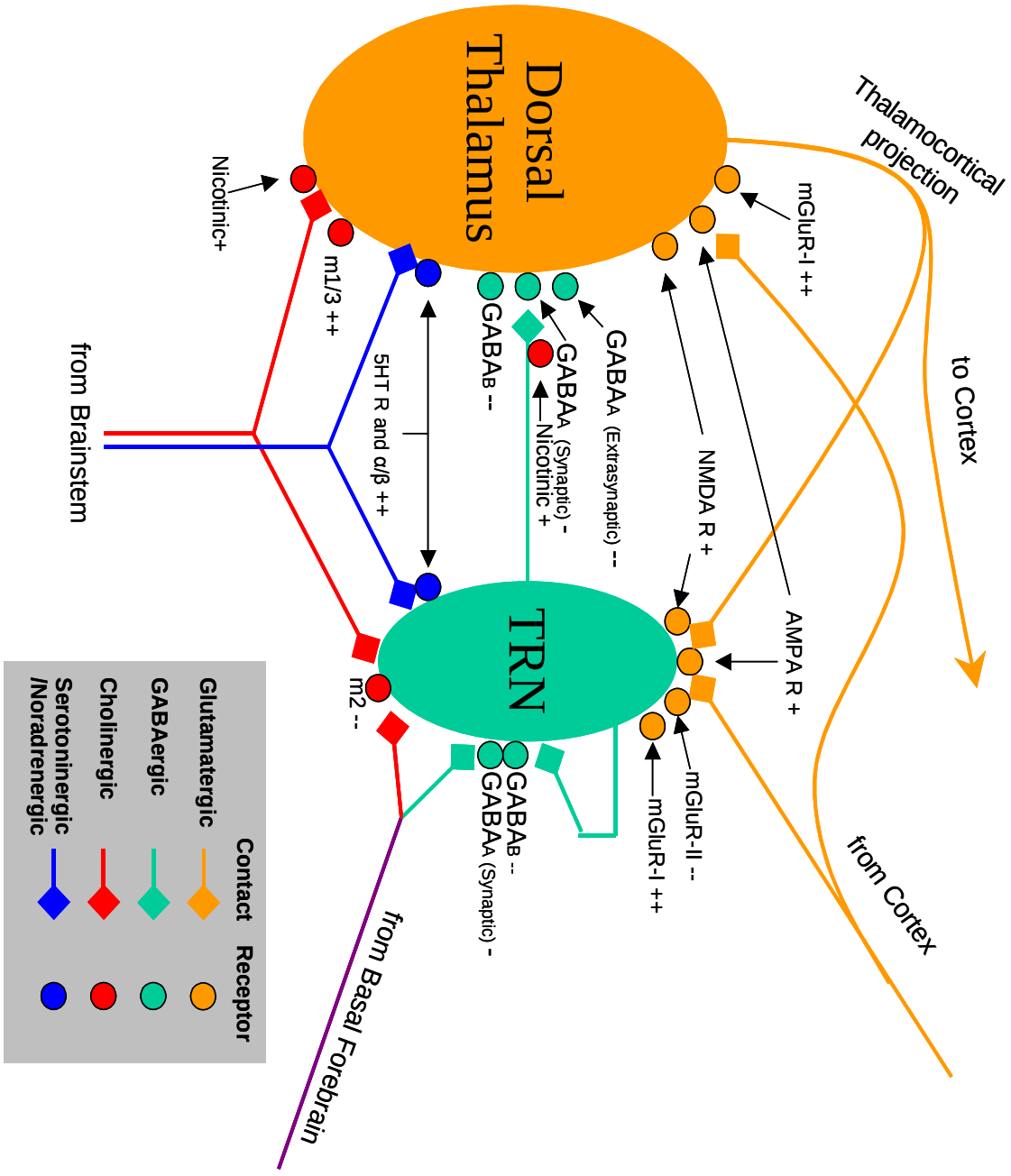

Figure 1.5. Schematic diagram of the main glutamatergic, GABAergic, cholinergic, serotoninergic and noradrenergic pathways innervating the dorsal thalamus and TRN, the receptors they act upon and the post-synaptic cellular effects the latter mediate. The symbols following the receptor names indicate the cellular effects of these receptors' activation: -: fast hyperpolarisation, --: slow depolarisation, +: fast depolarisation, ++: slow depolarisation. Noradrenergic and serotoninergic projections and receptors are shown together for simplicity, given they both mediate slow cell depolarisation effects on both dorsal thalamus and TRN cells. Abbreviations: m1,2,3: muscarinic acetylcholine receptors, 5HT R: Serotonin receptors, α/β: Noradrenergic receptors.

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1.3. Functional role of TRN

It is evident from the above anatomical data that TRN is far from a homogenous nucleus. TRN can be subdivided in numerous heterogeneous regions on the basis of a number of features and on a number of scales. Its various constituent subdivisions often feature unique anatomical, connectional and even neurochemical characteristics and as a result they are also likely to be functionally different. The broader functional subdivision within TRN is thought to be the one between its rostral and caudal halves (Jones, 1975). As mentioned earlier, caudal TRN (cTRN) is associated with sensory cortical and dorsal thalamic areas, whereas rostral TRN (rTRN) with non-sensory (e.g. limbic, motor and association) cortical and dorsal thalamic areas (see sections under *1.2*). As a consequence, cTRN is more suitably equipped to directly influence the thalamocortical flow of sensory information and it is therefore more likely to be involved in processes of sensory modulation and sensory filtering, which are requirements in attention.

1.3.1 Attentional processes as a concept

If we assume that cTRN is indeed involved in attentional processes, what exactly would these processes be? Defining attention can be a complicated task given that it does not represent a unitary process but a collection of associated cognitive processes that deal with a multitude of different behavioural demands. Even though the use of a single name (“attention”) to refer to these diverse processes can be misleading, it serves as a convenient tool to classify the behaviours these processes are responsible for (Parasuraman, 1998). The common feature of these processes is the

selection of certain environmental aspects, over others, in order for their processing to be enhanced (Duncan, 1984). At any given moment of an organism's life an enormous amount of information is received from the environment, most of which, however, is not relevant to its immediate behaviour. Consequently, in accordance to its immediate goals, an organism has to select to process only the information that is behaviourally relevant. Failure to be selectively attentive may lead to the additional processing of behaviourally irrelevant information, leading to behaviours that do not serve the organism's interests (Broadbent, 1957). Attention is, therefore, an important family of processes that enable an organism to deal with its environment in an as efficient way as possible.

Attention represents a pre-requisite element and the starting step of most other cognitive and motor processes. For example, the generation of most motor behaviours require the engagement of attention on the stimuli that these behaviours are directed towards or dependent upon. Attention enhances the perception of such stimuli, eliminating the influence of distracting stimuli, and thus enabling the optimal generation of the desired behaviour (Lu and Dosher, 1998; Carrasco, Penpeci-Talgar and Eckstein, 2000; Liu, Pestilli and Carrasco, 2005). Similarly, attention can facilitate learning acquisition by focusing sensory and cognitive processes on a task (Jiang and Chung, 2001; Rowland and Shanker, 2006). In a similar fashion, attention can enhance the creation of memories and lengthen their retention (Mackay and Ahmetzanov, 2005; Block, 2007; Chun and Turk-Browne, 2007).

The apparent involvement of attention in a multitude of other higher cognitive processes highlights the importance of being able to understand the brain mechanisms behind its operation. Attention is not “taking place” in a single brain area; rather it is a process carried out by an extended neural network involving areas as primitive as brainstem to as recent (in evolutionary terms) as neocortex (Colby, 1991). The thalamic stage of attention is of particular importance as it represents the first major stage of elaboration of sensory information following its collection by the sensory organs. As a consequence, thalamic processing may carry a considerable functional importance in the “preparation” of these sensory signals for the more elaborate attentional processing taking place at a later, cortical, level (Sherman and Guillery, 2001).

Attention can take a variety of different forms with regard to a number of parameters such as the sensory modality concerned, the number of sensory channels involved, the temporal and spatial characteristics of the attentional behaviours required, or the level of automaticity/voluntariness of these processes (see Parasuraman (1998), for a review on the multifaceted expressions of attention). Attention, for example, can be associated with one item, item feature, or sensory channel, at a time (selective attention), multiple items or sensory channels (divided attention), it can be transient or prolonged (e.g. sustained attention), voluntary (endogenous) or involuntary (exogenous), and even non-sensory, as it can also be applied to internal states rather than sensory signals, in a form of an executive function (controlled attention).

Selective attention represents the most basic form of sensory filtering and a constituent element of most other forms of attention. As its name suggests, it involves the selection of one piece of information, from the many available at a given time, in order to enhance its processing (see Hillyard, Vogel and Luck, 1998). Divided attention, on the other hand, is a form of selective attention that is applied to more than one informational sources (items, modalities, areas of sensory space, etc). Divided attention can only be generated through endogenous means (voluntarily), driven by the organism's behavioural needs and interests (Zentall, 2005). Selective attention, on the other hand, can be generated both endogenously but also exogenously, through its involuntary "capture" by sudden or salient stimulation representing potentially behaviourally-relevant events that may need to be acted upon (Jonides, 1980; Yantis, 2000). Performance in divided attention is typically poorer compared to selective attention given that the limited cognitive resources available at a given time have to be utilised simultaneously on multiple fronts, thus reducing their efficacy (Bonnel and Hafter, 1998). Whether a real *division* of attention is possible, or not, has been a matter of debate for several decades, with the alternative view suggesting that divided attention operates through a serial processing mechanism that moves selective attention rapidly between the two, or more, monitored (attended) sources (see Treisman, 1960; Deutsch and Deutsch, 1963; Braun, 1998).

The most-common form of selective attention is the one applied within visual space (visual spatial attention). Visual spatial attention is usually associated with the foveation of stimulation (i.e. the overt direction of the fovea - the area

of the eye used for acute vision - towards the area of visual space of interest). However, visual spatial attention can also be directed within space independently, or in the complete absence, of eye/head movements, in a form of *covert* orienting (Posner, 1980). This form of attention allows the rapid detection of potentially relevant stimuli at the periphery of visual space, before eye or head movements can be employed. In the rodent, where the ability for eye movements is minimal, covert orienting is the only possible form of visual spatial attention[1]. This makes it the ideal species for the investigation of covert orienting and its dissociation from oculomotor processes. Several discrete "stages" can be identified within a covert orienting behaviour. For example, a typical covert orienting behaviour may be initiated by the detection of a stimulus[2] in an area of visual space, the dis-engagement of attention from its previous location, its shift within space, the engagement of attention on the new location, and finally it may be completed with the analysis of the stimulation available within this location (see Posner, Walker, Friedrich and Rafal, 1984)

It is apparent from the above that attention represents a quite diverse variety of processes. Each of these processes is different and may be served by distinct, but nonetheless functionally related, anatomical substrate(s) (see Posner, *et al*., 1984, and also Ward and Brown, 1996). With regard to cTRN, this means its degree of involvement may also differ between different

[1] In the context of operant training, animals are usually required to hold their heads still during lateralised stimulus presentation, see chapters *IV and V,* thus eliminatiing head orienting.
[2] It should be noted that covert orienting can also be initiated in the absence of a stimulus (e.g. when *expecting* a stimulus to appear in a certain location).

expressions of attention. It becomes apparent, therefore, that the investigation around cTRN's potential involvement in attentional processes has to be narrowed down to these processes that the nucleus is more likely to be involved in. Because of its topographically precise connections, for example, cTRN is unlikely to act as an all-purpose attentional filter that regulates "general levels of attentiveness", which might include vigilance or concentration. Rather, its filtering function is more likely to involve spatially- or modality- specific actions, by which it can selectively allow certain parts of a sensory surface (e.g. visual field, somatosensory field) to be "attended" while preventing others from becoming so. This, however, remains to be determined experimentally.

1.3.2. cTRN's potential role in attentive behaviours

A common feature of the majority of cTRN's innervators (whether sensory or not) is that, to variable degrees, they are known to be involved in attentional processes, or alternatively that such processes modulate their activity. For example, cellular activities in sensory cortices become modulated by attention to their respective modalities. This holds true not only for higher-order sensory cortical areas but also for primary sensory cortices, albeit to a lesser degree (for evidence in primates see Motter, 1993, Roelfsema, Lamme and Spekreijse, 1998, for reviews see Posner and Gilbert, 1999; Treue, 2001). Moreover, basal forebrain, and more specifically its corticopetal cholinergic projection, is implicated in a multitude of attentional processes such as selective, divided, and sustained attention (Muir, Everitt and Robbins, 1994; McGaughy, Kaiser and Sarter, 1996; Sarter and Bruno, 1997; Turchi and

Sarter, 1997; Sarter, Bruno and Turchi, 1999; Risbrough, Bontempi and Menzaghi, 2002, also see Everitt and Robbins, 1997). As mentioned earlier, the cholinergic innervation that TRN (both rTRN and cTRN) receives from basal forebrain arises from collaterals of the abovementioned corticopetal projections (Jourdain, Semba and Fibiger, 1989) and is therefore very likely to also be functionally linked with attentional processes. Finally, brainstem function has been linked to general arousal and sleep processes (e.g. Sprague, 1967; Siegel, 1979; Vertes, 1984; Kayama and Koyama, 1998) but also to more specific sensory and attentional behaviours such as sustained attention (e.g. Inglis, Olmstead and Robbins, 2001; Kozak, Bowman, Latimer, Rostron and Winn, 2005). It appears, therefore, that cTRN could be acting as an integrator of all these diverse, yet attention-associated, sources and, in accordance to their combined effect, exert its selective, inhibitory, and thus modulating, effect upon the dorsal thalamus and its corticopetal (thalamocortical) projections. The exact mechanisms, however, by which cTRN could be intervening with thalamocortical activity remain by and large unclear. One theory postulates a lateral inhibition mechanism mediated by the cTRN upon thalamocortical cells, while another suggests a cTRN-mediated manipulation of the intrinsic electrophysiological characteristics of thalamocortical cells and the dynamic alteration of their responsiveness to sensory input. Evidence for both of these theories, with regard to a potential attention-mediating role for cTRN in the thalamus, will be discussed below.

1.3.2.1. cTRN as a generator of lateral inhibition in thalamus

Due to its anatomical, connectional and neurochemical features, cTRN is well equipped to mediate a lateral inhibition mechanism in the thalamo-reticulo-cortical network, which could form the basis of its modulatory role in the diencephalon (Pinault and Deschênes, 1998). Firstly, cTRN exerts inhibitory effects on thalamocortical cells, and moreover it does so in a precise, topographic, fashion that allows the selective inhibition of highly localised areas of the thalamic sensotopic field (see *1.2.4.* and *1.2.6*). Indeed, electric stimulation of cTRN cells can disrupt signal transmission of nearby, but not distant, dorsal thalamic cells (Yingling and Skinner, 1976). Furthermore, as mentioned earlier, cTRN tends to form open-loop circuits with thalamocortical cells (FitzGibbon, 1994; Pinault and Deschênes, 1998). This could contribute to the sharpening of the receptive fields of thalamocortical cells by inhibiting the influence of adjacent dorsal thalamic effects with overlapping receptive fields. Indeed, evidence suggests that lesions of TRN*som* result in a considerable enlargement of the receptive fields of VPm cells (Lee, Friedberg and Ebner, 1994a). Alternatively, or complementary, and depending on the pattern of synaptic architecture between cTRN and dorsal thalamus (see *1.2.4.2*), a lateral inhibition mechanism could enable activated cTRN cells to inhibit thalamocortical cells with adjacent, but different, receptive fields to their own. This could result in the weakening of the signal transmission of the latter, in favour of the signal transmission of thalamocortical cells with receptive fields similar to the activated cTRN cell (in other words, of its efferent thalamocortical cells) (see Sherman and Guillery, 2001).

For instance, a thalamocortical cell corresponding to a given area of the sensory field could become activated by a strong sensory input to its receptive field and, in turn, activate its post-synaptic cTRN cell. The activated cTRN cell would then inhibit thalamocortical cells in the vicinity of its afferent thalamocortical cell, but not its afferent thalamocortical cell itself (see Figure 1.6a). As a consequence, the activities of these thalamocortical cells would be inhibited, allowing the initially activated thalamocortical cell to make maximal post-synaptic impact. Such a bottom-up lateral inhibition mechanism could allow therefore the enhanced processing of strong or salient sensory signals, which are likely to be behaviourally relevant, at the expense of less-salient stimuli that are likely to be "noise" or distractors (Pinault and Deschênes, 1998; Sherman and Guillery, 2001).

A lateral inhibition mechanism could also operate in a top-down fashion. More specifically, a corticothalamic input could activate a pair of cTRN and thalamocortical cells that posses the same receptive field corresponding to an area of the sensory field with potential behavioural interest (see Figure 1.6b). This would result in the direct excitation of these two cells and the indirect inhibition of all the thalamocortical cells to which the activated cTRN cell projects (Tsumoto, Creutzfeldt, and Legendy, 1978; Montero, 2000). Once more, the outcome of such a mechanism would be the signal facilitation of the activated thalamocortical cell and the signal inhibition of nearby, attention-competing, thalamocortical cells. This would eventually result in a better signal-to-noise ratio transmission for the activated thalamocortical cell, with

implications for the better detectability and/or perception of its signal (Lavallée and Deschênes, 2004; McAlonan, Cavanaugh, and Wurtz, 2006).

Regardless of the nature of a TRN-mediated lateral inhibition, such a mechanism could, therefore, contribute to the creation of focal fields of enhanced activity surrounded by fields of suppressed activity, contributing to a differential promotion of sensory signals to cortex and a potentially differential allocation of attention to them.

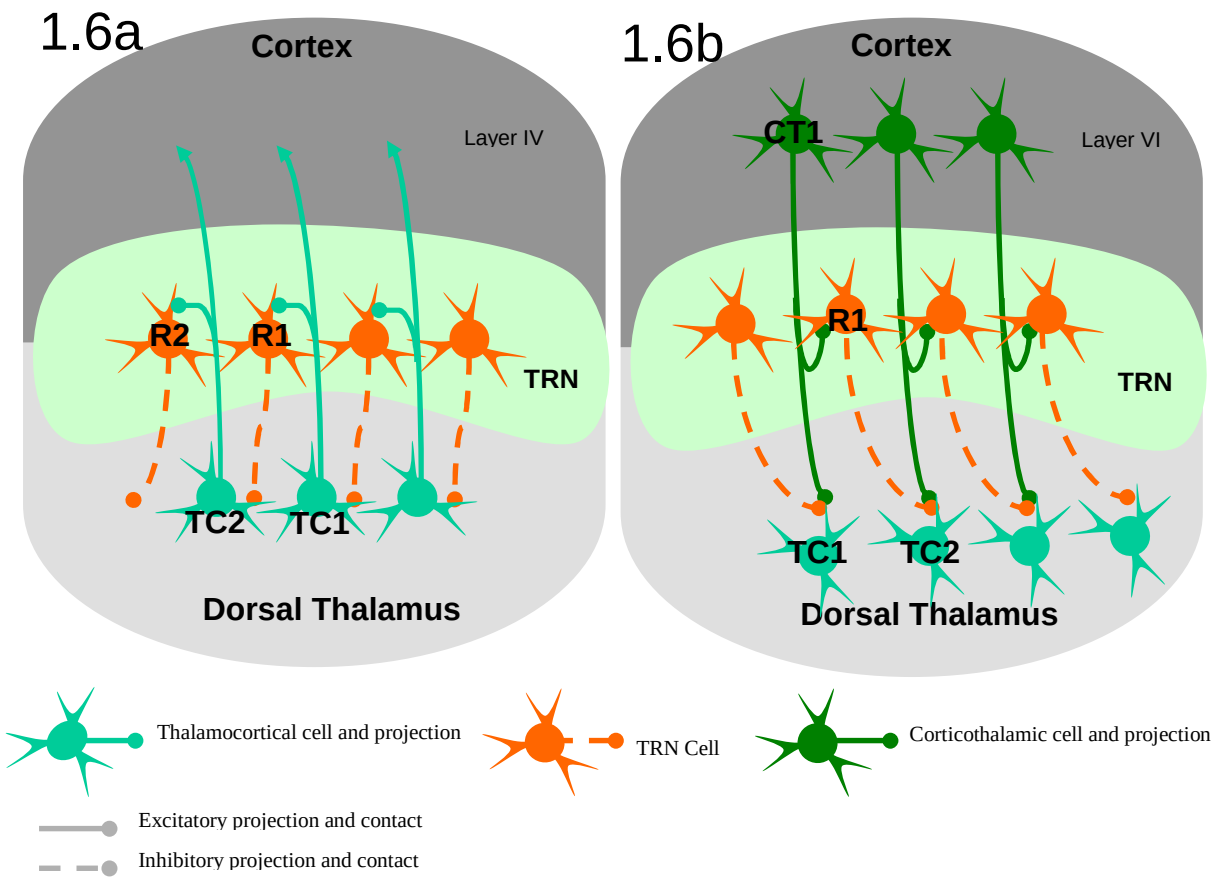


Figure 1.6:Two schematic examples of the functional cellular architecture of thalamo-reticulo-cortical lateral inhibition mechanisms (adapted from Sherman and Guillery, 2001). 1.6a: Bottom-Up mediated lateral inhibition network. A stimulus within the receptive field of the thalamocortical cell TC1 activates the cell, which in turn activates the R1 cell of TRN. The activated R1 cell then inhibits the thalamocortical cell TC2 (representing a different receptive field than TC1), the signal transmission of which is weakened. The overall result of this mechanism is that the signal TC1 sends to cortex will have greater post-synaptic effects on cortical cells compared to that of TC2, thus gaining an advantage at being processed. 1.6b: Top-Down mediated lateral inhibition. Activation of corticothalamic cell CT1 causes activation in TC1 and R1. The result of this activation is twofold. Firstly, the signal that TC1 projects to cortex (not shown here for reasons of simplicity) receives a boost and, secondly, the signal of the "competing" TC2 is inhibited through R1. As a result the signal carried by TC1 gains an advantage at being processed over that of TC2.

1.3.2.2. Alternative mechanisms of cTRN-mediated thalamocortical modulation

Advances in the study of the electrophysiological properties of thalamocortical cells revealed that these possess a diversity of manipulable intrinsic characteristics that can affect the way cells respond to incoming input and the nature of output they generate. Thalamocortical cells do not simply transfer signals in a rigid, “cable-like” fashion (Sherman, 2005), neither their output reflects faithfully the sum of their input. Rather, depending on their internal electrical state and the status of certain membrane conductances, these cells can respond to the same input in a number of different ways (Sherman and Guillery, 2001). Recognition of this point highlights the potential complexity of the mechanisms behind the attentional modulation of thalamocortical activity. As a consequence, in order to understand the potential functional role of cTRN in thalamocortical sensory transmission, and thus attention, we first have to comprehend the function of thalamocortical cells. More specifically, it is necessary to understand some of the electrophysiological properties of these cells, the functional significance of these properties with regard to the relay of sensory information, and how the cTRN-supplied inhibition can affect these.

1.3.3. Electrophysiological properties of thalamocortical cells

1.3.3.1.“Tonic” vs “Burst” mode

Thalamocortical cells can fire action potentials in two very distinctive fashions, namely the *tonic* and *burst* firing modes. When in tonic mode, cells fire successions of single action potentials (Figure 1.7a). When in burst mode, on

the other hand, cells fire closely packed groups of action potentials, each group separated by periods of relative inactivity (Figure 1.7b) (Jahnsen and Llinas, 1984). What determines the firing mode of a thalamocortical cell is the state of one of its many membrane conductances; namely, the Ca^{2+} conductance. This conductance (also known as I_T, because it involves T-type Ca^{2+} channels (Sherman, 2001)) is dependent on the voltage (and its duration) of the cell's membrane. I_T becomes *inactivated* at depolarised membrane potentials of above –55mV that last more than ~100ms. A strong depolarisation of the cell (e.g. due to a stimulus presentation in its receptive field) while I_T is inactivated results in the generation of trains of single action potentials (tonic firing mode) (Jahnsen and Llinas, 1984). When the cell's membrane, however, hyperpolarises at potentials below –65mV, and for periods longer than ~100ms, I_T becomes *de-inactivated.* While in that state, a strong enough incoming depolarisation can *activate* I_T, allowing the influx of Ca^{2+}, resulting in a low-threshold spike (a brief depolarisation of ~30mV) ridden by a burst of closely packed action potentials (burst firing) (Jahnsen and Llinas, 1984). In order for a cell to fire in bursts, therefore, the I_T has to first become de-inactivated, via a slightly lengthy hyperpolarisation, and then activated by a strong, and preferably brief, depolarisation.

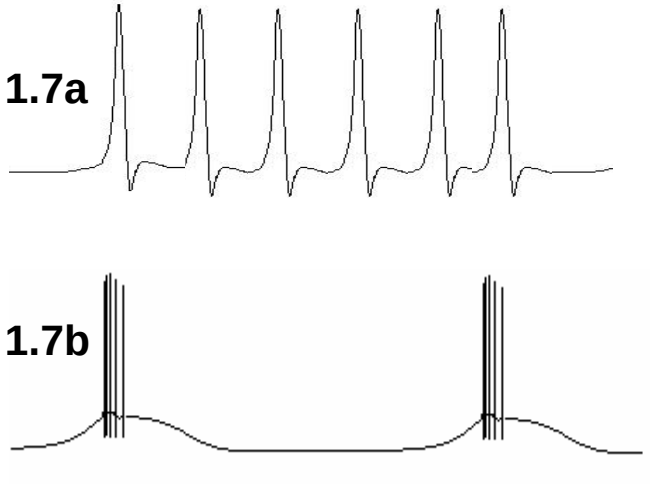


Figure 1.7: Schematic representation of tonic (1.7a) and burst (1.7b) activity

1.3.3.2. Functional role of firing mode in the thalamocortical pathway

Early *in vivo* investigations reported that tonic firing in thalamocortical cells was more prominent during periods of wakefulness or REM sleep, whereas burst firing was evident only during deep sleep stages or deep anaesthesia, when thalamocortical cells are typically hyperpolarised (e.g. Livingstone and Hubel, 1981; Steriade and McCarley, 1990; Steriade, Contreras, Curro Dossi and Nunez, 1993; Steriade, McCormick and Sejnowski, 1993). This led to the assumption that tonic firing is the main sensory-relaying firing mode of the thalamocortical relay system, whereas burst firing, and especially when rhythmic, represents a mode of sensory disengagement during which no sensory input is relayed to cortex (e.g. see Steriade *et al*., 1993). More specifically, the rhythmic bursting of thalamocortical cells was thought to represent a way by which the cortex was signalled that the relaying of information was suspended. Doing so by means of rhythmic bursts, rather than with a total cessation of firing, offers a clear functional advantage, for the

latter could also signal the lack of stimulation (Sherman and Guillery, 2001). Nonetheless, even though it is now generally accepted that the role of rhythmic bursting is associated with stages of sleep and the interruption of sensory transmission, more recent evidence suggests that burst firing in thalamocortical cells may also have an important functional role in awake states too (see below).

1.3.3.3. Mode of firing and transmission of sensory information

Contrary to initial belief that bursts only occur during non-REM sleep and anaesthesia, bursts have also been reported in awake animals (Guido and Weyand, 1995, Nicolelis, Baccala, Lin and Chapin, 1995; Ramcharan, Gnadt and Sherman, 2000, Fanselow, Sameshima, Baccala and Nicolelis, 2001). In the awake state, and in the absence of stimulation, bursts tend to be relatively infrequent and to possess little or no rhythmicity, occurring instead at random intervals. In the presence of stimulation, however, bursts stop occurring randomly and begin to follow the presentation rate of the stimulus (Sherman, 1996). This demonstrated that, similarly to tonic firing, burst firing is also capable of relaying sensory information. Indeed, burst firing in thalamocortical cells (LGN cells in particular) has been found to encode sensory information, and drive post-synaptic cortical cells, at least as effectively as tonic firing (Reinagel, Godwin, Sherman and Koch, 1999). Not surprisingly however, the properties of tonic and burst firing modes are quite different with regard to the relay of information. That is to say, even though the quantity of information relayed by these firing modes could be the same, the quality of the information relayed appears to be substantially different. When in tonic mode, cells

respond to sensory stimulation in a linear fashion reflecting faithfully the rate of the stimulus presentation (Sherman, 1996). However, the linear representation of the stimulus is accompanied by high levels of background activity. Burst firing, on the other hand, is characterised by much lower levels of background noise, but responses to sensory stimulation are less linear and considerably poorer in temporal resolution (Guido, Lu and Sherman, 1992; Guido, Lu, Vaughan, Godwin and Sherman, 1995; Mukherjee and Kaplan, 1995). As a consequence, whereas a cell when firing tonically would provide different responses for stimuli with different characteristics, such stimuli would generate highly similar responses in the same cell when bursting. A classic demonstration of the above comes from the responses of dLGN cells to the visual presentation of a drifting sinusoidal stimulus in the cat. Whereas the responses of tonically firing dLGN cells resemble the sine, bursting dLGN cells only respond to the beginning of each cycle of the sine, resulting in a much less sinusoidal pattern of responses (Sherman, 1996; also see Sherman and Guillery, 2001). This means that a cell in both tonic and burst firing can inform the postsynaptic cell of the occurrence of a stimulus, but, when in burst firing, most information about the characteristics of that stimulus is omitted.

Functionally, the above differences between tonic and burst firing result in certain advantages of the one over the other in the transmission of information, with regard to certain sensory parameters. For example, due to the faithful and linear representation of its input, tonic firing is optimal for the detailed analysis of sensory stimulation. On the other hand, burst firing may

lack linearity but, due to its much better noise-to-signal ratio, it is the ideal firing mode for the detection of weak or sudden stimuli that would be, most likely, missed by the “noisy” tonic firing. More specifically, because burst-firing cells respond better to middle- than high-frequency stimulus presentations, they are particularly good at the detection of abrupt, rather than gradual, changes in the environment (Guido, Lu, Vaughan, Godwin and Sherman, 1995; Guido and Weyand, 1995).

With regard to attention, it is apparent from the above that a continuous interchange between the two firing modes would be necessary in order for most attentional behaviours to take place. For example, when attention needs to be paid to a particular area of the sensory field where stimulation is expected, thalamocortical cells representing that area may initially start firing in burst mode so that to enhance the detection of the upcoming stimulus. As soon as the stimulus is detected, the firing mode can switch to tonic, which will enable the more detailed representation of that stimulus. In other words, even though tonic activity corresponds to the main and lengthier part of an attentional behaviour (the detailed perceptual analysis of the stimulation), it is burst activity that often initiates that behaviour. Burst firing, in this context, can be seen therefore as a “*wake up call*” (Sherman, 1996; 2005) to cortical sensory areas, informing them of new incoming information, thus preparing them for its subsequent delivery in tonic mode.

Further supporting evidence for the idea that burst mode acts as a “wake up call” comes from the observation that a thalamocortical burst, and more

specifically the first action potential in such a burst, is more effective at activating post-synaptic cortical cells than any individual thalamocortical tonic action potential (Swadlow and Gusev, 2001). This is because whereas post-synaptic effects of tonic action potentials are likely to suffer *paired-pulse depression*, this is not entirely the case for burst action potentials. Paired-pulse depression is the “weakening” of the post-synaptic effects of an action potential that arrives shortly after another action potential (see Castro-Alamancos and Oldford, 2002; Chung, Li, and Nelson, 2002; Nicolelis, 2002, for examples in the thalamocortcal circuits). The source of this effect is the inability of a cell to recover fully from the first action potential in time to generate an equally strong second one, which as a consequence ends up being weaker (depressed). Given that tonic action potentials come in long continuous sequences and with relatively brief interspike intervals, pair-pulse depression is in constant effect. On the other hand, the first action potential in a burst has to, compulsorily, be preceded by a silent hyperpolarized period of at least 100ms, which is enough time for the cell to recover from the effects of any preceding events (Sherman, 2001). As a consequence, the first action potential in a burst never suffers the effects of paired-pulse depression and can therefore exert maximal effects on post-synaptic cells. In addition, due to their extreme temporal proximity, the remainder of the action potentials in a burst can sum up their post-synaptic effects, and, despite suffering paired-pulse depression, provide a strong overall post-synaptic effect on cortical cells (Sherman, 2001; Swadlow and Gusev, 2001). The temporal proximity of tonic action potentials, on the other hand, is such that while paired-pulse

depression is inevitable, temporal summation of their post-synaptic effects is less likely.

1.3.4. Tonic and burst firing in TRN cells

Similarly to thalamocortical cells, TRN (both rTRN and cTRN) cells fire either in tonic or burst mode (Contreras, Curro Dossi and Steriade, 1993; Marks and Roffwarg, 1993). TRN bursts are usually observed during deep sleep stages, whereas tonic spikes are more prominent during wakefulness and some stages of REM sleep (Steriade, Domich and Oakson, 1986; Marks and Roffwarg, 1993). The intrinsic electrophysiological characteristics of TRN cells, however, differ from those of thalamocortical cells and, as a result, there are some marked differences in the way tonic and burst firing can be triggered in these two classes of cells. More specifically, the processes of activation and especially of inactivation of I_T in TRN cells are considerably slower compared to thalamocortical cells. As a result, TRN's I_T is referred to as "I_{TS}", with the additional "$_S$" standing for "slow" (Huguenard and Prince, 1992). In addition to its slower speed, and in marked contrast to thalamocortical I_T (Coulter, Huguenard and Prince, 1989), the inactivation of I_{TS} is voltage independent, meaning that regardless of the size of depolarisation, the slow rate of its inactivation remains constant (Huguenard and Prince, 1992). Finally, I_{TS} activation appears to occur at more depolarised membrane potentials than I_T activation, which, in part, may explain its slower course (I_{TS} activation may have to wait for the peak of the incoming depolarisation). This is possibly due to the larger size of TRN cells, their longer dendritic arbours, and also the distant dendritic site of their Ca^{2+} channels (most of its inputs arrive at

proximal dendrites of TRN cells) (Destexhe, Contreras, Steriade, Sejnowski and Huguenard, 1996).

Not only the dynamics of burst generation but also the intrinsic profiles of the bursts themselves differ between TRN and thalamocortical cells. Due to the slower inactivation of I_{TS}, TRN bursts last longer (50-100ms) than those of thalamocortical cells (5-25ms) and they also contain at least twice the number of individual spikes (Domich, Oakson and Steriade, 1986). In addition, and possibly due to the combination of slow activation and inactivation of I_{TS} (Huguenard and Prince, 1992), TRN burst spikes exhibit an "early acceleration-late deceleration" pattern with regard to their inter-spike intervals (ISIs). This means that there is an initial ISI decrease for the first few burst spikes followed by an ISI increase for the remaining spikes (Domich, Oakson, and Steriade, 1986; Avanzini, de Curtis, Panzica, and Spreafico, 1989; Contreras, Curro Dossi and Steriade, 1993; Huguenard and Prince, 1994; Hartings, Temereanca, and Simons, 2003). Finally, another interesting feature of TRN bursts is that they can be directly passed from one TRN cell to another via gap junctions. This is particularly the case for slow burst activity, due to the low-pass filter of TRN electrical synapses (Landisman *et al.*, 2002, also see Deleuze and Huguenard, 2006).

1.3.5. Control of response mode in thalamocortical cells

Given the potential functional significance of burst and tonic firing modes (see *1.3.3.3*), what is the mechanism that controls their interchange in thalamocortical cells in accordance to the immediate behavioural, and thus

attentional, demands? For such a mechanism to be effective it would be necessary to posses a topographic map of each modality's sensory surface. By being sensotopically precise, this mechanism could then selectively promote burst firing in cells corresponding to areas of the sensory surface for which there is a requirement for signal detection or analysis under noisy conditions, and tonic firing in cells corresponding to areas of the sensory surface that require detailed stimulus analysis. As a consequence, this mechanism should also be able to exert both excitatory and inhibitory effects of enough duration and intensity to enable the respective inactivation and de-inactivation of I_T in thalamocortical cells. There are two suitable candidates for such a role, namely the corticothalamic feedback projection and the miscellaneous brainstem innervation of dorsal thalamus. Both these mechanisms, as will be described below, may require to a great degree the involvement of cTRN. Despite the potential importance of these two mechanisms, the available evidence regarding their plausibility is remarkably limited.

1.3.5.1. Cortical Control

The first, and perhaps most obvious, candidate for a role in the control of sensory thalamocorticial firing mode is the sensory cortical feedback innervation of the thalamus (Sherman, 1996). These corticothalamic projections possess the required degree of topography in order to selectively and accurately promote tonic and burst firing in the thalamocortical cells representing the desired areas of the sensory field. This holds particularly true for the projections rising from the primary sensory cortices, but also, albeit to a

lesser degree, for those from the higher-order sensory cortices.

Corticothalamic fibres contact thalamocortical relay cells exclusively with glutamatergic synapses (de Curtis, Spreafico and Avanzini, 1989; McCormick, 1992; Salt and Eaton, 1996). Only corticothalamic input activates metabotopic glutamate receptors in thalamocortical cells (McCormick and von Krosigk, 1992; Godwin, Vaughan and Sherman, 1996). Therefore, these glutamatergic projections possess the privileged ability to create long enough post-synaptic depolarisations to inactivate I_T and facilitate tonic firing generation. How can cortical inputs however provide the necessary hyperpolarisation to thalamocortical cells in order to generate burst firing, given that they only supply the latter with glutamatergic (and thus depolarising) innervation? This can only be done indirectly, via either the GABAergic interneurons present in the dorsal thalamus or via the cTRN. The latter route possesses a precise topographical sign of its own (as described in *1.2.4* and *1.2.6*) and could, thus, more accurately, compared to the less strictly topographic local interneurons, inhibit the desired thalamocortical cells. According to the latter scenario, therefore, corticothalamic cells could excite cells of cTRN associated with the area of the sensory field of interest, which would then selectively inhibit the dorsal thalamic cells that correspond to that same area, de-inactivating their I_T and creating the grounds for I_T activation and burst firing generation. cTRN cells are particularly well-suited for de-inactivating I_T in thalamocortical cells as they contact them mainly at distal dendrites (Wang, Bickford, van Horn, Erişir, Godwin and Sherman, 2001), which is the area where the majority of Ca^{2+} (T) channels are situated (Kim and McCormick, 1998). Following the I_T de-inactivation, any sufficiently strong depolarising input (either from a

sensory stimulus or from a monosynaptic corticothalamic contact) could then initiate burst firing in these thalamocortical cells. Having said that, the long-lasting, cTRN-mediated, inhibitory post-synaptic potentials (IPSPs) necessary to de-inactivate I_T and initiate burst firing in thalamocortical cells could be achieved in two ways: either via the activation of the metabotropic $GABA_B$ (or the extrasynaptic $GABA_A$) receptors, with their prolonged inhibitory post synaptic effects, or alternatively via the ionotropic (synaptic) $GABA_A$ receptors provided that there is enough temporal summation of their resultant IPSPs. Either of these alternatives, nevertheless, is hypothesised to be more likely if the cTRN fires in burst mode. This is because, firstly, $GABA_B$ receptors appear to require a large release of GABA in order to activate, possibly due to their, often, extra-synaptic location in thalamocortical cells – i.e. similarly to extrasynaptic $GABA_A$ receptors (Dutar and Nicoll, 1988, as cited in Huguenard and Prince, 1992). A large release of GABA is more likely when there is burst activity in cTRN (Kim and McCormick, 1998). Indeed, whereas tonic activity in cTRN cells results in brief and small amplitude synaptic $GABA_A$-mediated IPSPs in thalamocortical cells (Kim, Sanchez-Vives and McCormick, 1997), burst firing in cTRN results in strong and long-lasting IPSPs in thalamocortical cells, mediated by both $GABA_A$ and $GABA_B$ receptors (Kim *et al.*, 1997; Kim and McCormick, 1998). Secondly, in comparison to thalamocortical bursts, cTRN bursts can be long in duration and with particularly short ISIs (see *1.3.4*). Therefore the IPSPs of individual spikes within a cTRN burst can be temporally summated to exert a long enough hyperpolarisation on thalamocortical cells to de-inactivate their I_T, regardless of the type of GABA receptor implicated (Huguenard and Prince, 1992).

If we assume however that cTRN bursts are indeed the source of inhibition that promotes burst firing in thalamocortical cells, then what is the source of inhibition that allows cTRN cells to hyperpolarize and thus start firing in bursts themselves? Even though intra-reticular inhibition could contribute to such an effect (Zhang and Jones, 2004), given the presence of functional $GABA_B$ receptors in TRN (Sanchez-Vives, Bal, and McCormick, 1997), the most likely mechanism behind cTRN's burst-generating hyperpolarisation involves the neuromodulation from other subcortical sources. These are likely to stem from brainstem and basal forebrain, which are areas rich in neurotransmitters that are known to inhibit cTRN activity (e.g. acetylcholine and/or GABA, see *1.2.9*). The role of these projections with regard to the generation of bursts in cTRN will be described in more detail below (see *1.3.5.2*). Finally, another possible source of cTRN hyperpolarisation could arise from its glutamatergic thalamocortical and corticothalamic inputs, through the involvement of mGluRII receptors, exclusively found in TRN (Cox and Sherman, 1999, see *1.2.9.1*). This possibility, however, has not been yet directly investigated.

1.3.5.2. Brainstem control

In addition to cortical input, brainstem input can also affect thalamocortical cells' firing mode, both directly and also indirectly through the cTRN. Cholinergic (Morrison and Foote 1986; de Lima and Singer, 1987), noradrenalinergic (de Lima and Singer, 1987) and serotinergic (de Lima and Singer, 1987; Gonzalo-Ruiz, Lieberman and Sanz-Anquela, 1995) projections arising from different areas of brainstem terminate throughout dorsal thalamus

and cTRN. These projections operate only through metabotropic receptors of their respective neurotransmitters (with the exception of acetylcholine, which operates through both metabotropic and ionotropic receptors) and, consequently, are capable of exerting prolonged post-synaptic effects on thalamocortical cells; a necessary feature for the control of the latter's firing mode. Indeed, both *in vivo* and *in vitro* studies in rats and cats have demonstrated slow depolarising effects of cholinergic (Lo, Lu, and Sherman, 1991; Lu, Guido and Sherman, 1993), noradrenergic (McCormick and Prince, 1988; Funke, Pape and Eysel, 1993; Holdefer and Jacobs, 1994) and serotinergic (McCormick and Pape, 1990) brainstem action on thalamocortical cells, which result in the suppression of burst activity. Brainstem, however, also supplies the above neurotransmitters to the cTRN (see *1.2.8.2*) and could therefore, indirectly, affect thalamocortical firing mode through the latter. For example, brainstem acetylcholine slowly hyperpolarises cTRN cells, mainly through m2 muscarinic receptors (found in abundance in cTRN; see McCormick and Prince, 1986; Carden and Bickford, 1999), and it is likely to de-inactivate I_{TS} and create the conditions for the generation of bursts in cTRN (McCormick *et al*., 1986), and thus possibly in thalamocortical relays too (basal forebrain acetylcholine and/or GABA could also have a similar effect on cTRN cells, e.g. see Pinault and Deschênes, 1992). On the other hand, the long-lasting excitatory effects of serotonin and noradrenalin on cTRN cells, in combination with their similar effects on thalamocortical cells, are more likely to promote tonic activity in both nuclei (Rogawski and Aghajanian, 1980; McCormick and Wang, 1991, but also see Kayama, Shimada, Hishikawa and Ogawa*,* 1989).

Unlike most sensory corticothalamic projections, however, brainstem thalamic projections are not topographic. Whereas corticothalamic input is organised so that it can operate on certain areas of the thalamic sensory field with precision, brainstem innervations appear to be more diffuse and without sensotopic organisation (see Sherman and Guillery, 2001 for details). Therefore, unlike corticothalamic input, which could switch highly specific areas in burst or tonic mode, brainstem input is likely to have a more general impact on firing mode of cTRN. Nonetheless, it is possible that brainstem input works in conjunction with cortical and cTRN input to modulate the firing mode within highly specific dorsal thalamic areas. More specifically, brainstem projections could neuromodulate extensive zones of cTRN, creating large platforms of depolarisation or hyperpolarisation upon which corticothalamic inputs could subsequently operate, exerting their topographically-specific effects. For example, in a hypothetical scenario, cholinergic brainstem inputs could hyperpolarise large areas of cTRN for a relatively long period of time, therefore de-inactivating the local cells' I_{TS}. A strong, localised, cortical input upon some of these cTRN cells (those corresponding to a sensory area of interest) could then initiate bursts. The localised bursting cTRN cells would then provide an equally localised area of thalamocortical cells with prolonged hyperpolarisation to de-inactivate their I_T and allow them to burst next time they receive strong excitation. In such a way, the brainstem, alongside cTRN and corticothalamic projections, could contribute to the creation of a locus of bursts within dorsal thalamus (e.g. in dLGN), which may correspond to an attentional hotspot of increased stimulus detectability (personal

communication with Lee, C.C. and Sherman, S.M., also see Sherman and Guillery, 2001).

1.3.5.3. Summary

It is evident from the above that cTRN could play a key role in the mechanisms underlying thalamocortical firing mode control, as these are dictated by both corticothalamic feedback and brainstem influences. cTRN constitutes the primary means by which sensory thalamic loci can be selectively and topographically hyperpolarised and therefore the most likely means by which burst firing can be selectively induced in particular thalamocortical relays representing sensory areas of interest. Indeed, lesions of the cTRN (and more specifically TRNvis) have been found to abolish bursts in the dLGN of the anaesthetised rat (French, Sefton and Mackay-Sim, 1985). Similarly, the periods of hyperpolarisation that normally precede dLGN bursts were also abolished. This demonstrated that the necessary hyperpolarisation for the de-inactivation of the I_T and the commencement of burst firing in thalamocortical cells may indeed arrive from the cTRN.

1.4. Investigations of caudal TRN's attentional role

Despite the strong theoretical likelihood that cTRN could be involved in the modulation of thalamocortical sensory transmission and, by extension, in attentional processes, very little behavioural research has been devoted in investigating this possibility. It would be reasonable to assume that, at least to some degree, this is due to the practical difficulties associated with the behavioural investigation of cTRN through most conventional experimental

techniques. For example, due to its unusual thinness, elongated and crescent-like shape, and its proximity to sensory areas of the dorsal thalamus, selective lesions restricted within the boundaries of the nucleus are hard to achieve. Injections of neurotoxins are likely to spread to nearby brain areas and/or leave the extreme ends of cTRN unaffected, resulting therefore in non-selective and/or incomplete lesions (personal communication with Tait, D.S. and Lukoyanov, N.V. and personal observations). *In vivo* electrophysiological recordings from the cTRN of awake animals present similar challenges. Due to TRN's curved -in all orientations- shape, it is difficult to sample from more than a few cells with the same electrode without attempting multiple electrode penetrations (that is, without causing additional mechanical damage). Moreover, cTRN cells are not only hard to isolate but they also demonstrate large individual variation with regard to their receptive fields properties and response characteristics, which make attempts for *in vivo* recordings all the more frustrating (Funke and Eysel, 1998, and personal observations). As a consequence of the above practical difficulties, and as it will be described below, early behavioural studies looked for alternative, less invasive, methods to investigate the role of cTRN in attention.

1.4.1. Fos studies

One such alternative method involved the use of *c*-fos immunocytochemistry. *c*-fos, a proto-onco gene, and its product protein, Fos, (for a review see Herrera and Robertson, 1996) are present in most brain cells but in low, undetectable, levels. However, after the persistent excitation of their host cell, c-fos and Fos levels increase and become detectable (Dragunow and

Robertson, 1987). As a consequence, both the gene and its product protein can be used as markers of neuronal activation to help identify, with single-cell spatial accuracy, the brain regions that activate during the performance of certain behaviours (Campeau, Hayward, Hope, Rosen, Nestler and Davis, 1991; Imaki, Shibasaki, Hotta, and Demura, 1992; 1993; Nikolaev, Werka and Kaczmarek, 1992).

One of the first attempts to demonstrate the involvement of cTRN in attention through Fos immunocytochemisty was a series of studies by Montero (1997, 1999). In these studies, Montero looked at the expression of Fos in sensory nuclei of the dorsal thalamus and the cTRN of rats, following the performance of attention-demanding exploratory behaviours. In the first study, Montero (1997) examined the distribution of Fos immunoreactive cells in the brains of healthy and blind rats that had explored a complex novel environment immediately prior to their sacrifice. It was assumed that healthy rats would perform the exploration mainly by means of vision while blind animals would do so by vibrissae whiskering and other tactile means. Montero observed considerable inequalities in the distribution of Fos immunoreactive cells in the TRN*vis* and TRN*som* of the healthy and blind animals. More specifically, healthy (i.e. presumably visually-exploring) rats illustrated rich and selective TRN*vis* activation, while blind (i.e. presumably tactilely-exploring) rats showed selective TRN*som* activation. Furthermore, no Fos activation was found in the TRN*aud* of either group, despite the generation of various sounds during the exploratory behaviour, which were presumed to have been ignored. Finally, a control group of healthy animals that was exposed to a familiar environment

(i.e. that did not induce exploratory behaviours) did not demonstrate activation anywhere in the cTRN.

These results provided the first evidence for a role of the modality-specific cTRN sectors in attentional processes of their respective modalities. This is because despite the stimulation through multiple modalities, only the cTRN sectors associated with the primary exploratory modality (i.e. the presumably attended one) were activated. However, there are alternative interpretations of the results that cannot be ruled out. In order to claim that differential activation is the result of attention specifically, it is necessary that the sensory stimulation received by the compared populations is identical. Given that one group of rats was blind and that another did not experience the novel complex environment, this is not the case for this study. Thus, rather than attributing the inequalities of TRN*vis* and TRN*som* activation to attention, they could be ascribed to differential sensory stimulation. Moreover, the absence of Fos in the TRN*aud* of all animals could be accounted for not by the lack of attention, but, rather, because of the insufficiency of auditory stimulation: the level of auditory stimulation was likely to have been considerably lower than the level of visual and tactile stimulation.

In a follow up experiment, Montero (1999) showed that monocular amblyopic rats exposed to a complex novel environment demonstrated unequal levels of Fos activation in the TRNvis of their two hemispheres. More specifically, greater Fos expression was observed contralaterally to the normal eye, compared to contralaterally to the amblyopic one, despite the identical

stimulation of both eyes, which was reflected by equal Fos activation of their two dLGNs. Thus, in spite of the equal stimulation of both eyes, only the TRNvis corresponding to the non-amblyopic, that is, the presumably attentive, eye became activated. Again however, the strength of this evidence can be challenged because the visual input received by the two hemispheres cannot be assumed to have been equal. Although the retinal stimulation was identical, the quality of the visual signal from the amblyopic eye differed from that of the healthy eye. Consequently, it could not be said with confidence that the observed Fos inequalities in TRNvis reflected differences in attention, rather than in the quality of sensation. Although Montero's approach resulted in strongly suggestive data, nevertheless, both of his studies could not ensure the equality of stimulation between the compared populations. Therefore, this evidence, with regard to cTRN's role in attentional processes, can be regarded as circumstantial, but it does not provide a strong test of the hypothesis that cTRN is involved in attention. A stronger demonstration was attempted by McAlonan, Brown and Bowman (2000). McAlonan *et al*., conditioned rats to either visual or auditory stimulation, by pairing brief light or tone presentations with food. On the test day, the two groups of rats were given simultaneous light and tone presentations paired with food. Even though food was predicted by the compound light/tone stimulus during this session, it was assumed that animals would continue paying attention only to the stimulus they had been previously conditioned to (see "blocking effect", Kamin, 1969). Therefore, unlike Montero's studies, McAlonan *et al*. were able to compare two populations of animals that were attentive to different modalities, but which at the time of testing had received identical stimulation.

Following the animals' sacrifice, McAlonan *et al*. observed an uneven distribution of Fos-labelled cells in the TRNvis and TRNaud of the visual and auditory attentive animals. More specifically, light-attentive animals demonstrated an almost selective TRNvis activation (TRNaud activation was negligible) and tone-attentive animals demonstrated an almost selective activation of TRNaud. Given that the stimulation delivered to the two populations of rats was identical, the possibility that the observed Fos inequalities were due to differences in sensation was ruled out. Nonetheless, it has been argued that these results could be explained by means other than the differential allocation of attention. More specifically it has been argued that the uneven cTRN activation could be the result of differential excitatory effects of the test stimuli due to the differential length of their previous exposure. In other words, the conditioned stimuli that had been repeatedly encountered during training could more strongly excite cTRN cells compared to stimuli that had been experienced only during the final testing session (Jones and Gonzalez-Lima, 2001). This could explain, for example, why visual stimuli excited TRNvis in animals conditioned (pre-exposed) to them but not in animals that were not. This "associative" interpretation of McAlonan *et al.*'s findings remains an unaddressed possibility.

1.4.2. Lesions of cTRN and attention

The above mentioned immunocytochemical studies provided the first experimental behavioural evidence for the involvement of the caudal TRN segments in attentional behaviours. To date, equivalent evidence from lesion studies is limited to only one report. The only behavioural evidence for the

involvement of cTRN in attentional behaviours through lesion techniques arrives from a study by Weese, Phillips and Brown (1999). In this study, Weese *et al*. investigated the effects of unilateral TRNvis lesions on covert attentional orienting using a rodent adaptation of the Posner task (see Posner, 1980). More specifically, they trained rats in a choice reaction time where a nose-poke response was required towards the location (right or left) of a visual target. Preceding each target, a visual cue (brief dim light) would appear in either location. It was found that when the cue and the target appeared at the same location (valid trial), rats were faster to respond compared to trials where the cue was presented at the location opposite to that of the subsequent target (invalid trial). The difference in reaction times between the valid and invalid trials is called the "validity effect". The validity effect is thought to be the result of the attraction of attention to the location of the cue, which either speeds up or slows down the detection of the subsequent target depending on the latter's presentation at, or away, the current location of attention. Weese *et al*. found that after the infliction of unilateral exitotoxic lesions of the dorsal cTRN (TRNvis), the validity effect for responses to contralateral targets was abolished. This was not the case for responses to ipsilateral targets. According to the authors, this demonstrated that the lesion impaired the animals' ability to move covert orienting (attention) within the visual field, an interpretation in line with the idea that cTRN is involved in the creation of attentional hotspots within sensory space (Crick, 1984). However, a potential criticism of this study has to do with the fact that besides TRNvis damage, which was found to be complete, dorsal thalamic damage was not thoroughly examined. As a consequence, the behavioural

effects observed following the lesions could not be attributed, with certainty, to TRNvis damage only.

1.4.3. cTRN electrophysiology in behaving animals

Until recently, no electrophysiological data was available from the cTRN of awake behaving animals performing attentional tasks. McAlonan, Cavanaugh and Wurtz (2006) recorded from the TRNvis of monkeys while performing a cross-modal attention task. Animals were required to fixate in the centre of a visual display while a visual stimulus (a spot of light at a fixed peripheral location of their visual field) was presented simultaneously to an auditory stimulus (a tone). The colour of the fixation point informed the animals to which of the two stimuli they must attend for that particular trial. Either, both, or neither of the two stimuli would then gradually change (dim in intensity). The animals had to report a change in their attended stimulus by performing a saccade (upwards for a dimming light and downwards for a dimming tone), regardless of a change in the non-attended stimulus. This allowed the comparison of neuronal activity between situations where visual stimulation was presented and attended and situations where the same visual stimulation was presented but not attended (auditory stimulation attended instead). It was found that while the latency and duration of visual responses of TRNvis cells remained identical between the attended and not-attended conditions, the amplitude of the visual responses was significantly enhanced in the former scenario. This evidence, in line with the findings of earlier Fos investigations, demonstrated what was already suspected, namely that the activity of

individual cTRN cells increases when attention is directed to stimuli of the modality they represent.

Functionally, the attention-mediated increase of its activity may represent the means by which cTRN could maximise its inhibitory effects upon thalamocortical cells, thus either increasing its influence upon the latter's firing mode or optimising the implementation of lateral inhibition. Which of the two mechanisms is in effect depends on the particular synaptic architecture of the thalamo-reticular loops (open- vs closed) and also on the relative intensity of cTRN's output on thalamocortical cells. For example, assuming a closed-loop thalamo-reticular synaptic architecture, cTRN's hyperpolarisation of its afferent thalamocortical cells would have to be strong enough to activate post-synaptic $GABA_B$ receptors (Dutar *et al*., 1988) and promote I_{TS} de-inactivation, but not too strong, in order to avoid "shutting down" these cells. On the other hand, in an open-loop thalamo-reticular synaptic scenario, cTRN's inhibitory output would have to be of enough intensity to dampen down the relay of information in its post-synaptic thalamocortical cells.

1.5. Aims of present Thesis

Following the early demonstrations of a potential involvement of cTRN in attentional processes (see *1.4.*), it has become necessary to investigate this role in more depth, in behaving animals. The aim of the present thesis is to take the investigation of the functional significance of cTRN a step further and examine its involvement in a broader range of attentional behaviours through the use of functional immunocytochemical (Fos) and lesion techniques,

combined with tests of behaviour. By identifying the attentional processes that cTRN is involved and not involved in, we aim to create a functional profile for this area that would aid our understanding of its functions in thalamocortical circuits.

In chapter *II*, we address the methodological problems of previous investigations that have demonstrated selective immunocytochemical (Fos) activation of cTRN sectors following attentive behaviours to their respective modalities. More specifically, we address the inequality of sensory stimulation (Montero, 1997, 1999) and unevenness of pre-exposure to test stimuli during behavioural training (McAlonan, Brown and Bowman, 2000), which prevented the confident attribution of the observed cTRN activation patterns to attention. In this chapter, we use a behavioural task that ensures both equal sensory stimulation and equal pre-exposure to all test stimuli during training (in addition to equal stimulation during testing), for all compared groups of subjects, and predict that only cells of the cTRN sectors associated with the attended modality would activate, demonstrating a direct link between attention and cTRN activation.

In chapters *III-V* we implement lesioning techniques and investigate the effects of cTRN lesions in the performance of various attentional tasks. Through the use of fine glass micropipettes and of ibotenic acid of carefully selected degree of molarity (toxicity) we plan to inflict selective lesions of the desired cTRN areas (TRNaud and/or TRNvis) with minimal dorsal thalamic damage and with a lesser degree of demyelination of traversing

thalamocortical and corticothalamic fibres compared to lesioning techniques used in the past. This will allow us to attribute any behavioural effects following the lesion(s), specifically, to the lack of cTRN. The choice of behavioural tasks used in these investigations was based on the attentional processes that cTRN is thought to be more likely to be involved in, as determined by the available, albeit limited, information regarding its potential functional significance. We generally hypothesise that cTRN lesions will result in deficits with regard to the performance of the attentional behaviours examined.

More specifically, in chapter *III*, we look at the effects of combined TRNvis and TRNaud lesions on the performance of a cross-modal divided attention task that requires the division of attention between the visual and auditory modalities. The main hypothesis is that following the destruction of TRNvis/aud, animals' performance in the divided attention task will deteriorate, due to an impaired ability to split attention between the two modalities corresponding to the two lesioned cTRN sectors.

In chapter *IV*, we focus on visual attention only, and we seek to investigate the ability of animals to covertly move attention within visual space following the selective bilateral lesioning of TRNvis. We employ a test of endogenous covert orienting that manipulates visuospatial attention on the basis of learnt spatiotemporal probabilities (asymmetric spatial probability task – ASP). Given the previous demonstration of apparently impaired exogenous covert orienting after TRNvis lesions (Weese, Phillips and Brown, 1999), and the apparent

functional dependence of TRNvis on top-down signals arriving from visual cortex (see Montero, 2000), we predict that endogenous covert orienting will also be impaired after TRNvis destruction.

Chapter *V* explores further the effect of selective TRNvis lesions (unilateral this time) on the ability to orient covertly within visual space, using a task that guides attention by means of exogenous spatial cueing (Posner task). As mentioned earlier, this has been investigated in the past by Weese *et al*., but their findings were compromised by uncertainty regarding the selectivity and severity of their lesions. Similarly to their findings, however, we predict that our selective TRNvis lesions will abolish the effect of the attention-grabbing cues thus preventing covert orienting behaviour. Finally, in the same chapter, we also investigate animals' ability to learn the ASP task, and in conjunction with our findings in chapter *IV*, we seek to determine whether the laterality of the TRNvis lesion (bilateral or unilateral) affects differentially performance on this task.

Overall, we will use a multitude of behavioural tasks combined with either functional immunocytochemistry or lesions and the results we obtain will be discussed in relation to the potential role of cTRN in the mediation of attentional behaviours.

Chapter *II.* Enhanced Fos expression in the visual segment of the thalamic reticular nucleus by rats attending to visual versus tactile aspects of the same stimuli

2.1. Abstract

Previous reports have suggested that the modality specific sectors of cTRN may selectively activate as a result of attention being drawn to their respective modalities, but the evidence they provided remains dubious (Montero, 1997, 1999, McAlonan *et al*., 2000). Here we used a task that required the discrimination of digging bowls on the basis of their visual (colour) or tactile (external texture) characteristics. We trained animals to perform both modality discriminations, ensuring the equity of exposure to both visual and tactile aspects of the stimuli. On the final day, animals had to perform only one of the modality discriminations for a 1-hour period prior to being transcardially perfused and their brains removed and processed for Fos immunocytochemistry. We found that animals that performed the visual discrimination prior to sacrifice demonstrated a selective activation of cells in the TRNvis. On the other hand, animals that had performed the tactile discrimination, despite having received equal visual stimulation as the visually discriminatory animals, did not feature TRNvis activation. This evidence suggests that activation of TRNvis does not reflect visual stimulation but it is more specifically reflective of attention being drawn to visual stimulation. The accompanying absence of TRNsom activation in the texture-attentive animals is suggested to be related either to a higher Fos-inducibility threshold for TRNsom cells or to a less significant involvement by TRNsom in the processes underlying the tactile attentive behaviour examined in our task. The latter may be considered as a reminder of the multiple expressions of attention and the potential differential involvement of cTRN in each of these.

2.2. Introduction

The thalamic reticular nucleus (TRN) is a thin layer of GABAergic neurons that surrounds most of the dorsal thalamus. By virtue of its neurochemistry and precise anatomical connections with sensory cortex and dorsal thalamus the caudal half of TRN (cTRN) has been implicated in processes of sensory modulation and thus attention (Yingling and Skinner, 1976; Crick, 1984). Its position and connections make the cTRN a perfect candidate for such a role, but decisive and convincing evidence for this has been elusive. Due to its reticulated shape and particular thinness, it has been extremely difficult for researchers to investigate the nucleus' functions by means of lesion or electrophysiology. Early attempts in this and other labs to lesion the cTRN often resulted in incomplete and/or extending to the dorsal thalamus lesions (Tait, D.S.; Lukoyanov, N.V., *personal communication*).

Due to the difficulty in inducing selective and complete lesions in the cTRN, an alternative way to investigate its potential involvement in attentional behaviours has been the use of Fos immunocytochemistry. Fos is a protein product of the immediate-early gene *c*-fos, and has been used as a marker of neuronal activation (e.g. Dragunow and Robertson, 1987). Studies using the expression of Fos have suggested that particular modality-associated segments of the cTRN become selectively activated when attention is directed to their associated modality (Montero, 1997, 1999). The evidence provided by these studies however, has been regarded equivocal, given that the subjects were not visually "normal" (blind or amblyopic) resulting in possible inequalities in the sensory stimulation they received. As a consequence, it

cannot be said with confidence that the selectivity of activation in the various segments of the cTRN was due to inequalities in attention, as opposed to inequalities in stimulation. Later, McAlonan, Brown and Bowman (2000) used a classical conditioning blocking paradigm (Kamin, 1969) in order to compare cTRN Fos activation levels in visually attentive and auditorily attentive rats. They demonstrated selective activation of either the visual (TRN*vis*) or auditory (TRN*aud*) segment of the cTRN according to the attended modality, this time using healthy, in tact, animals and ensuring the identical audiovisual stimulation of all subjects during the pre-sacrifice test session. However, in order to elicit a blocking effect during testing (i.e. to selectively direct attention to only one of the two modalities), a pre-conditioning stage was required during training in which animals were exposed to only one of the two stimulus modalities. This meant that arriving at the testing session the rats had experienced differential exposure to the test stimuli of the two modalities. This raised the possibility that the subsequent Fos inequalities observed in the TRN*vis* and TRN*aud* following testing may have been due to the differential excitatory effects of these stimuli and not the result of the differential allocation of attention to the two sensory modalities (Jones and Gonzalez-Lima, 2001).

The aim of the present investigation was to demonstrate differential activation of sensory cTRN sectors as a function of differential attention to their corresponding modalities, using a task that would ensure equal sensory stimulation between the compared populations but also equal exposure to the test stimuli, both during training and testing. We trained animals in a task that

required discrimination of food-baited digging bowls according to their visual or tactile properties. We used bowls that differed both in colour and texture, but otherwise were the same shape, size and odour. During training, all animals were trained to perform both a visual discrimination and a tactile discrimination (in separate, counterbalanced in order, blocks), shifting their attention to the currently informative modality and ignoring the other, irrelevant, difference between the bowls. During a final testing session, animals were tested on one of the discriminations for an extended period of time (1 hour) prior to being sacrificed. We expected to demonstrate a selective Fos activation of TRN*vis* in the animals that performed the visual discrimination task (visually attentive) and a selective TRN*som* activation in the animals that performed the tactile discrimination task (tactilely attentive) in the final test session. In contrast to Montero's studies, there was no interference with the normal functioning of the visual or any other sensory system of the animals and as a consequence the quality of sensory stimulation was invariant between experimental groups. Moreover, given that our animals were introduced simultaneously and exposed equally to both the *attended* and the *ignored* dimensions of the test stimuli during training, we intended to rule out the possibility that a potential differential activation of TRN*vis* and TRN*som* could be due to differential excitatory effects of these stimulus dimensions.

2.3. Methods

2.3.1. Animals

Eight, experimentally naïve, male Lister hooded rats (Harlan, UK), weighing 390-450g, were used in the investigation. Rats were pair-housed in a 12-hour

dark/light cycle (lights on at 7am) with *ad libitum* access to water. Animals were initially allowed free access to food (standard laboratory chow), but 2 days prior to training food was restricted to 15-18g a day, per rat.

2.3.2. Materials and Apparatus

Ceramic bowls with a diameter of 7cm and a height of 4cm were used as stimuli. There were four types of bowls, which differed according to their colour and texture: black bowls with glazed smooth surface; black bowls with rough surface; white bowls with glazed smooth surface; and white bowls with rough surface. Rough-surfaced bowls were made by applying a thin layer of glue mixed with coarse sand onto the outside of smooth-surfaced bowls. Subsequently, white or black spray paint was used to give the bowls the desired colour (smooth-surfaced bowls were painted using the same sprays). Despite using odourless glue, and in order to absolutely ensure that bowls with different textures did not differ odour-wise, we applied glue and sand on the inside walls and bottom of the smooth-surfaced bowls. Four bowls of each type were available for use so that the trial-to-trial variation in the use of these minimized the possibility that the rat might use other subtle cues to perform the discrimination. The bowls were filled with fine white sand. Half-pieces of a 'Honey Nut Loop' (Kellogg, Manchester) were used to bait the bowls.

Training and testing took place in a specially designed box, as previously described in Birrell and Brown (2000), (see Figure 2.1). Briefly, the box was a rectangular (40 x 70 x 18cm) plastic box, with sawdust on the floor and a flat lid made of transparent Plexiglass that allowed the monitoring of the animal

inside. The box was partitioned into an area comprising two-thirds of the length of the box, (to which the rats were confined during inter-trial intervals) and an area further sub-divided into two half-widths of the box (within each of which one bowl was placed). The rat was given access to the compartments with the bowls by lifting independently-removable doors, and this signalled the start of each trial. The trial ended when the rat moved to the larger compartment: the rat was never 'ushered' away from the bowls, even after having recovered the bait, but rather the experimenter waited for the rat to return voluntarily to the larger compartment. The bowls were then rebaited through two doors on the top of the box.

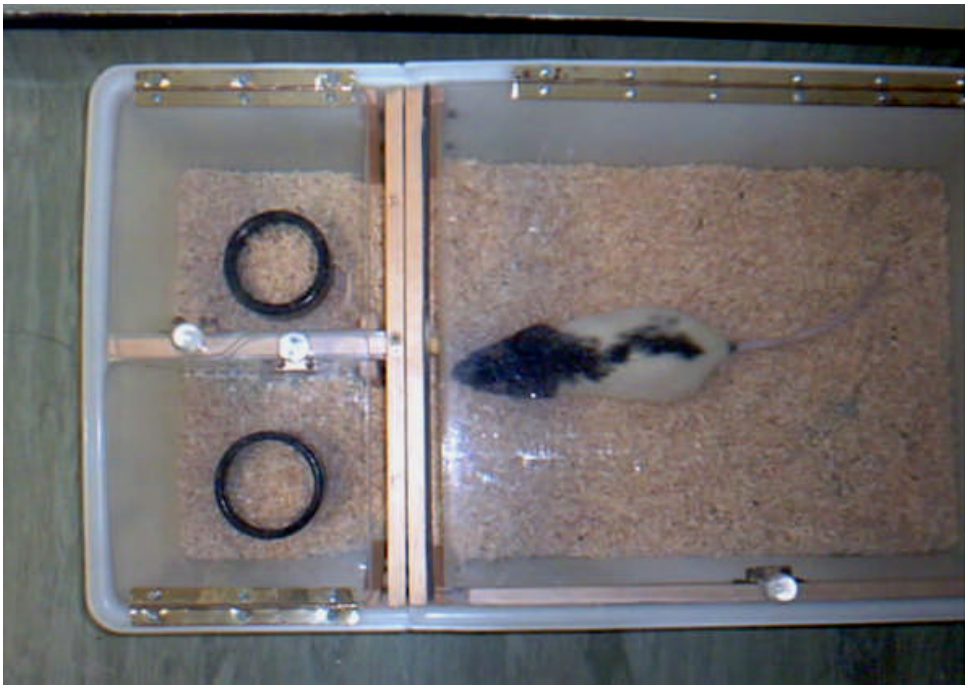


Figure 2.1: The bowl-digging kit used

2.3.3. Training regime

Training involved the performance of a series of visual and tactile bowl discrimination tasks. A visual discrimination task involved the discrimination of digging bowls on the basis of their colour (black or white), while a tactile

discrimination task required the discrimination of bowls' external surface texture (rough or smooth). Animals were assigned pseudo-randomly to two groups (n = 4 in each), each performing discriminations based on a different modality as described below (see also Table 2.2).

The introductory training stage involved the exposure of the animals to the digging bowls with the intent to elicit digging behaviour. Animals were presented with two identical (brown ceramic, filled with white sand) baited bowls and were allowed free access to both. As digging behaviour is natural to rodents, it was elicited rapidly and only 4 trials (8 bowl digs in total) were necessary to establish reliable responding. The two subsequent training stages involved two *simple discriminations* (SD), of colour and texture, with other aspects of the bowls being non-discriminable. The particular colour, or texture, of the baited bowl was counterbalanced across animals within each group. A trial was recorded as correct if the rat refrained from digging in the incorrect (unbaited) bowl, and obtained the reward by digging in the correct (baited) bowl. If a rat dug into the unbaited bowl, the trial was recorded as incorrect. For the initial four trials of each training stage, after incorrectly digging in the unbaited bowl, the rat was given access to the other bowl to recover the bait. Subsequently, the rat was not given access to the baited bowl following an incorrect dig. "Digging" was defined as any attempt to use the forepaws and/or the snout in order to examine the presence of reward underneath the sand surface. Sniffing or simply touching the sand was not considered a "dig". For each animal, the first SD (stage SD1) was followed by a second SD in the other modality, thus requiring a modality shift (stage SD-

MS). At the third stage, compound stimuli were introduced– i.e., the bowls of each pair differed in both colour and texture. There were two further modality shifts within this *compound discrimination* (CD) context: the first (stage CD-MS1) was back to the originally rewarded modality of SD1 but with a different rewarded exemplar (i.e. if black bowls were rewarded in SD1, white bowls were rewarded in CD-MS1), and the second (stage CD-MS2) was back to the rewarded modality of SD-MS (similarly, with a different rewarded exemplar). In that way all animals responded at some point of training to each of the four exemplars. We need to note that for some, randomly selected, trials after the achievement of performance criterion both bowls were baited in order to examine the possibility that animals were performing the discriminations by being able to smell the reward through the sand. Animals did not dig in the incorrect - yet baited - bowl, in no single trial, thus demonstrating an inability to smell the bait and/or a focus on the discrimination task at hand.

We know from previous investigations (e.g. Birrell and Brown, 2000) that, healthy rats typically require fewer than 20 trials, in a training stage, in order to reach a level of discrimination performance that is below chance (i.e., in order to perform 6 consecutive correct trials, the probability of which to happen by chance is less than 0.016). This is the case even when they must switch their attention (either within the same dimension, as in reversal learning, or from one dimension to another). However, here it was important to ensure that exposure to each stimulus was absolutely equal between animals and experimental groups. Therefore, all animals performed 30 trials

at each stage of training, regardless of how early on they achieved below-chance performance indicative of learning.

All training stages and the final testing session took place in a dimly illuminated room (~5 Lux). All training stages (i.e., two simple discriminations – one of each modality – and two compound discriminations – again, one of each modality) took place on a single day. Testing was conducted the following day.

	Group 1 (Visual)		Group 2 (Tactile)	
	Attended feature	Irrelevant feature	Attended feature	Irrelevant feature
Day 1				
Intro	**-**	-	**-**	-
SD1	**Texture**	-	**Colour**	-
SD-MS	**Colour**	-	**Texture**	-
CD-MS	**Texture**	Colour	**Colour**	Texture
CD-MS2	**Colour**	Texture	**Texture**	Colour
Day 2				
Test	**Colour**	Texture	**Texture**	Colour

Table 2.2: Order of discriminations, showing the attended and irrelevant stimulus features (modalities) for each of the two experimental groups.

2.3.4. Behavioural Testing

The day following training, animals were tested in a final session wherein they were required to perform the same discrimination task as they had in the final training stage (CD-MS2), the previous day. Testing consisted of 120 trials and lasted no fewer than 60 and no more than 75 minutes. Rats were tested in pairs. Each pair consisted of one visually and one tactilely attentive animal tested simultaneously in two separate boxes, with trials alternating between

rats, giving mean inter-trial intervals of just under 30 secs, with a range of about 10 secs to about 2 minutes. Testing started approximately 7 hours into the dark circle (~2:00 a.m.) meaning that the rats had not been exposed to light for 7 hours prior testing. After the end of the testing session, rats were left in the room with the lights off for 1 hour and 15 minutes, before being transcardially perfused with 4% paraformaldyhyde in 0.1M phosphate buffer, and their brains removed and stored in 20% sucrose solution.

2.3.5. Behavioural data analysis

The number of trials required to reach below-chance performance (i.e., 6 consecutive correct trials), as well as the total number of error trials, was recorded for each training stage and a repeated measures analysis of variance was carried out (SPSS v.12), with *discrimination type* (simple *vs* compound), and *discrimination modality* (visual *vs* tactile) as within subjects factors and *experimental group* as a between subjects factor (Group 1: visually attentive in the testing session *vs* Group 2: tactilely attentive during the testing session). An independent groups t-test was also carried out to compare performance (% correct trials) in the testing session between the two groups of animals.

2.3.6. Immunocytochemical procedures

Approximately 36 hours following perfusion, 40µm coronal sections were cut through the thalamus (approximately between bregma –1.10 and bregma –5.00) using a freezing microtome. The sections were then processed for *c*-fos protein immunoreactivity. The sections were treated with 0.9% PBS before

blocked in blocking solution and then incubated in *c*-fos antibody at 1 in 20.000 over two nights. Following incubation in vector IgG solution, sections were put in avidin-biotin complex and then incubated in DAB substrate. Following mounting onto gelatinised slides, sections were lightly stained with cresyl violet.

Brain sections were examined using light microscopy (at x250 magnification) and Fos immunoreactive cells in the cTRN were identified (by the dark black appearance of their somata) and counted. More specifically, we looked for labelled cells in the dorsal 3/5^{ths} of the nucleus from about –2.7 to –4.0 from bregma, where the TRN*vis* is located (Shosaku, Kayama, Sumitomo 1984; Coleman and Mitrofanis, 1996•), and also at the whole dorsoventral extent of the nucleus from about –1.5 to –2.7 from bregma; the TRN*som* (Soshaku, Kayama, and Sumitomo, 1984). Particular care was taken in order for labeled cells of the, adjoining to the dorsal cTRN, ventral segment of the lateral geniculate nucleus (vLGN) not to be included in the count. Finally, labeled cells were also counted in the dorsal segment of the lateral geniculate nucleus (dLGN) (from –3.6 to –5.0 from bregma) and from the ventrobasal complex (VB) (from –2.5 to –4.4 from bregma) including both the ventroposterior lateral (VPL) and ventroposterior medial (VPM) nuclei. Due to the large number of Fos immunoreactive cells in the dLGN, two counts (by two independent observers) were performed for each section. If the difference between the two counts was less than 10%, then the first count was used. If the difference

• Co-ordinates were deducted, with the help of the stereoscopic atlas of Paxinos and Watson (1998), from the photomicrographs in Coleman et al., (1996) illustrating retrogradely and anterogradely labelled cTRN cells, following dLGN, V1 and V2 injections of biotinylated dextran.

exceeded this limit then two more counts were performed, which typically satisfied the above criterion. Separate independent samples t-tests were performed to compare the levels of Fos activation in dLGN and VB of the two experimental groups.

2.4. Results

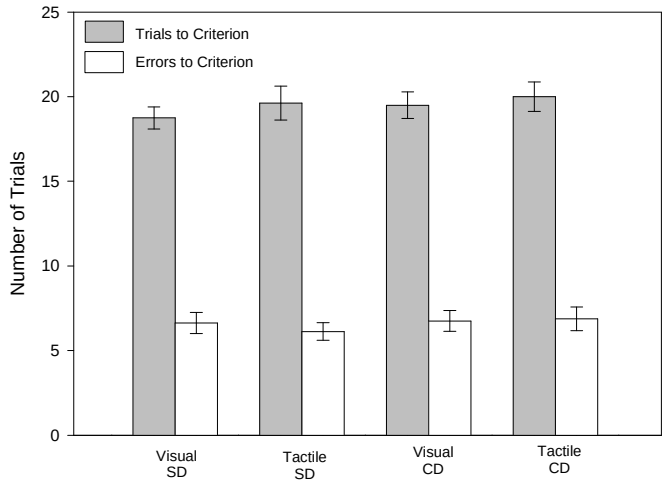
2.4.1. Behavioural Results

2.4.1.1.Training

All rats reached behavioural criterion within 30 trials for each of the four training stages. Behavioural performance in the visual and tactile discrimination tasks was highly comparable across stages and between groups (see Figure 2.3A). Rats demonstrated an equal ability to switch attention from the visual aspects of the stimuli to their tactile aspects and vice versa (no main effect of modality for trials to criterion, $F(1,6)=3.7$, $p>.05$, and errors to criterion, $F(1,6)=.25$, $p>.05$). Moreover, and perhaps somehow surprisingly, performance during the simple discriminations was comparable to performance during compound discrimination tasks (no main effect of type of discrimination for trials to criterion, $F(1,6)=.72$, $p>.05$, and errors to criterion, $F(1,6)=.93$, $p>.05$). Finally, the different order that the discrimination tasks of the two modalities were performed did not have an effect on performance, (no main effect of group for trials to criterion, $F(1,6)=.52$, $p>.05$, and errors to criterion, $F(1,6)=0$, $p>.05$), and also lack of group by modality interaction, for both trials to criterion, $F(1,6)=3.27$, $p>.05$, and errors to criterion, $F(1,6)=1$, $p>.05$). Overall these results suggest that the two tasks (and/or the shift of attention from one to the other) were comparable in

difficulty, and also that the two groups of animals directed attention to the relevant modalities at equivalent points within each training stage.

2.3A



2.3B

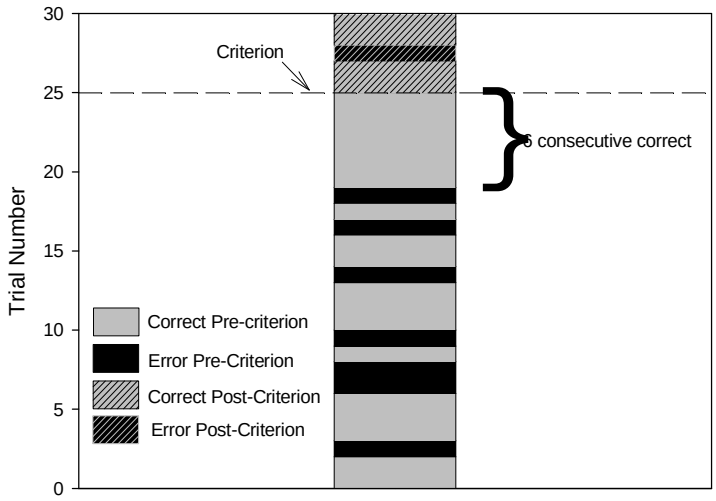


Figure 2.3.A: Mean number of trials performed before reaching criterion (grey bars) and mean number of erroneous trials prior to criterion (white bars) for the SDs and CDs of both modalities. Error bars represent SEM. B: Example of trial-by-trial performance in a training stage.

2.4.1.2. Testing

During the final testing session, and despite the strict criteria used for defining a “dig” (see above), rats rarely dug in the incorrect bowl. Every individual rat’s performance consisted of 97% or more correct trials. The performance of the two groups in the final testing session was highly comparable t(7)=.37, *p*>.05.

2.4.2. Fos expression in TRNvis and TRNsom

A selective activation of TRN*vis* was observed in visually attentive animals (see Figures 2.4 and 2.5A), while TRN*vis* was completely devoid of Fos immunoreactive cells in tactilely attentive animals (see Figure 2.5B). Surprisingly, there were no Fos immunoreactive cells in the TRN*som* of either experimental group (Figures 2.5C, 2.5D).

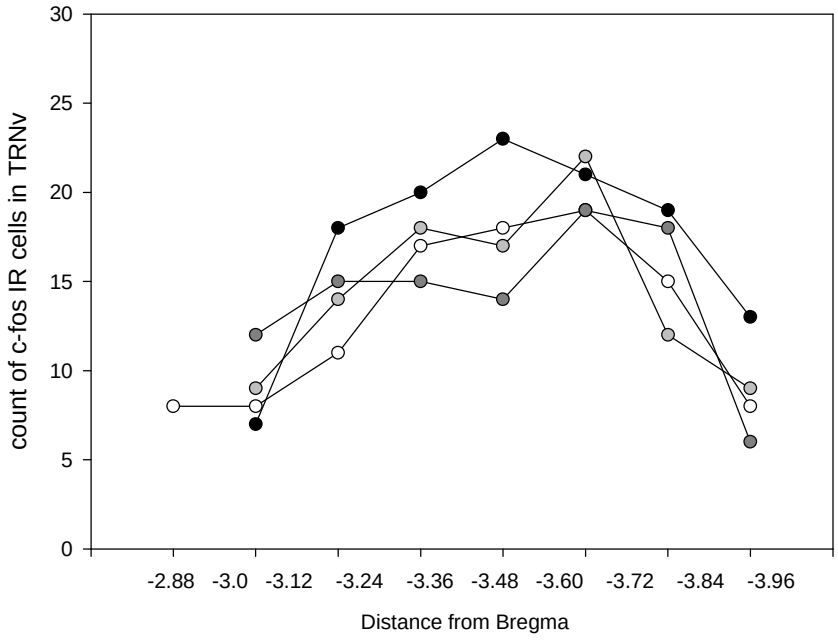


Figure 2.4: Count of Fos immunoreactive (IR) cells in the TRNvis of each visually attentive rat, at different distances from bregma.

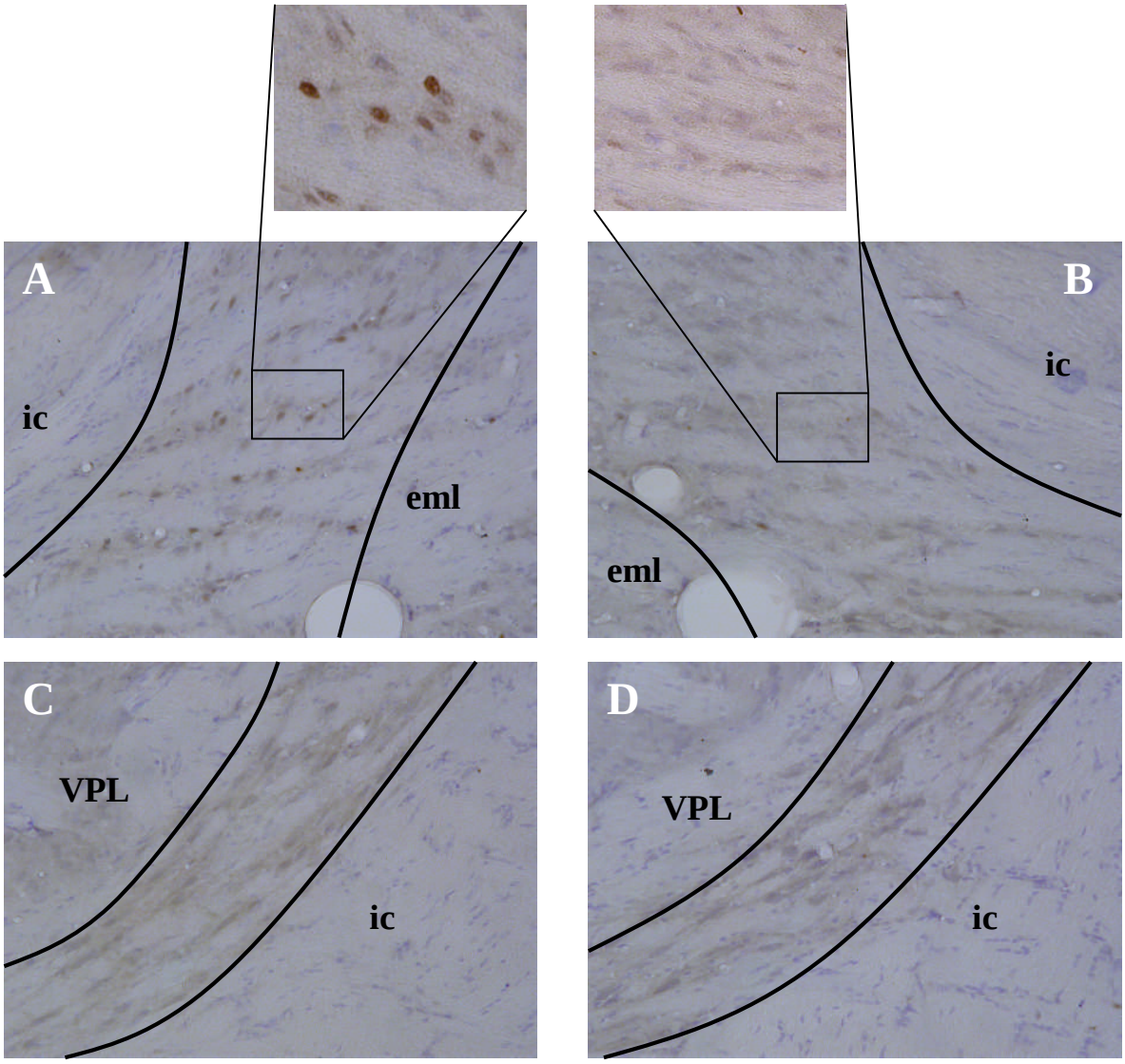


Figure 2.5: Microphotographs of Fos-processed coronal sections through the TRNvis and TRNsom of visually and tactilely attentive animals (x160 magnification). A & C: TRNvis and TRNsom, respectively, of a colour-attentive animal. The dark-labeled cell somata in TRNvis represent Fos immunoreactive cells. Notice the absence of such cells in TRNsom. B & D: TRNvis and TRNsom, respectively, of a texture-attentive animal. No Fos immunoreactive cells are evident in either area. Insets in A and B represent selected areas, magnified at x250, the magnification level at which cell counting took place. (abbreviations: ic: internal capsule, eml: external medullary lamina, vpl: ventroposterolateral nucleus)

2.4.3. Fos expression in dLGN and VB

Both groups of animals illustrated relatively rich Fos activation levels in dLGN (see Figure 2.6), which however did not differ from one another t(7)=1.02, *p*>.05. In contrast, Fos activation in the VB was poor and was confined mainly in the VPM, whereas the VPL was, by and large, devoid of Fos immunoreactive cells in both groups (see Figure 2.6). The activation levels of VB for the two groups of rats were comparable, t(7)=2.27, *p*>.05.

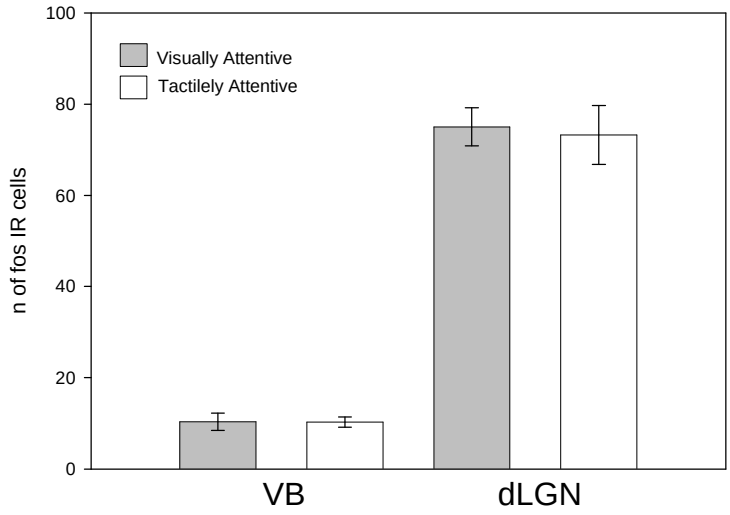


Figure 2.6: Mean counts (and SEM) of Fos immunoreactive cells in the VB and dLGN, for visually and tactilely attentive animals.

2.5. Discussion

2.5.1. Selective TRNvis activation in visually attentive animals

We compared Fos activation in the TRN*vis* and TRN*som* of rats that performed a visual or a tactile bowl discrimination task. Rats that used visual information to discriminate between the bowls illustrated a selective Fos activation of their TRN*vis,* which was not seen in rats that performed the discrimination on the basis of tactile information. On the other hand however, no difference was evident between the two groups with regard to TRN*som*

Fos activation levels, as no Fos immunoreactive cells were found in that area in either group.

During the testing session and prior to sacrifice, both experimental groups of rats were presented with similar series of stimuli, under identical environmental conditions, and for an equivalent number of trials. The only differentiating aspect between the animals of the two groups therefore was that they were attentive to different properties of the test stimuli at the time of testing. Whereas one group attended to the colour of the bowls in order to discriminate between the baited and unbaited, the other group was required to attend to the covering texture of the bowls. We believe, therefore, that the marked Fos activation of TRN*vis* in colour-attentive animals reflected the involvement of that area in processes of visual attention and did not therefore reflect prior history of exposure, or differential exposure, to the attended stimulus.

2.5.2. Equality of exposure and stimulation

Our results resemble those of Montero, (1997, 1999) and McAlonan *et al.* (2000) in that they illustrate selective activation of TRN*vis* in animals that performed a visual attention-demanding task. Unlike the above investigations however, our results cannot be accounted for by sensory abnormality or differential exposure to the test stimuli. Firstly, in contrast to Montero's investigations in which he used blind and amblyopic animals respectively, we used healthy animals with no sensory impairments. This ensured that the two groups had the same sensory means to experience the available visual and

tactile stimulations. Indeed, the equal levels of dLGN and VB Fos activation in the two groups of animals support the equity of visual and tactile stimulation across groups. Furthermore, contrary to McAlonan *et al.*, in our task there was no differential exposure to the two stimulus properties of interest (colour and texture of the bowls). Both visual and tactile stimulation was available for every trial on the final test day, regardless of the task being performed, and had been equally exposed on the training day with both groups responding to each of colour and texture for 60 trials (30 simple discriminations and 30 compound) and having each colour and texture being irrelevant for 30 trials (compound discriminations). Finally, and perhaps most importantly, behavioural performance in the two tasks during training was equivalent for both groups, as the number of trials to criterion and the overall error rate was not different for the two discrimination modalities at any training stage for either group. This suggested that the two tasks were comparable in difficulty and therefore also potentially comparable in the attentional load required in their performance. As a consequence, having ensured the equity of stimulation and exposure across groups with regard to the two stimulus dimensions, we are more confident that the selective TRN*vis* activation we report here reflects visual attentional processes rather than differential sensory effects.

2.5.3. Lack of TRNsom activation

In contrast to the excitatory effects of visual attention on TRN*vis*, and contrary to our expectations, we found that attention to the tactile features of the test stimuli did not induce an equivalent selective Fos activation in TRN*som*. One

potential reason for this lack of a double dissociation may be related to the fact that even though both the visual and the tactile discrimination tasks required attention to be directed to their respective modalities, the precise attentional behaviours required within each of them were not analogous, thus requiring differential involvement by the cTRN segments of their respective modalities. This possibility will be discussed later with regard to the specific ways that cTRN could be involved in sensory and attentional processes.

In an alternative, or complementary, scenario, the lack of TRN*som* activation could be related to immunocytochemical rather than behavioural factors. More specifically, in addition to the complete lack of TRN*som* Fos activation, we also observed extremely low Fos activation levels in the VB, despite the continuous tactile stimulation that the animals received. It is possible therefore that the lack of TRN*som* Fos activation was fully or partially due to an overall uncharacteristic poor Fos induction in cells of the somatosensory thalamic pathways. Indeed, previous studies that looked at Fos activation in somatosensory areas of dorsal thalamus, have reported similarly low Fos activation levels in tactilely active animals (Bullitt, 1990; Pertovaara, Bravo and Herdegen, 1992). As a consequence, a potential involvement of TRN*som* in the tactilely attentive behaviours of our task may have not been possible to detect through Fos immunocytochemistry. The only study yet to demonstrate Fos expression in the TRN*som* (Montero, 1997) has been carried out on young, blind, animals. As the blindness was inflicted at a relatively early developmental stage in these animals (2 months old), it is possible that the Fos activation observed in their TRN*som* was due to plasticity mechanisms

that increased the sensitivity of TRN*som* cells (as well as of cells elsewhere in the somatosensory pathways) to somatosensory input.

2.5.4. cTRN's potential involvement in attentive processes

As mentioned in the general introduction, our knowledge of the precise functions of the thalamoreticulocortical circuit is surprisingly limited. As a consequence, the exact ways cTRN could be involved in sensory or attentive processes remain poorly understood. Nonetheless, one of the most plausible functional roles within thalamocortical sensory systems for the cTRN is one of firing mode control in thalamocortical relay cells. Thalamocortical relay cells typically illustrate two types of firing: *tonic* and *burst (*Jahnsen and Llinas, 1984; Sherman and Guillery, 2001). Tonic firing relays sensory information in a linear fashion, thus more accurately, whereas burst firing transmits input with a much better signal-to-noise ratio, ideal for the detection of abrupt changes in the sensory environment or the sensory transmission under "noisy" conditions (Guido and Wayand, 1995; Wayand, Boudreaux, and Guido, 2001). What determines the firing mode of a relay cell is the potential of its membrane. A slightly hyperpolarized cell membrane (~ -65mV) lasting for more than 100ms contributes to burst firing, whereas a slightly depolarised one (~ -55mV or more positive) of the same duration, to tonic. The inhibitory cTRN innervation onto, and its subsequent withdrawal from, thalamocortical relay cells is thought to provide the latter with the necessary hyperpolarisation and depolarisation respectively for switching their firing between the two modes (French, Sefton and Mackay-Sim, 1985; Wang, Bickford, van Horn, Erişir, Godwin and Sherman, 2001). Having in mind the different sensory

transmission properties of the two firing modes, it becomes apparent that cTRN may be in the privileged position of controlling the thalamocortical relay of sensory information according to the immediate attentional demands. Of course, cTRN is not expected to do so autonomously. Its output on thalamocortical cells is thought to be dictated by its multiple inputs coming from a diversity of sources in cortex, dorsal thalamus and brainstem (Montero, 2000; Sherman and Guillery, 2001).

In the present case, therefore, the enhanced Fos activation seen in the TRN*vis* of visually attentive animals may reflect the inhibitory innervation of its cells upon the cells of the visual thalamocortical relay so that the latter fire in burst mode. Even though our visual task was one of visual discrimination and was therefore dependent on the *analysis* of the stimuli (better carried out by tonic firing) it has to be noted that it was carried out under low lighting conditions that may have favoured burst firing. More specifically, given its better signal-to-noise ratio, burst firing may have been necessary during the performance of the visual task in order to optimise signal transmission in the dim lighting conditions under which it was carried out. On the other hand, the attentive behaviour required by the tactile discrimination task may have not been reliant on burst activity, as it involved the analysis of texture under conditions unaffected by the luminance levels. This could explain why no activation was observed in TRN*som* of the tactilely attentive animals. Notwithstanding the potential differences in the induction of Fos in visual and somatosensory pathways, the lack of a double dissociation in our findings may, therefore, imply that the various sensory sectors of cTRN become

involved, and thus activated, only during some particular forms of attention within their respective modalities. Attention represents a wide range of diverse behavioural process and as a consequence the involvement of cTRN may differ amongst its various expressions.

Chapter *III.* Selective lesions of the rat visual thalamic reticular nucleus do not affect performance in a non-spatial, cross-modal, divided attention task

3.1. Abstract

Certain anatomical features suggest that cTRN may be functionally related to processes of cross-modal division of attention. The proximity between, and the occasional overalp of, its constituent sensory sectors, in addition to its innervation by collaterals of the cortically-projecting, cholinergic, basal forebrain fibres (known to be involved in divided attention processes, see Turchi *et al.,* 1997), postulates that cTRN may represent a functional branch of the neural mechanism underlying cross-modal divided attention. We trained animals in a visual and an auditory discrimination task using an operant paradigm. During testing, rats received blocks of either visual or auditory stimuli (unimodal condition), or blocks where the modality of the stimuli was unpredictable from trial to trial (bimodal condition). Reaction times to stimuli were faster during unimodal blocks compared to bimodal blocks. This reaction time difference is assumed to reflect the cost of dividing attention between the two modalities. Following combined lesions of TRNvis and TRNaud we did not observe any behavioural detriments related to the animals' ability to divide attention. However, animals manifested a decrease in their response accuracy, both in the divided and the undivided attention conditions, an effect potentially reflective of an overall poorer discriminatory ability. These results suggest that cTRN may not be involved in the generation of divided attention behaviours but that it may, nonetheless, be involved in processes of sensory processing enhancement within some forms of attention.

3.2. Introduction

In the previous chapter we demonstrated that attention to visual aspects of a stimulus elicits Fos activation in TRN*vis* cells. This suggested that TRN*vis* may be involved in processes related to visual attention. The same is likely to be the case for other sensory sectors within caudal TRN (cTRN) but the available evidence regarding these is limited (see Montero, 1997; McAlonan *et al*., 2000). Even though cTRN appears to be involved in some attentional processes, it is unlikely that its role in attention is one of a general attentional filter or sensory enhancer that contributes to all forms of attention. In order to better appreciate the involvement of cTRN in attention we need to look at the nucleus' role in a broader range of attentional behaviours. By determining what processes the cTRN is involved in (or not involved in), we could potentially understand the nature of the region's precise undertaking in attentional mechanisms.

To date, investigations on the functions of cTRN have focused primarily on its potential role in attentional behaviours within a single modality. For instance, Weese *et al.* (1999) demonstrated deficits in visual covert orienting following TRN*vis* lesions in rats. Furthermore, Montero provided evidence, albeit ambiguous, suggesting a TRN*vis* involvement in visual exploratory behaviours (Montero, 1997, 1999) and a TRN*som* involvement in tactile exploratory behaviours (Montero, 1997). Similarly, McAlonan *et al.* (2000) showed activation of TRN*vis* and TRN*aud* following visual and auditory attentive behaviours respectively. Interestingly, however, McAlonan *et al.* also showed that when attention is directed to both modalities simultaneously then both

TRN*vis* and TRN*aud* become activated. Although not touched upon at the time, this observation is suggestive of a potential cTRN involvement in the cross-modal division of attention. The possibility of cTRN being involved in attentional processes that require the simultaneous monitoring of two or more informational channels (e.g. modalities) is something that has not yet been looked upon.

Cross-modal divided attention is known to deteriorate following the administration of muscarinic receptor antagonists or the infliction of cholinergic lesions in the corticopetal neurons of basal forebrain (McGaughy, Turchi and Sarter, 1994; Turchi and Sarter, 1997). This suggests that the normal functioning of cross-modal divided attention is dependent on cortical cholinergic innervation from basal forebrain. TRN (mainly rostral TRN but also cTRN) also receives cholinergic input from basal forebrain and more importantly from collaterals of its corticopetal fibres (Jourdain, Semba and Fibiger, 1989). This, combined with the close anatomical and functional relationship of the sensory sectors of cTRN with their corresponding sensory areas of the dorsal thalamus, raises the possibility that the divided attention deficits observed following basal forebrain damage may be, to a degree, also attributable to abnormal cTRN function. To add to this possibility, the existence of dually-responsive areas at the borders of the various sensory cTRN sectors (Sugitani, 1979, Pollin and Rokyta, 1982; Stehberg, Acuña-Goycolea, Ceric and Torrealba, 2001, also see Sherman and Guillery, 1996) suggests that there might be an interplay of cross-modal activity within the nucleus (something that does not happen anywhere else in the thalamus at

such an early stage), which could be associated with cross-modal expressions of attention.

In the present study, therefore, we decided to investigate the potential role of cTRN in non-spatial, cross-modal, divided attention behaviours. More specifically, we attempted to investigate the effects of excitotoxic lesions of the auditory and visual segments of the TRN (TRN*aud* and TRN*vis*, respectively) on the performance of an audiovisual divided attention task.

As mentioned earlier in this thesis (see *1.4.*), a major obstacle in the behavioural investigation of cTRN's functions has been the difficulty to induce selective and/or complete lesions. In the only known attempt to induce such lesions, Weese *et al.* (1999) were highly successful at destroying the entirety of TRNvis but failed to examine thoroughly the extent of dorsal thalamic damage, which as a result remained unknown. The problem in creating complete and selective lesions in rodent cTRN is related not only to its relative thinness but also to its curved, in all orientations, shape (see *1.2.1.* in *general introduction*). In order to tackle this problem we decided to attempt the use of fine glass micropipettes for the injection of neurotoxins in cTRN. More specifically, we manufactured custom-made micropipettes with a tip less than 40 microns across, which could release neurotoxins with high spatial specificity and with minimal spread. Similar micropipettes have been used previously for the microiontophoretic injection of tracers (Zahm, Jensen, Williams and Martin, 1999; Zahm, Williams, Latimer and Winn, 2001) but also for the injection of neurotoxins in lesion challenging structures such as the

nucleus accumbens (Chapman and Zahm, 1996; Alderson, Parkinson, Robbins, Everitt, 2001) and the laterodorsal tegmental nucleus (Alderson, Latimer and Winn, 2005), with great success.

Divided attention is believed to work in two possible ways, namely either through the simultaneous monitoring of multiple informational channels (parallel processing theory) or through the rapid and continuous switch of attention between informational channels (serial processing theory) (see Treisman, 1960 and Deutsch and Deutsch, 1963). Compared to undivided expressions of attention, divided attention leads to behavioural detriments related to the speed of response to stimulation (Bonnel and Hafter, 1998). These behavioural costs of divided attention are thought to be either due to the limited cognitive resources to deal with the multiple simultaneous monitoring or due to the limited speed with which the continuous serial switch of attention could take place (Bonnel *et al*., 1998; Parasuraman, 1998).

The task we used to measure divided attention was a modification of a divided attention task originally developed by McGaughy, Turchi and Sarter (1994). The purpose of the task was to assess the animals' ability to monitor two modality channels (visual and auditory) for the detection and identification of a stimulus that was equally likely in either modality (bimodal condition). Performance in this task was compared to performance in the visual and auditory tasks when these were performed on their own (unimodal condition). The difference in performance between the bimodal and unimodal tasks represented the measure of divided attention. We wanted to determine

whether combined TRN*vis* and TRN*aud* lesions would result in deficits with regard to the division of attention between the two modality channels. We decided to lesion both cTRN areas in order to ensure that the bimodally-responsive area at their borders (which may be central in a cross-modal divided attention mechanism) was also lesioned. We therefore compared the measure of divided attention (i.e. the behavioural costs of divided attention) of TRN*vis/aud*-lesioned rats against that of surgical control rats. Based on the available evidence suggesting a cTRN role in some forms of selective attention (Weese *et al*, 1999; McAlonan *et al*, 2000) and also the theoretical likelihood for its involvement in divided attention, we predicted that TRN*vis*/*aud* lesions would result in a slowed ability to respond to the test stimuli during the unimodal condition, reflecting a selective attention deficit, and in a disproportionaly greater slowing in responding to the test stimuli in the divided attention context, reflecting a decreased ability to cope with the monitoring of two modality channels.

3.3. Methods

3.3.1. Animals

Twenty-four adult male Lister-hooded rats (Harlan, UK) approximately 3 months of age on starting the experiment were used in this study. During the training and testing periods, animals were kept under a restricted diet with access to 15-18g of standard laboratory chow per day in addition to sucrose pellets earned during the training sessions (~5g, per day). Water was available *ad libitum*. Animals were kept in a 12 hours light/dark cycle with lights off at 7:00pm. They were pair-housed in 25x45x15cm solid-base plastic

cages with wire-mesh ceiling, in a temperature and humidity controlled room. All procedures used in this study were performed under licence in accordance with the UK Animals (Scientific Procedures) Act 1986.

3.3.2. Apparatus

Behavioural training and testing was carried out in eight standard 9-hole operant chambers (CeNes Ltd., Cambridge, U.K., see Figure 3.1). The chambers were situated within sound attenuated cubicles supplied with fans. A 9-hole array was situated at the rear wall of each chamber. For the purpose of the present experiment only the central 3 holes of the 9-hole array were used. A light bulb was situated deep inside each hole. Behaviour (nose pokes and withdrawals) was recorded through infra-red beams at the entrance of each hole. A food magazine, accessible via a plastic flap, was situated on the wall opposite the 9-hole array. The floor of the chamber was comprised of a metal grid. A speaker and a 6V, 3W, house light were situated on the ceiling of the chamber. An automatic pellet-dispenser delivered sucrose pellets (Noyes Precision Pellets, PJAI-0045, Formula A/I, Noyes, New Jersey) into the food magazine following correct responses. A PC486 computer equipped with a SPIDER extension for online control (Paul Fray Ltd, Cambridge, UK) controlled the equipment and recorded responses.

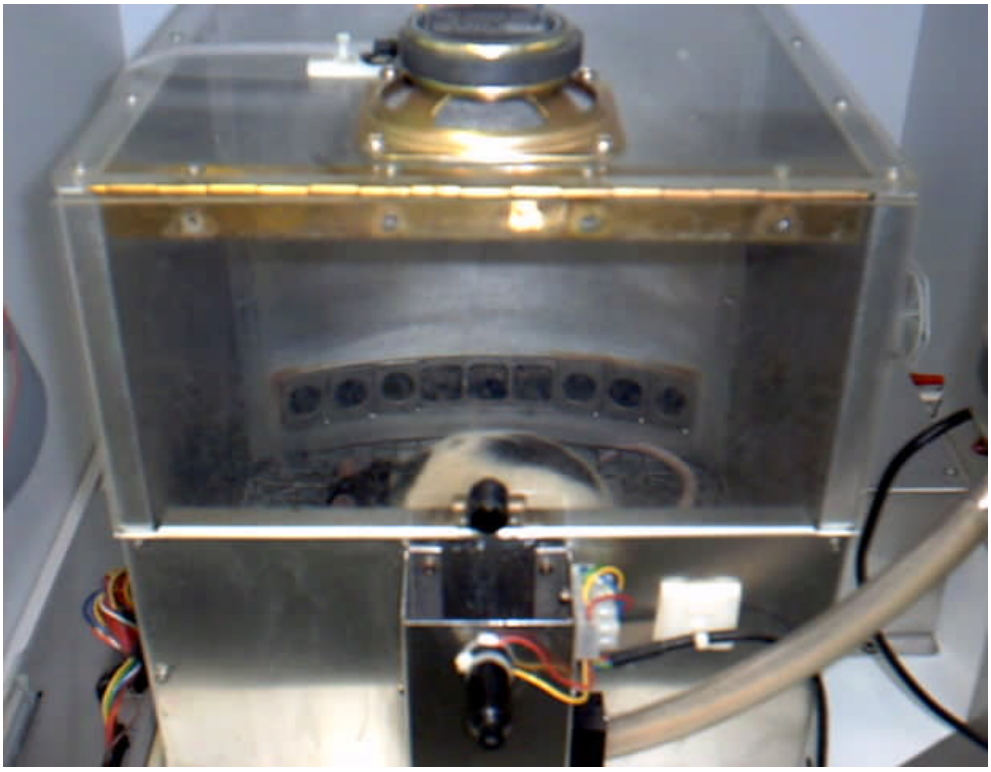


Figure 3.1. Photograph of the 9-hole box chamber

3.3.3. Behavioural Training Regimen

Rats had to go through several stages of training before the final testing protocol could commence. The first stage of training involved the habituation with the operant chambers. Rats were introduced to the chambers, the food trays of which were filled with 20-25 sucrose pellets, in order to accustom the animals with pushing the panel to gain access to the food. During the second stage of training, rats were trained to make sustained nose pokes for a standard delay of 0.2 seconds in the central hole of the array when illuminated (the remaining 8 holes were covered). After each successful nose poke, the light in the central hole was switched off and a sucrose pellet was delivered in the, now illuminated, tray behind the animal. After the animal made a panel push to gain access to the pellet, the tray lamp was switched off and the one

in the central hole was switched on again, indicating the beginning of a new trial. Early withdrawals from the central hole (i.e. before the lapse of the 0.2 second foreperiod) were penalised with a 2-seconds timeout during which all lights in the chamber were kept switched off. When all animals demonstrated a reliable ability to sustain successful nose-pokes for more than 70% of the trials per training session, they were moved to the next training stage.

The third training stage involved a brightness discrimination task (see Figure 3.2A). After each successful central nose-poke, a pair of lights was presented for a 1.5-second period in the two (left and right) side holes, which were now open. On the basis of the brightness of these lights rats were required to make a response by poking either to the left or right hole. More specifically, a nose-poke to the right hole was required when the lights were bright, whereas a left nose-poke was required when the lights were dim. A sucrose pellet was delivered in the tray after the completion of a correct trial. Parallel[*] to the brightness discrimination task, all rats were also trained in a tone discrimination task (see Figure 3.2B). For this task, rats were required to make a left nose poke when presented with a continuous tone and a right nose poke when presented with an intermitted tone (both presented for a 1.5-second period, following a successful central nose-poke). Initially, the visual and auditory discrimination tasks were carried out in separate, *unimodal*,

[*] It has to be noted however that auditory discrimination training started 2 weeks after training for the visual discrimination task started. The reason we chose not to introduce the two tasks simultaneously was to avoid overwhelming the animals. The abrupt introduction of two difficult tasks could have resulted in a reluctance on the animals' behalf in performing them. We chose to introduce the visual discrimination task first due to the fact that the visual stimuli were presented inside the response holes and would therefore establish a stimulus-response association more readily than the auditory stimuli. Once this association was established for the visual task, animals would more easily adopt it for the auditory task too.

blocks of 120 trials. That meant that animals were trained in the visual and auditory tasks on alternating days (visual-auditory-visual-auditory, etc). When animals reached, on average, a stable performance of more than 70% accuracy for light discrimination blocks and 60% for tone discrimination blocks, they moved to the final stage of training which involved blocks of randomly alternating visual and auditory trials (*bimodal* blocks). More specifically, within a bimodal block the modality of a given trial was randomly determined and thus unpredictable. This final manipulation intended to "divide" rats' attention between the two modalities in order to detect the stimulus as quickly and efficiently as possible. When performance was stable and accuracy was at least 70% for the visual and 60% for the auditory discrimination trials, in both the unimodal and bimodal condition, rats proceeded to surgery. For all training stages, a session was terminated after 120 successful trials or otherwise after 30 minutes. Training was carried out daily, during the light cycle. Training lasted 21 weeks in total.

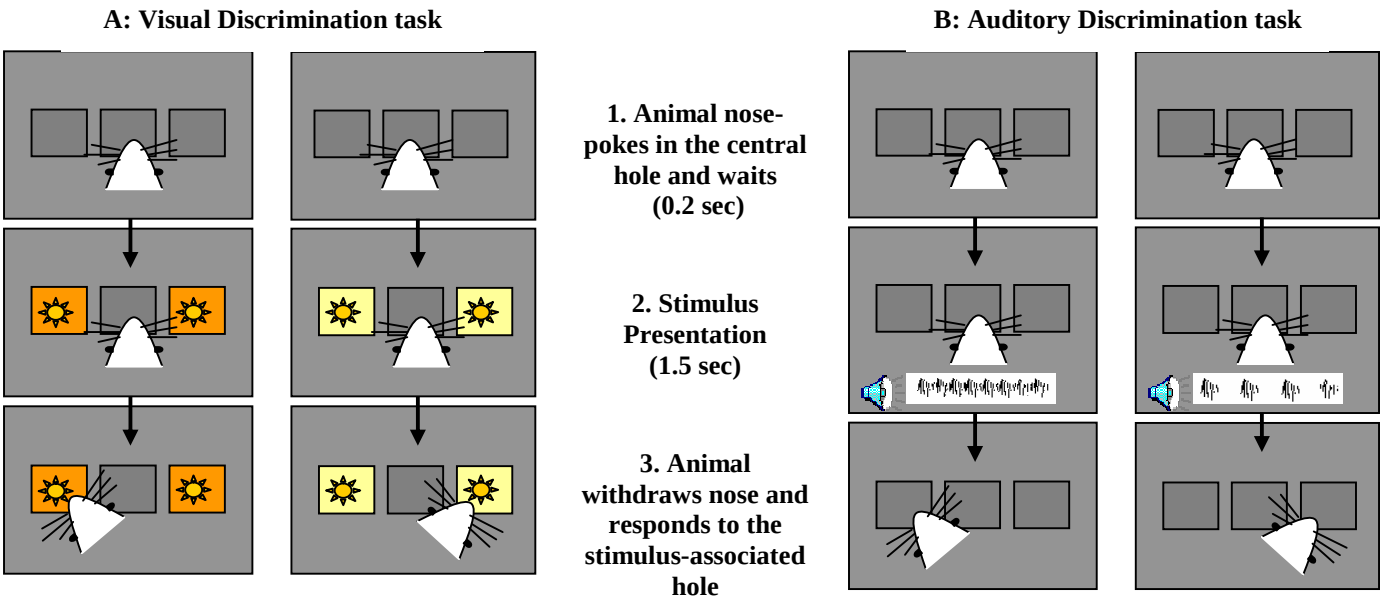


Figure 3.2. Graphic representation of the visual (A) and auditory (B) discrimination tasks

3.3.4. Surgery

Surgery was performed under isoflurane anaesthesia (induction: 5%, maintenance 2%). Rats were placed in a stereoscopic frame (Kopf, Tujunga, CA) with atraumatic ear bars. The incision bar was adjusted at 3.3 (flat skull). The head was shaved, loose hairs removed with duct tape, and the shaved area was cleaned with saline and 70% alcohol solution. Prior to any invasive procedures, a 0.05ml injection of the anti-inflammatory Carprofen (Rimadyl™) was given sub-cutaneously. A midline incision was made on the scalp to expose the skull. Using a dental drill (Volvere GX, NE22L, Tokyo, Japan) a 0.3 x 1 cm rectangular craniotomy perpendicular to the sagittal suture was made from about –1.5 to –4.5mm from bregma. The piece of skull within the craniotomy was removed and the area underneath was cleaned carefully with saline-soaked cotton buds and pieces of absorbable gelatine sponge (Spongostan, Ferrosan, Denmark). In addition, the edges of the craniotomy

were carefully inspected under the microscope for traces of bone flakes, which were then removed. Glass micropipettes with an external tip diameter of ~30μm were used to deliver the injections. Anterior-posterior (AP) measurements were taken from bregma, mediolateral (ML) measurements from the exposed central sinus, and dorsoventral (DV) measures from the brain dura. Injections of 0.3μl of 0.04M ibotenic acid were made bilaterally at -3.4mm AP, +/-3.9mm ML, and -5.7mm DV from bregma. The pipette was left *in situ* for 4 minutes following each injection. The same procedure was followed for the surgical control animals but sterile phosphate buffered saline was injected instead of the neurotoxin. Finally, metal suture clips (B.Braun, Sheffield, UK) were used to close the wound. Immediately following the completion of the surgery, all animals were given 0.3ml of diazepam intraperitonealy.

3.3.5. Behavioural Testing

Following surgery, a 7-day recovery period was allowed for both surgical groups. Prior the post-operative collection of data, rats were given 3 further days of training (one with a bimodal block, one with a unimodal/visual block and one with a unimodal/auditory block) in order to establish responding in the task. Data from these initial post-surgical days were not included in the analysis. Rats were then tested for 10 days in bimodal blocks of trials, and for another 10 days in unimodal blocks of trials (5 days of visual discrimination and 5 days of auditory discrimination) in alternating days (i.e. bimodal, unimodal visual, bimodal, unimodal-auditory, etc)

3.3.6. Data analysis

Data from the first 10 trials of every session, regardless of condition or modality, were excluded from the analyses, as these initial trials represented a period of uncertainty with regard to which type of block of trials (bimodal or unimodal, and of which modality) the animals were in. The remaining responses in the session were categorised into 4 types: *correct responses*: (responses towards the stimulus-associated hole), *incorrect responses*: (responses to the hole opposite the stimulus-associated hole), *early* or *anticipatory errors* (withdrawal from the central hole before the lapse of the 0.2 delay), and *late errors*, (failure to produce a side nosepoke following 2 seconds from a successful withdrawal from the central hole). *Mean reaction times* were calculated for each animal, for each condition and modality, from correct trials only. Reactions times were measured with centisecond accuracy and represented the time between the presentation of a stimulus and the animal's withdrawal from the central hole. Data were analysed using a repeated measures ANOVA with *condition* (unimodal/bimodal) and *modality* (lights/tones) as within-subjects variables, and *surgery group* (lesion/surgical control) as a between-subjects variable, using SPSS (v.12) for Windows.

3.3.7. Histological analyses:

The day following perfusion, one in six series, 40μm coronal sections were cut through the thalamus (between bregma –1.10 and bregma –4.10) using a freezing microtome. Brain sections from 2 consecutive series were then separately processed, staining for parvalbumin and NeuN. Parvalbumin is a calcium ion binding protein that is expressed particularly heavily in healthy GABA-containing cell areas (like TRN) and can thus be used to assess the

extent of neurotoxic lesions within such areas (Celio, 1986). NeuN, on the other hand, is a general neuronal marker (see Mullen, Buck and Smith, 1992) that was mainly used to delineate non-TRN areas and, in conjunction with parvalbumin, assess the overall extent of lesioned tissue outside TRN. The sections were treated with 0.9% PBS before blocked in blocking solution and then incubated in parvalbumin or NeuN antibody at 1 in 20.000 and 1 in 5.000, respectively, overnight. Following incubation in vector IgG solution, sections were put in avidin-biotin complex and then incubated in DAB substrate. Following mounting onto gelatinised slides, sections were lightly stained with cresyl violet.

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3.4. Results

3.4.1. Histology

Histological analyses showed that, there was complete damage of the cTRN between approximately –2.7 and –4.0mm from bregma, an area that contains both the visual and auditory sectors, in all animals. Dorsal thalamic damage was restricted to a relatively thin zone at the dorsolateral borders of cTRN, in an area of the ventroposterolateral nucleus (VPL), a somatosensory relay structure (see Figure 3.4 for minimum and maximum extent of the lesions).

Data from 17 animals (8 lesioned and 9 surgical controls) were analysed. The remaining 7 animals were excluded: 2 failed to make a satisfactory recovery following surgery and were euthanized. A further 5 rats sustained lesion damage extending into dLGN, ventrolateral nucleus and/or the internal segment of the globus pallidus.

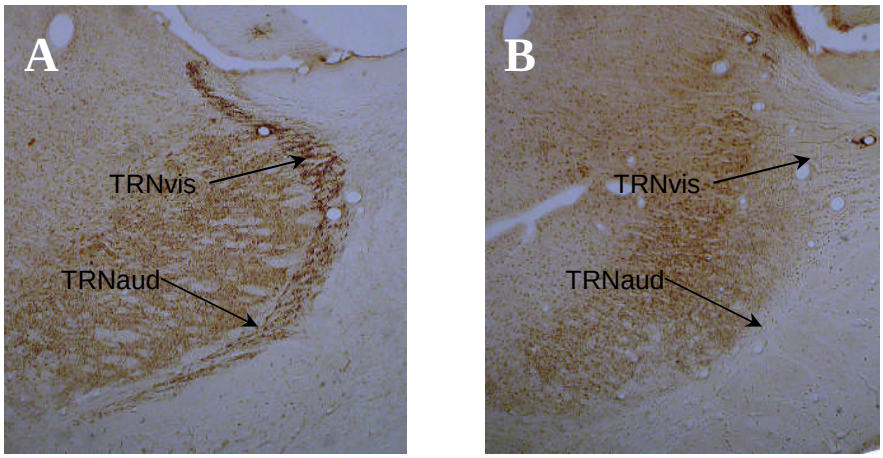

Figure 3.3. *Microphotographs (magnification x25) of parvalbumin-stained sections through the right TRNvis/TRNaud complex at about –3.6mm from bregma in a control animal (A) and in a lesioned animal (B). The dark patch of stained cells in A (see arrows) represents the whole dorsoventral extent of cTRN (dorsal: TRNvis; ventral: TRNaud). In B, the cells of TRNvis/TRNaud have given way to an empty, unlabelled, space representing the area of the lesion.*

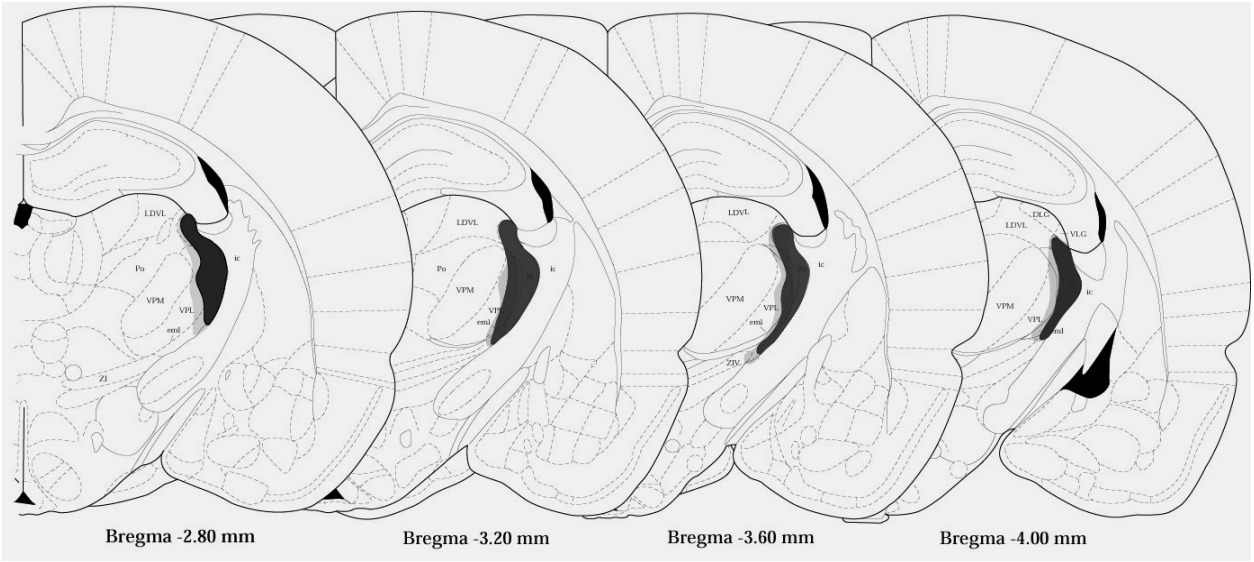

Figure 3.4: Minimum (black) and maximum (grey) extent of lesions at 4 sequential anterior-posterior co-ordinates. Numbers represent distance from bragma. ic: internal capsule, eml: external medullary lamina, ldvl: ventrolateral laterodorsal nucleus, Po: Posterior nucleus, vpl: ventroposteriolateral nucleus, vpm: ventroposteromedial nucleus, zi: zona inserta, ziv: ventral zona inserta

3.4.2. Behavioural results

3.4.2.1. Response Latency

Response latencies for visual discrimination trials were faster than for auditory discrimination ones (main effect of modality: F(1,15)=94.2, *p*<.001, see Figure 3.5). As predicted, reaction times in the unimodal condition (where the modality did not change from trial to trial) were faster than in the bimodal condition in which the animals had to divide attention between the visual and auditory channels (condition main effect: F(1,15)= 23.04, *p*<.001). This difference reflects the "cost" of dividing attention. This cost (reaction time difference between conditions) was evident only for visual discrimination but not for auditory discrimination (modality by condition interaction: F(1,15)=13.29, *p*<.005). However, the cost was unchanged by lesion (no surgical group by condition interaction: F(1,15)= .591, *p*>.05*;* and no surgical group by modality by condition: F(1,15)=4.45, *p*>.05, see Figure 3.6).

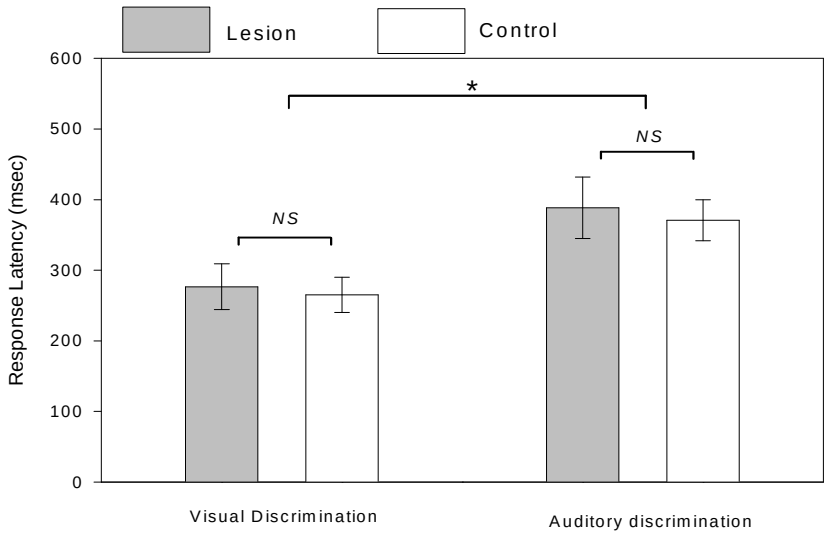

*Figure 3.5. Response latencies for visual and auditory discrimination trials for lesioned (grey bars) and surgical control (white bars) groups (collapsed across conditions). Auditory discrimination trials were slower than visual discrimination ones (*p<.001). The lesion did not affect response latency in either modality task (NS).*

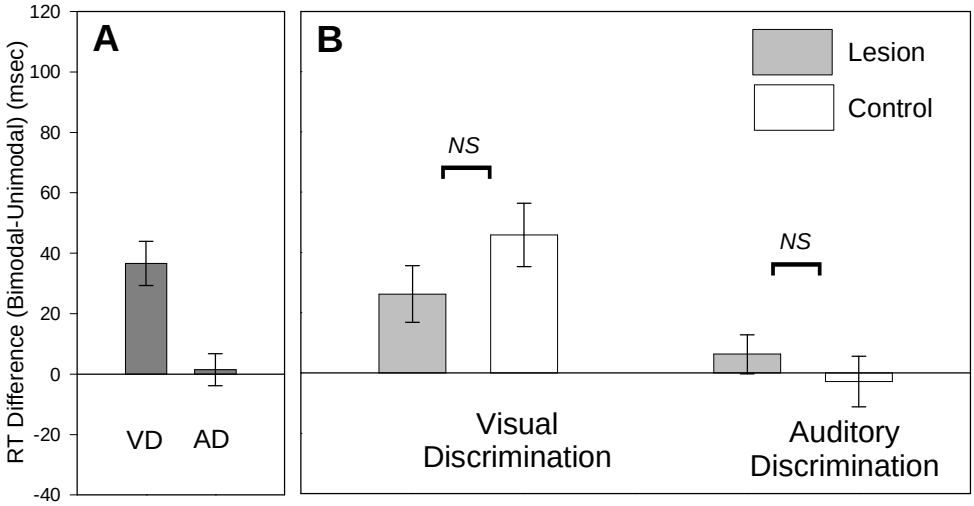

Figure 3.6: A: Subtracted unimodal from bimodal reaction times for visual and auditory discrimination trials collapsed across surgical groups. The difference in response latency between bimodal and unimodal trials was greater for the visual discrimination trials (VD) compared to the auditory discrimination trials (AD) for which the difference was negligible. B: Subtracted unimodal from bimodal reaction times for visual and auditory discrimination trials, for lesioned (grey bars) and control (white bars) animals. The infliction of the lesions did not affect response latencies for either modality task.

3.4.2.2. Incorrect responses

Accuracy (reflected by the % of incorrect trials) was lower for auditory compared to visual discrimination (main effect of modality: $F(1,15)=63.37$, $p<.001$, see Figure 3.7), but there was no effect of condition (unimodal vs bimodal) on accuracy ($F(1,15)=3.11$, $p>.05$). Lesioned animals were less accurate than control animals (main effect of surgical group: $F(1,15)=11.11$, $p=.005$, see inset in Figure 3.7) although overall they continued to perform above chance.

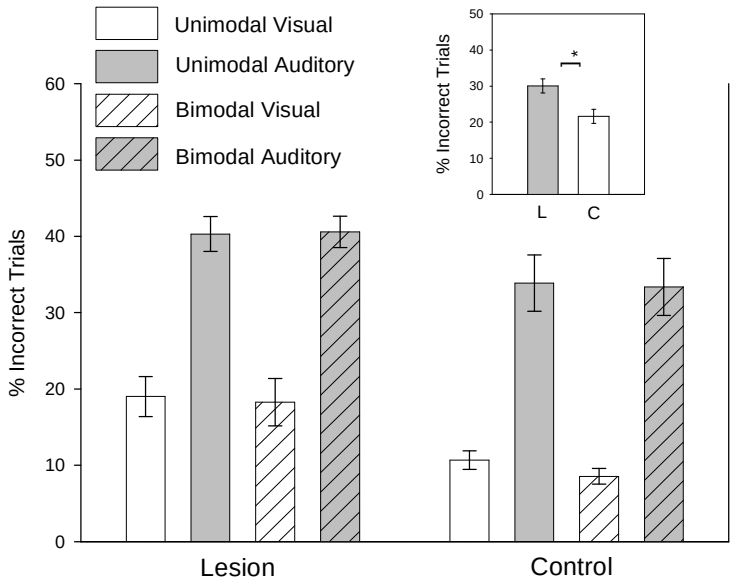

Figure 3.7: Percentage of incorrect responses across conditions and modalities for both surgical groups. Inset: Lesioned animals were overall less accurate compared to surgical controls, p<.005

3.4.2.3. Late Errors

Overall late errors were relatively infrequent (see Figure 3.8) and did not increase even when the animal was required to divide attention. Late errors were more frequent during auditory discrimination trials (main effect of modality: $F(1,15)=14.42$, $p=.002$). The infliction of the lesions had no effect on late responding: (no main effect of surgical group $F(1,15)=3.1$, $p>.05$ and no condition by group interaction $F(1,15)=0.41$, $p>.05$).

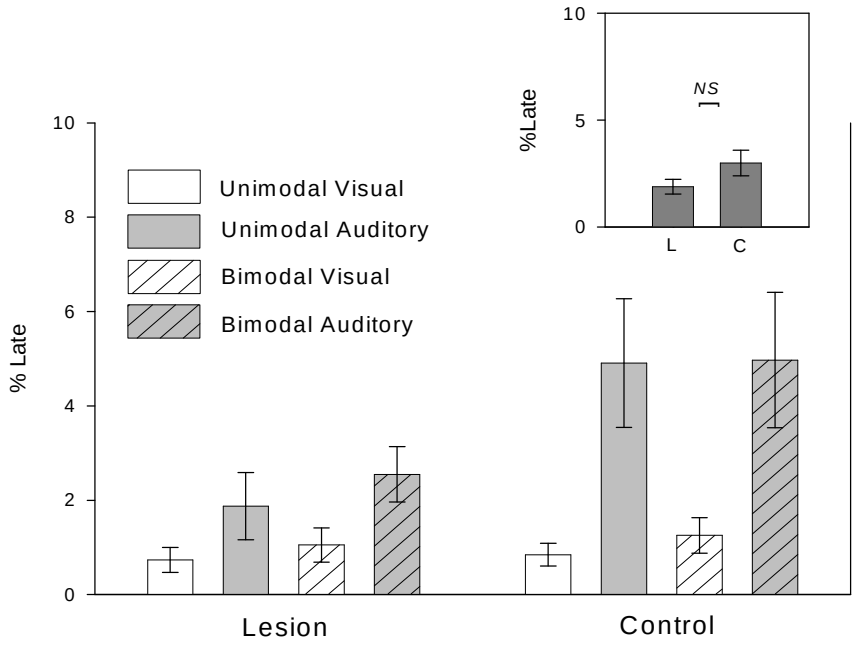

Figure 3.8. Late responses across modalities and conditions for both groups. Auditory trials produced more late errors than visual ones. Inset: TRNvis/aud lesions did not interfere with late responding

3.5. Discussion

3.5.1. Measure of divided attention (Uni- vs. Bimodal condition effects)

Divided attention was measured by comparing performance in the unimodal against the bimodal blocks of trials. Differences in performance between these two conditions represented the cognitive costs for dividing attention between the two modalities. Divided attention costs were observed only with regard to response latency but not for response accuracy. The latter is not surprising as there was no reason for the identification of the stimuli, and the subsequent generation of the appropriate response, to suffer from the prior division of attention. Response latency on the other hand was slower in the bimodal compared to the unimodal blocks of trials reflecting the detriments of simultaneously processing two information channels. However, this overall

slowing down during the bimodal condition was specific to the visual trials and was not observed for auditory trials. A possible explanation for this may be related to the temporal-dependency of the auditory discrimination. More specifically, whereas visual discrimination could be performed immediately upon the presentation of the pair of lights, auditory discrimination required a short wait for the animals to determine whether the stimulus was a continuous or an intermitted tone. This was reflected in the reaction times, where visual discrimination was quicker than the auditory one by approximately 100ms. It is possible, therefore, that the additional time needed for discriminating the tones (~100msec) overlapped to some extent with the additional time required to perform the task in a divided attention context (bimodal task). This could have resulted therefore in the absence of a discrepancy in reaction times between unimodal and bimodal blocks of trials for the auditory condition. As a consequence, although the task was efficient in assessing the behavioural costs of the division of attention with regard to the visual discrimination, it was not able to detect those costs in the context of an the auditory discrimination. The behavioural costs of the bimodal condition reflect either the limited recourses to deal with the parallel processing of the two modality channels or the time required for shifting attention between the two modalities (Bonnel *et al*., 1998; Parasuraman, 1998).

3.5.2. Lesion effects on cross-modal divided attention

We expected that TRN*vis/aud* lesions would result in a selective attention deficit expressed by a slowed response latency in unimodal trials and, most importantly, in a divided attention deficit expressed by disproportionaly

increased reaction time costs during bimodal blocks of trials. However, we found that the infliction of the lesions had no effect in response latency neither in the unimodal nor in the bimodal condition. More specifically lesioned and control animals were equally fast in unimodal blocks of trials and equally slower in bimodal blocks of trials. Contrary to our predictions, therefore, our findings suggest that TRN*vis*/*aud* might not be involved in processes of audiovisual cross-modal divided attention.

One of the main contributions of cTRN in attentional processes is believed to be the switch of firing mode between burst and tonic in thalamocortical cells (see general introduction). Tonic and burst firing modes have distinctive transmission properties yielding, as a result, different functional significances with regard to attentional processes. More specifically, burst firing, which is generated at hyperpolarised membrane potentials, is the optimal firing mode for the detection of sudden or weak sensory stimuli. For that reason it is believed to represent an alerting signal to the cortex, informing it of new incoming information that potentially needs to be attended (Guido *et al.*, 1992, 1995, Mukherjee and Kaplan, 1995, also see Sherman *et al.*, 2001, Sherman, 2005). Tonic firing on the other hand, which is generated at relatively depolarised membrane potentials, is less good at detecting stimuli (especially when these are challenging to detect), but it transmits sensory information more linearly, enabling the detailed perception of stimuli once they have been detected (see Sherman and Guillery, 2001 for a review). In a selective attention context, therefore, cTRN's main role could be to hyperpolarise thalamocortical cells in order to promote burst firing in them and enhance

stimulus detection. Once a stimulus of interest is detected, cTRN inhibition could be withdrawn from thalamocortical cells thus allowing them to fire in tonic mode and enhance the analysis of the stimulus. Similarly, for attention to be divided cross-modally, in order for visual or auditory stimuli to be detected and then analysed/identified, both modality channels would have to fire simultaneously, or in close alteration, in burst mode so that to boost signal detection. This could possibly be achieved by the parallel or interchanging supply of inhibitory feedback by TRN*vis* and TRN*aud* onto visual and auditory thalamocortical cells respectively. Once the stimulus of interest is detected in one of the two channels, the inhibitory TRN*vis* or TRN*aud* feedback could be reduced or discontinued, allowing its efferent thalamocortical cells to switch their firing into tonic mode, thus enhancing the analysis of the now-detected and attended stimulus.

The destruction of TRNvis/aud in lesioned animals was, therefore, expected to prevent the generation of bursts in thalamocortical cells (French, Sefton and Mackay-Sim, 1985), thus delaying the detection of the test stimuli, which would then have to be carried out by the, non-optimal for detection, tonic mode. Our finding that TRN*vis*/*aud* lesion did not affect response latency, both in a selective and in a divided attention context, reflects an intact stimulus detection ability. In turn, an intact stimulus detection ability despite the presumed inability to induce burst firing in thalamocortical cells suggests a reduced requirement for burst firing in these cells during the performance of our task (see below).

Even though burst firing is optimal for the detection of transient, unexpected, stimuli, tonic firing can also carry out stimulus detection, provided that the stimulus is salient enough, long enough in duration, or temporally predictable (Sherman, 1996). The test stimuli used in our investigation featured some of the above characteristics, which may have made their detection equally achievable by tonically-active thalamocortical cells. More specifically, the test stimuli were both relatively predictable in the temporal domain (they appeared invariably 0.2 seconds after the initial nose poke), and they were presented for a duration of time (1.5 second) that was relatively too long to challenge detection. As a consequence, the presumed inability of lesioned animals to implement burst firing in their visual and auditory thalamocortical cells, may have not constituted a disadvantage in detecting the stimuli. What's more, it is not unlikely that due to the temporal predictability and long duration of the test stimuli that their detection had been carried out by tonically-firing thalamocortical cells even in surgical control animals.

3.5.3. Response accuracy and omissions

Although we had not predicted it, we observed a poorer response accuracy of lesioned animals compared to surgical controls. More specifically animals that sustained selective lesions of the TRN*vis* and TRN*aud* produced more incorrect responses compared to surgical controls for both modality tasks and during both the unimodal and bimodal conditions. It is not unlikely that this overall poorer accuracy of lesioned animals reflects an attentional deficit, albeit unrelated to the division of attention. Failure to produce the correct response may have resulted from a failure to engage attention sufficiently in

the task, leading to erroneous stimulus identifications. However, a more parsimonious explanation for the poorer accuracy of lesioned animals would involve a deficit related to the stimulus discrimination ability rather than an attention-engagement deficit. Such a deficit could simply reflect a poorer ability to perceive/analyse and thus discriminate the two types of stimuli within each modality. Despite the absence of damage in the visual and auditory dorsal thalamic nuclei, the cessation of TRN*vis*/*aud* feedback onto these areas may have affected sensory transmission in such a way as to render the perception of the test stimuli more ambiguous. The increased ambiguity in the perception of audiovisual stimulation could have then resulted in more incorrect identifications of the test stimuli. Even though receptive field elaboration is not believed to take place to a great extent at a thalamic level (e.g. see Sherman, 2005) there is sufficient evidence to suggest that destruction of cTRN cells results in a significant receptive field increase in their afferent thalamocortical cells (Lee, Friedberg, Ebner, 1994a). It is possible therefore that the cessation of TRN*vis*/*aud*-delivered inhibition on thalamocortical cells following the lesions resulted in the “blunting” of their receptive fields and in the relay of sensory information of poorer discriminative quality to the sensory cortices.

In an alternative, or complementary, scenario, the poorer accuracy of lesioned animals could be related to deficiencies in the generation of tonic firing in thalamocortical cells as a result of TRN*vis*/*aud* damage. Even though cTRN is believed to be mainly involved in the generation of bursts in thalamocortical cells, its role in the generation of tonic firing in these is much less understood.

Whether it is simply the withdrawal of cTRN's inhibition combined with depolarising effects from other afferents or a more complex, not as yet identified, mechanism that allows tonic firing in thalamocortical cells remains to be determined (Sherman and Guillery, 2001). If cTRN is indeed involved in the generation of "healthy" tonic activity in thalamocortical cells then TRN*vis/aud* lesions could have affected the presumably tonic-dependent stimulus analysis, deteriorating the quality of signal transmission resulting often in erroneous stimulus identifications and incorrect responses. This line of reasoning may somehow conflict with our earlier argument that tonic activity in lesioned animals may have been equally effective to tonic and/or burst activity in surgical control animals at detecting the test stimuli. It must be said, however, that the processes of detection and discrimination are substantially different and may therefore be differentially affected by abnormal tonic activity. More specifically, whereas stimulus detection is instantaneous and less likely to be affected by the poor analytical quality of the sensory signal, stimulus discrimination is by definition dependent on the good quality of the sensory signal and thus the efficient operation of tonic activity.

3.5.4. Conclusions

To sum up, we did not find evidence for a role of TRN*vis*/*aud* in cross-modal audiovisual divided attention as lesioned and control animals demonstrated analogous behavioural costs when required to divide attention between the visual and auditory modality channels. However our results cannot be generalised across cross-modal divided attention behaviours as the lack of a behavioural effect following the lesions may have been related to the choice

of test stimuli. Given the potential involvement of cTRN in thalamocortical burst firing generation and thus in stimulus-detection ability, cTRN lesions may differentially affect cross-modal divided attention behaviours that require the detection of briefer, less salient, or temporally more unpredictable stimuli than the ones used here. On a different note, we found that the lesions induced a potential stimulus discrimination impairment, which could reflect a role for cTRN in normal sensory perception, with or without implications to attention.

Chapter *IV. Lesions of the visual thalamic reticular nucleus do not impair endogenous covert orienting in the rat*

4.1. Abstract

One of the earliest proposals of TRNvis's functional importance suggested that it may represent the neural substrate of the spotlight of visual spatial attention (Crick, 1984). Indeed, some behavioural evidence indicates that damage to TRNvis results in detriments of covert orienting of attention when the latter is triggered by exogenous means (Weese *et al.*, 1999). Attention can also be covertly oriented voluntarily, on the basis of cognitive or endogenous cues. Because of the heavy, retinotopically-organised, cortical input it receives, which could represent the top-down drive for the endogenous covert orienting behaviour, we wanted to investigate the role of TRNvis in these behaviours. We trained rats in a visual target detection task, where we cued targets' location by manipulating their spatial probability as a function of time: at short foreperiods, left targets were more probable but, with increasing foreperiods, right targets were increasingly more probable. Pre-operative reaction times were faster for left targets at early foreperiods and faster for right targets at late foreperiods, indicating that attention was covertly oriented. Lesions of TRNvis did not alter the above pattern of responding, which continued to reflect the spatiotemporal probability of the targets. These results indicate that visual covert attention, when mediated by endogenous cues, may be shifted within space without the involvement of TRNvis.

4.2. Introduction

One of the earliest proposals for the potential functional role of caudal TRN (cTRN) postulated that it is the generator and controller of the “attentional searchlight” (Crick, 1984). More specifically, through its precise topographic inhibition upon sensory thalamocortical relay cells, Crick proposed that the cTRN could selectively act upon groups of thalamocortical neurons creating hotspots of optimised sensory processing within a sensory surface, which would correspond to the attended sensory space. The mechanism behind the creation of these attentional hotspots was speculated to be the generation of burst firing in subsets of thalamocortical relay cells and the relative suppression of activity of others, resulting in an overall signal “promotion” for the former. The sensory mapping of cTRN’s thalamocortical and corticothalamic inputs, in conjunction with its equally topographic inhibitory output on corticopetal thalamic (thalamocortical) cells (*see 1.2.4.2. and 1.2.6. in general introduction*), could allow high spatial specificity in the creation of these attentional loci and also in their movement within sensory space.

Experimental support for the idea that cTRN could be involved in the creation of spotlights of attention was provided by Weese, Phillips and Brown (1999) with regard to the visual modality. Using a rodent equivalent of the Posner task, a cued visuospatial task (Posner, 1980) they measured rats’ ability to attend to peripheral locations of visual space while fixating centrally, a behaviour known as covert orienting. Rats were required to sustain a nosepoke for a variable delay and then respond towards a visual target that could appear on their left or right. Immediately prior to the presentation of the

target stimulus, a cue in the form of a brief dim light was presented in one of these two locations. Responses to targets presented at the same location as a cue (validly cued targets) were faster compared to responses to targets presented at the location opposite that of a cue (invalidly cued targets). It was thought that attention was involuntarily attracted to the location of the cue, resulting either in more rapid detection of targets appearing at the, now attended, cue location or a slower detection of targets appearing away from that location and to which attention had to be redirected. The difference in reaction times between validly and invalidly cued trials, known as the “validity effect”, represents a measure of covert orienting, as it reflects the movement of visual attention within visual space. Weese *et al*. found that unilateral TRN*vis* lesions resulted in the abolition of the validity effect for targets contralateral to the lesion. In other words, valid cues no longer accelerated the detection of targets appearing in the visual hemifield corresponding to the lesioned TRN*vis*. Even though contralateral targets could still be detected and responded to, the priming element offered by their preceding valid cues was removed. This suggested that TRN*vis* may be involved in the selection, and shifting, of the areas of visual field within which sensory processing is enhanced.

In the Posner task, covert orienting is manipulated by pre-target spatial cues, which direct attention towards or away from subsequent target stimuli. In other words, both the generation and the direction of attentional orienting are stimulus-driven (*exogenous*) and involuntary. However, both overt and covert attentional orienting is commonly performed not just to exogenous cues but

also due to voluntary decisions. One could, for example, intentionally move attention within visual space in order to enhance perception in areas of the visual field with potential behavioural interest. This form of *endogenously* generated orienting can be performed either in the absence of sensory guidance or it can be directed by sensory input without, however, being a direct consequence of it. For example, and with regard to the latter, covert orienting can be guided by arbitrary cues that posses no intrinsic spatial information but which have been associated (through learning) with particular spatial meanings (e.g. arrowheads are associated with the directions they point to). Unlike exogenous orienting, endogenously cued orienting is not dependent on the sensory stimulation of the cue but on top-down signals that contain the “translated” spatial meaning of the endogenous cue (Coull and Nobre, 1998).

The present investigation intends to examine the possibility that TRN*vis*, in addition to exogenous covert orienting, is also involved in the endogenous, top-down mediated, control of visual spatial attention. Our reasoning stems from the fact that the vast majority of TRN*vis* input comes from corticothalamic projections (Guillery, 1967, Jones 1975), which are the main conveyors of top-down communication to the diencephalon. Moreover, there is some evidence suggesting that the Fos activation seen in TRN*vis* following voluntary attentive behaviours is heavily dependent on top-down influences arriving from visual cortex (Montero, 2000). As a consequence, TRN*vis*, by virtue of its visuotopic corticothalamic input and equally topographic thalamocortical output, could be ideally suited for the generation and

movement of the attentional spotlight within visual space (i.e. for the generation of covert orienting), in an endogenous context.

To test the above hypothesis, we decided to investigate the effects of TRN*vis* lesions on the performance of an endogenous attentional task in rats. Appreciating the practical difficulties of training rodents to associate abstract sensory cues with spatial locations we employed a task where visuospatial attention within each trial was manipulated not by sensory endogenous cues but by certain learnt spatiotemporal parameters. More specifically, likewise the Posner task, our *asymmetric stimulus probability* (ASP) task required a sustained nosepoke of a variable delay (foreperiod delay), which was followed by the presentation of a visual stimulus either on the left or right of the animal's head. However, the probability of the stimulus being presented to each side was manipulated within the trial as the foreperiod elapsed (see Figure 4.1 in *Methods*), such that at early foreperiods targets were more likely to occur on the left while at late foreperiods targets were more probable on the right. Target stimuli in the ASP were therefore expected to appear at certain locations at certain points in time, and as a result attention was directed to these locations as a function of elapsed foreperiod. This resulted in faster response latencies for left, compared to right, targets early in a trial and, conversely, faster reaction times for right, compared to left, targets late in a trial, in a pattern that resembled the validity effect of the Posner task (see *Results*).

On the basis of the abolition of the Posner validity effect following TRN*vis* lesions (Weese *et al.*, 1999) we tested the hypothesis that such lesions would

also eliminate the ASP-generated spatiotemporal validity effect. More specifically, we predicted that there would be no discrepancy between reaction times for “spatio-temporally valid” and “spatio-temporally invalid” targets following lesions of the TRNvis, which would reflect an inability to endogenously engage and direct the attentional spotlight within visual space.

4.3. Methods

4.3.1. Animals

24 adult male Lister hooded rats (Harlan, UK) were used for this experiment. Animals were paired-housed in 25x45x15cm sawdust-filled wire-mesh cages, and kept in a light/dark regime changing every 12 hours (lights on at 7 am). Access to food in the home cage during training and training was limited to 15-18g, per day, per animal. Water was available *ad libidum*.

4.3.2. Training

Similarly to the previous chapter, all training and testing took place in eight 9-hole operant boxes, only the 3 central holes of which were used. Training took place in several stages, gradually shaping the desired behaviour in the animals. For all stages, training consisted of daily 30-minute sessions (otherwise terminated upon the completion of 120 correct trials).

After being habituated to the operant boxes (see *3.3.3.* in previous chapter for details), animals were trained to maintain sustained nose-pokes in the illuminated central hole of the 3-hole array for a variable foreperiod delay of between 0.2 and 0.6 seconds (0.2, 0.3, 0.4, 0.5 or 0.6 seconds). A successful

nose-poke (that is, a nose poke lasting at least as long as the current trial's required foreperiod) resulted in the delivery of a food pellet in the food magazine. *Early withdrawals* (nose pokes lasting less than the current trial's required foreperiod) resulted in a 2-second dark timeout and in the delivery of no food. When every individual animal's performance consisted of approximately 70% successful trials per session, training proceeded to its final stage where the ASP task was introduced. In ASP, a successful central nose poke was followed by the presentation of a bright light (0.5 sec in duration) in one of the adjacent left or right holes. Animals had to nose-poke in the hole where the stimulus appeared. The probability of a target appearing on the left or the right hole varied as a function of the lapsed foreperiod. More specifically, targets were more likely to appear in the left hole during the early foreperiods and increasingly more likely to appear on the right hole as foreperiod time increased. The exact *a priori* stimulus probabilities for each side can be seen in Figure 4.1. At this stage, two new types of errors were introduced and recorded, both of which resulted in a 2-second dark timeout. *Incorrect errors* were responses in the hole opposite the one where the stimulus appeared. In addition, a *late error* represented the failure to produce any response following 2 seconds from the stimulus' onset of presentation. Reaction times were recorded as the time between the presentation of the stimulus and the withdrawal of the snout from the central hole.

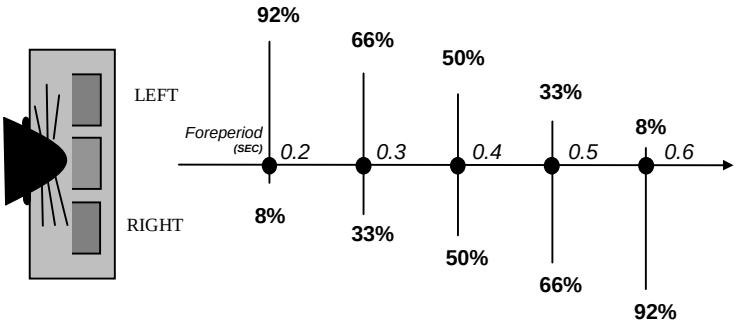

Figure 4.1: The absolute, a priori, stimulus probabilities for each side, for each of the 5 foreperiods (redrawn from O'Neill, unpublished PhD Thesis).

4.3.3. Pre-operative data collection

Animal's performance had to satisfy two criteria before pre-operative data could be collected. Firstly, animals had to reach a stable performance of above 70% correct trials per session. Secondly, their reaction latency had to reflect the spatiotemporal probabilities established by the ASP. That is, animals had to respond faster to left targets during early foreperiods but faster to right targets for late foreperiods (refer to Figure 4.3 in *Results*). When all animals satisfied the above criteria, pre-operative data collection commenced. Pre-operative data was collected over 14 days.

4.3.4. Surgery

Of the 24 animals used, 16 received excitotoxic lesions of TRNvis while the remaining 8 received sham surgery and were used as controls. Animals were anaesthetised by inhalation of 5% Isoflurane in an anaesthetic induction chamber. They were then placed in the stereoscopic frame (Kopf, Tujunga, CA), while isoflurane anaesthesia was maintained (at 2.5-3%) through a facemask fitted to the nosepiece of the frame. While in the frame, animals

were given 0.05ml of the anti-inflammatory analgesic Carprofen (Rimadyl™). Invasive procedures commenced with an incision at the surface of the scalp to expose the skull so that both bregma and lambda were well visible. A 0.3 x 1cm craniotomy was made perpendicular to the saggittal suture between approximately –1.5 and –4.5mm from bregma, to expose the brain underneath. Anterior-posterior (AP) measurements were taken from bregma, mediolateral (ML) measurements from the exposed central sinus, and dorsoventral (DV) measures from the brain dura. Bilateral injections of 2µl of 0.04M ibotenic acid were made using glass micropipettes (external tip diameter: ~ 30µm) at coordinates AP: -3.4, ML:+/- 3.7, DV: -5.2mm. Pipettes were left *in situ* for 4 minutes following the injection to allow the absorption of the injected liquid and minimise its upward suction during their retraction. The wound was sealed using 4 metal suture clips (B.Braun, Sheffield, UK). Surgical procedures for control animals were identical to those for lesioned animals, with the exception of the injection of sterile phosphate buffered saline instead of the neurotoxin. Following surgery, animals were given 0.3ml of diazepam intraperitonealy for the prevention of seizures and also for the containment of the excitotoxic effect of ibotenic acid. Animals were allowed to recover for 1 week before testing commenced. Food-control was interrupted during recovery (food was available *ad libitum*) and was reinstated the day before the first day of testing.

4.3.5. Post-operative data collection

Following the 1-week recovery period, animals were trained in the ASP task for 3 to 5 days in order to establish responding in the task (data from these

sessions was not used in the analyses). Finally, post-operative data were collected over the following 14 days over equinumerant sessions.

4.3.6. Behavioural data analysis

Behavioural data were analysed using a repeated measures analysis of variance (SPSS v.12) with *response side* (left;right), *foreperiod* (0.2; 0.3; 0.4; 0.5; 0.6 sec) and *surgery* (pre-operative; post-operative) as within-subjects variables, and *surgery group* (surgical control; lesion) as a between subjects variable. Separate analyses were carried out for reaction times (expressed as modal response latency), incorrect errors, late errors and early withdrawal errors.

4.3.7. Histological procedures

Following the completion of behavioural testing, all animals were transcardially perfused with 4% paraformaldyhyde in 0.1M Phosphate Buffered Saline (PBS), their brains removed and post-fixed overnight in 20% sucrose in PBS. 1 in 4, coronal, sections through the thalamus were cut using a freezing microtome, and free-floating slices were processed separately for parvalbumin and NeuN to examine the extent of the excitotoxic damage in cTRN and dorsal thalamus. After processing, sections were mounted onto gelatinised slides and stained with cresyl violet to enhance the delineation of brain structures. Sections were examined under the microscope and the extent of the lesions was assessed and recorded.

4.4. Results

4.4.1. Extent of lesions

Histological analyses revealed that TRNvis was completely removed in 15 of the 16 lesioned animals. More specifically, the lesion included the whole dorsal half of cTRN between –2.7 and –4.0mm from bregma. In most animals there was also complete or partial damage to the ventrally adjacent TRNaud. Dorsal thalamic damage was restricted to a thin zone within the dorsal and lateral ventroposterolateral nucleus (VPL). Two animals in total were excluded from the analyses; one due to incomplete TRNvis damage, and another because of extensive dorsal thalamic damage. Figure 4.2 shows the minimum and maximum extent of the lesions of the 14 lesioned animals used in the analyses.

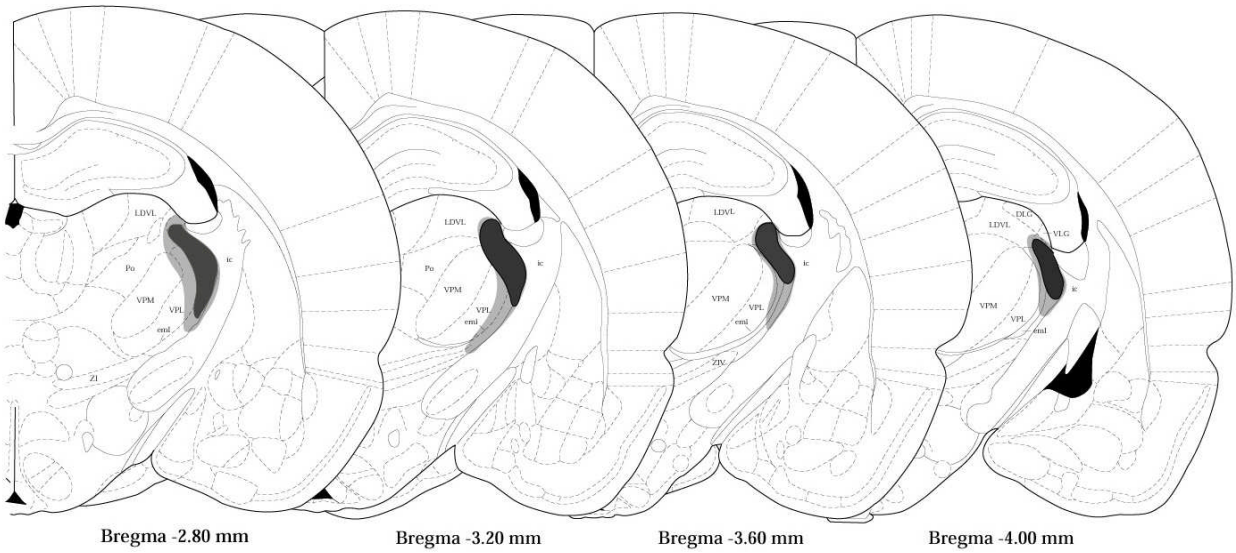

Fig 4.2. Minimum (black) and maximum (grey) extent of lesions. Numbers represent distance from bragma. ic: internal capsule, eml: external medullary lamina, ldvl: ventrolateral laterodorsal nucleus, Po: Posterior nucleus, vpl: ventroposteriolateral nucleus, vpm: ventroposteromedial nucleus, zi: zona inserta, ziv: ventral zona inserta.

4.4.2. Reaction times

Modal response latency became faster with increasing foreperiod (main effect of foreperiod: F(4,80)=50.21, *p*<.001). Furthermore, reaction times reflected the spatiotemporal probability of the targets, as animals were faster in responding to left targets during early foreperiods and faster for right targets at late foreperiods (foreperiod by side interaction: F(4,80)=11.34 *p*<.001, see Figure 4.4, also refer to Figure 4.3). However, this spatiotemporal pattern of responding was not altered by the lesion (no foreperiod by side by surgical group interaction: F(4,80)=.64, *p*>.05, see Figure 4.4) and was not different post- from pre-operatively (no surgery by foreperiod by side interaction: F(4,80)=1.92, *p*>.05).

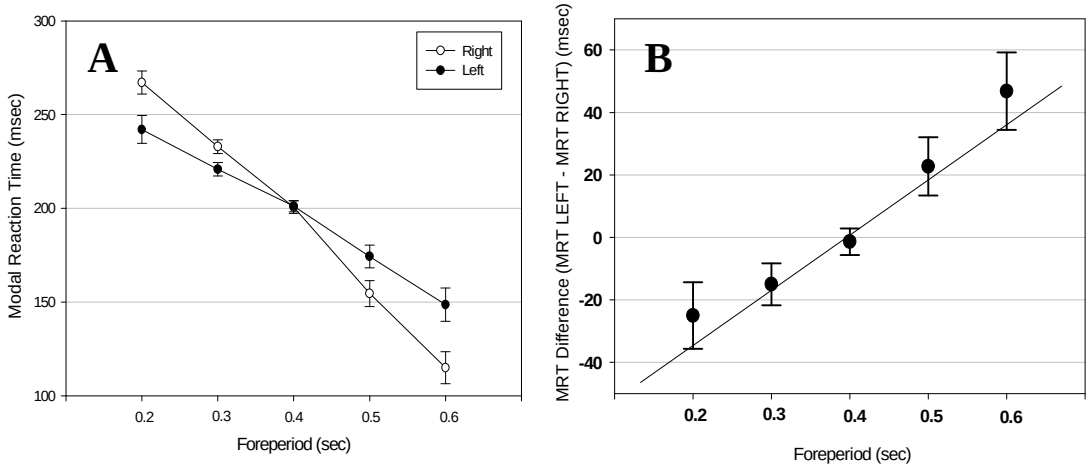

*Figure 4.3: **A**: Pre-operative modal reaction times for left and right targets across all foreperiods. **B**: Subtracted right from left modal reaction times for each foreperiod. Error bars represent SEM.*

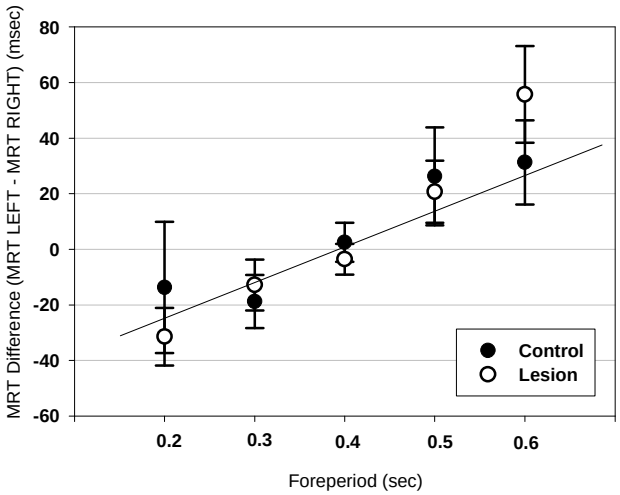

Figure 4.4. Subtracted right from left modal reaction times for each foreperiod for both control and TRNvis lesioned animals, post-operatively. Error bars represent SEM.

4.4.3. Incorrect errors

The number of incorrect responses increased with increased foreperiod latency (main effect of foreperiod: $F(4,80)=8.58$, $p<.001$) and were more frequent for left targets (i.e. more erroneous right responses after a left stimulus presentation than erroneous left responses after a right stimulus presentation) (main effect of side: $F(1,20)=7.46$, $p=.013$). However, the frequency of left and right errors was differentially affected by foreperiod latency. More specifically, while the percentage of right errors remained relatively stable across foreperiods, left errors sharply increased for the latest two foreperiods (foreperiod by side interaction: $F(4,80)=18.59$, $p<.001$, see Figure 4.5). In other words, at the later foreperiods the animal was most likely to make a right response, which resulted in errors to the (improbable) left target. Even though one might have also expected increased right errors for the early foreperiods (reflecting the *a priori* higher spatiotemporal probability for left targets in these foreperiods), it appears that the animals' pattern of

incorrect responding reflected an *instantaneous conditional* probability. This means that at the beginning of a trial, and despite the initial higher probability for a left target, the probability of a left or right *response* being required in the trial was equal. However, as foreperiod progressed, and no stimulus appeared, the conditional probability for a right response gradually increased, resulting in the sharp increase of incorrect responses to the improbable left targets for the last two foreperiods. The lesion had no effect on this pattern of incorrect responding (no main effect of surgery group: $F(1,20)=0.55$, $p>.05$; all surgery interactions, *NS*).

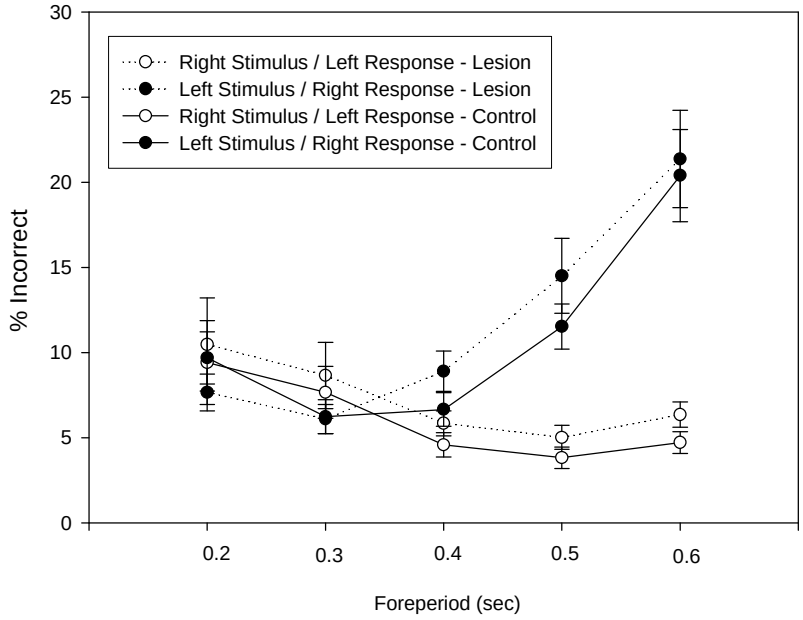

Figure 4.5: Post-operative frequency of left and right incorrect responses across foreperiods, for both control and TRNvis lesioned animals.

4.4.4. Early Withdrawals and Late Errors

The number of early withdrawals increased with increased foreperiod (main effect of foreperiod: $F(4,80)=277.1$, $p<.001$) but there were no lesion effects or interactions. No significant patterns were observed with regard to late errors, which were extremely infrequent.

4.5. Discussion

4.5.1. Response latency in ASP and the effects of TRNvis lesions

Response latency in the asymmetric stimulus probability task (ASP) reflected the spatiotemporal probability of the targets, as responses were faster to the side where a target was more likely to appear. The difference in reaction times between spatiotemporally probable and improbable targets created a *spatiotemporal validity effect* equivalent to the validity effect of cues seen in the Posner task (Weese *et al*., 1999; also see Ward and Brown, 1996). Contrary to our prediction, however, the infliction of the TRN*vis* lesions did not abolish the spatiotemporal validity effect, as animals remained faster to the left during early foreperiods and faster to the right at late foreperiods. In other words, the beneficial effects of directed visuospatial attention were preserved despite the loss of TRN*vis*. Our results, therefore, suggest that the ability to covertly shift attention within visual space is independent of normal TRN*vis* functioning. Attention could still be gradually moved from left to right hence affecting the speed by which targets were detected at these locations.

Assuming that the exogenous covert orienting deficit previously observed following TRNvis lesions (Weese *et al*., 1999) was due to an inability to shift

covert orienting within visual space, our results may be suggesting a differential role for this area in endogenous and exogenous attentional processes. More specifically, whereas TRNvis appears to be involved in the generation of exogenously-driven covert orienting (Weese *et al*., 1999), it may not constitute a necessary structure for its endogenous manipulation. This could add to the increasing evidence suggesting different neuroanatomical substrates for endogenous and exogenous visuospatial attention (e.g. Hahn, Ross and Stein, 2006).

However, the differing results of the two studies may be unrelated to whether the cue is endogenous or exogenous. A major difference between the Posner and the ASP task, notwithstanding the nature of the cues, lies in that while visuospatial cueing in the former is accomplished by a sensory cue, in the latter no sensory cue is implicated. As a consequence the generation of a covert orienting behaviour in the Posner task is first and foremost dependent on the detection of the cue. Without the detection of the cue attention could not be directed/attracted to the location of its appearance thus failing to influence subsequent responses to target stimuli. In the ASP, on the other hand, covert orienting does not rely on the detection or perception of a stimulus cue, but on the learnt knowledge of the spatiotemporal probabilities. It is therefore possible that the deficit observed following TRNvis lesioning in the Posner task had risen not from a reduced ability to move visuospatial attention, but from a reduced ability to detect the brief visual cue, which would have initiated the orienting.

Detection of brief or non-salient stimuli is better carried out by burst firing in thalamocortical cells due to its low signal-to-noise ratio of transmission (Guido *et al*., 1992; 1995; Mukherjee and Kaplan, 1995). cTRN is the most likely generator of such activity in thalamocortical circuits by supplying them with the necessary prolonged hyperpolarisation to de-inactivate their I_T membrane conductance and trigger bursts (French, Sefton and Mackay-Sim, 1985, also see *1.3.5.*). It is possible, therefore, that the unilateral discontinuation of TRNvis inhibition in Weese *et al.* abolished thalamocortical burst firing unilaterally, thus impairing the detection of contralateral cues and preventing a covert orienting response. While the presumed absence of burst firing prevented the detection of the brief (0.1sec) and dim cues, the longer duration of the target stimulus (0.15 sec) in combination with its greater saliency (bright light as opposed to the dim cue light) may have allowed its detection by means of tonic firing (which can be equally effective to burst firing in the detection of relatively long in duration and/or salient stimuli (Sherman, 1996; also see Sherman and Guillery 2001, Sherman, 2005). This could also explain why the detection of targets in our task (also bright and of a relatively long duration: 0.5 sec) was similarly unaffected by the lesion. Therefore, an alternative or complementary reason behind the differential effects of TRNvis lesions in the Posner and the ASP tasks may be related to the cue detection requirement in the former and the absence of such a requirement in the latter.

4.5.2. Response accuracy

In addition to the absence of a response latency effect, response accuracy in the ASP task also remained unaffected by the TRNvis lesions. In the ASP

task, incorrect errors were particularly prominent to the side opposite that of a spatiotemporaly highly probable (according to the instantaneous conditional probability) target. Incorrect errors for these types of targets, therefore, represented a failure to inhibit a motor preparatory response. Incorrect errors, especially to highly probably targets, in addition to anticipatory errors, which also reflect motor inhibition failure, have been found to increase in ASP-performing animals sustaining lesions of the subthalamic nucleus, an area associated with motor preparatory and inhibitory processes (Thomson and Brown, 2006). Given the lack of TRNvis connectivity with motor and motor association areas, it comes as no surprise that our lesions did not interfere with the above mentioned aspects of performance.

4.5.3. Extent of lesion

When comparing the behavioural effects of the TRNvis lesions in our study with those in Weese *et al*., it should also be borne in mind that the nature and extent of the lesions of the two studies may have been dissimilar. In Weese *et al.*, despite the creation of complete TRNvis lesions, the extent of dorsal thalamic damage was not examined thoroughly and, as a consequence, remained unknown. Considering that injections were performed using needles of approximately equivalent tip diameter to TRNvis's width, (thus resulting in a wider spread of toxin compared to the one of micro pipettes), then damage in other, including visual, dorsal thalamic areas adjacent to TRNvis remains a realistic possibility. In addition, by using more than twice the concentration of neurotoxin than we did (0.09M vs 0.04M), it is possible that the thalamocortical and corticothalamic axon damage caused (more specifically

the demyelination of these cells' axons) was more severe (Coffey, Perry, Allen, Sinden and Rawlins, 1988; Stellar, Hall and Waraczynski, 1991). This does raise the possibility that the behavioural deficits observed in Weese *et al.* were related not only to TRNvis dysfunction but also to additional dorsal thalamic damage and/or abnormal corticothalamic feedback transmission. The final experimental chapter of this thesis will seek to investigate this possibility by examining the effects of verifiably selective TRNvis lesions (similar to the ones inflicted in the present study) on performance in the Posner task.

4.5.4. Non-cTRN mechanisms implicated in ASP performance

While the present study was underway, another experiment, also employing the ASP task, was being conducted examining cholinergic lesions of the basal forebrain. These lesions were found to abolish the spatiotemporal validity effect by eliminating the accelerated response latencies to spatiotemporally probable targets (Farovik and Brown, 2006). This study adds to the already extensive evidence linking abnormal basal forebrain cholinergic function with attentional deficits (e.g. Muir, Everitt and Robbins, 1994; McGaughly, Kaiser and Sarter, 1996; Everitt and Robbins, 1997; Sarter and Bruno, 1997; Turchi and Sarter, 1997; Sarter, Bruno, Turchi, 1999; Risbrough, Bontempi, Menzaghi, 2002). Even though there are numerous recipients of cholinergic basal forebrain output (see Semba, 2000 for a detailed review), the various attentional deficits seen following basal forebrain damage are believed to be primarily due to the corticopetal cholinergic projections. More specifically, evidence suggests that the loss of cholinergic input to prefrontal and parietal

cortical areas, in particular, results in dysfunctional top-down influences upon sensory cortical and thalamic areas giving rise to attentional abnormalities (McGaughy, Everitt, Robbins and Sarter, 2000; Burk and Sarter, 2001; Arnold, Burk, Hodgson, Sarter and Bruno, 2002; Burk, Herzog, Porter and Sarter, 2002; Kozak, Bruno and Sarter, 2006). Despite the apparent role of basal forebrain cholinergic projections in the ASP-generated covert orienting and the fact that cTRN (including the TRNvis) receives collaterals from these projections (Jourdain, Semba and Fibiger, 1989), the data we present here suggests that the involvement of the latter in these processes may not be significant.

Chapter *V.* Effects of unilateral TRNvis lesions on performance in an endogenous and exogenous covert orient task

5.1. Abstract

The evidence presented in the previous chapter contrasts to some degree with that of Weese *et al.* (1999) in suggesting that TRNvis is not involved in processes of visual covert orienting. These contrasting results were attributed to a number of factors such as the different nature of the covert orienting in the two studies (endogenous vs exogenous), the different duration of the stimuli used, the laterality of the lesions (bilateral vs unilateral) and/or the severity of the lesions. We sought to provide a more specific answer to this by investigating the effects of TRNvis lesions (similar to the ones used in the previous chapter, albeit unilateral) on performance in the Posner task (used by Weese *et al*.) and the ASP task (used in the previous chapter). In the Posner task (see Posner, 1980) targets preceded by spatially valid cues were responded faster and more accurately than targets preceded by invalid cues, which misdirected attention away from the location of the subsequent target. In the ASP task, where left targets were more probable at short foreperiods and right targets were more probable at long foreperiods, response latencies were faster to the side of each foreperiod's most probable side. The discrepancy in reaction times between validly and invalidly cued targets in the Posner task and between spatiotemporaly probable and improbable targets in the ASP task reflected the movement of covert attention within visual space in each of these tasks. We found that the unilateral TRNvis lesions did not affect any aspects of performance in either the Posner or the ASP task, suggesting that visual covert orienting, regardless of its nature, is possible even without the involvement of TRNvis. We also suggest that the impaired covert orienting

observed following TRNvis lesioning in the past may have been accounted for by non-TRNvis damage.

5.2. Introduction

In the previous chapter we demonstrated that covert orienting (the covert movement of attention within visual space) triggered by learnt spatiotemporal stimulus probabilities was unaffected by bilateral lesions of the TRNvis. Taken into consideration alongside the findings of impaired exogenously triggered covert orienting following unilateral TRNvis lesions (Weese, Phillips and Brown, 1999), these results suggested that TRNvis may be differentially involved in endogenous and exogenous forms of covert orienting. However, factors unrelated to the source (endogenous vs exogenous) of covert orienting may have also contributed to the contrasting findings of the two above-mentioned studies. One such factor may be the nature of the TRNvis lesions inflicted in the two investigations. More specifically, the lesions in the two studies differed with regard to their laterality (Weese *et al.*: unilateral lesions; chapter *IV*: bilateral lesions) and possibly also, given the different surgery protocols followed, with regard to their extent and severity. A propos the latter, the different neurotoxin injection medium used (Weese *et al*.: metal syringe; chapter *IV*: fine glass micropipette) and the different toxicity of the ibotenic acid injected (Weese *et al*.: 0.09M, chapter *IV*: 0.04M) may have contributed to dissimilarities concerning both the degree of dorsal thalamic (or non-TRNvis in general) damage and also the degree of demyelination of thalamocortical and corticothalamic axons traversing the lesioned area. As a consequence of the above, a comparison of the behavioural results between the two studies in question may not be feasible.

In order to allow a more direct comparison of the effects of TRNvis lesions in the covert orienting behaviours triggered by the two tasks used in the two above-mentioned studies (i.e. the Posner and ASP tasks), it is essential to ensure that the lesions implemented are identical and, furthermore, that they are both selective to TRNvis. Given the success of inflicting highly selective TRNvis lesions in chapter *IV*, we decided to examine the effect of similar lesions, albeit unilateral, on the Posner task, in an attempt to replicate the findings of Weese *et al.* (1999). A replication of Weese *et al*.'s findings with these lesions would rule out the possibility that the different effects of TRNvis damage on covert orienting generated by the Posner and ASP tasks were due to lesion-specific differences and would indicate instead that these are more likely to be concerned with the mechanisms that trigger covert orienting in each of the two tasks. If, however, a replication were not achieved, it would have to be attributed to the different nature of TRNvis lesions implemented by the two studies. In accordance to Weese *et al.*'s findings (see *1.4.2.*), therefore, we predicted that, following the infliction of unilateral TRNvis lesions, the validity effect caused by the pre-target cues in the Posner task will be abolished for responses to targets on the contralateral to the lesion side.

In a second part of the experiment and in order to investigate the role of lesion laterality (unilateral vs bilateral) on ASP-generated covert orienting, we will use the same, unilaterally TRNvis-lesioned, animals and examine their ability to learn and perform the ASP task. Even though it may seem unnecessary to examine the effect of unilateral lesions, given that we have previously shown that bilateral (i.e. greater in overall volume) TRNvis lesions do not affect

performance in the ASP, this comparison may be useful in ruling out the possibility that the hemispheric imbalance (with regard to damaged TRNvis tissue), caused by unilateral lesions, does not bias covert orienting towards one side. In other words, provided that Weese *et al*.'s findings are replicated in the first part of the experiment, a demonstration that the same animals can perform the ASP task would suggest that lesion laterality was not a factor that contributed to the observation of covert orienting changes in Weese *et al*. and not in chapter *IV*. Additionally, this final experimental manipulation will also allow a direct comparison between the behavioural effects of unilateral TRNvis lesions on performance in the Posner and ASP tasks in the same animals. Likewise the bilaterally lesioned animals in chapter *IV*, we expect that the unilaterally lesioned animals would learn the spatiotemporal probabilities of the ASP task and move visuospatial attention according to them.

5.3. Methods

5.3.1. Animals

Twenty-three adult male Lister hooded rats (Harlan, UK) were used for this experiment. Animals were paired-housed in 25x45x15cm sawdust-filled wire-mesh cages, and kept in a light/dark regime changing every 12 hours. Access to food was limited to 15-18g per day per animal. Water was available *ad libidum*.

5.3.2. Apparatus

The training/testing apparatus used was the same as for chapters *III* and *IV* (see *3.3.2.* or *4.3.2.* for more details), and consisted of six 9-hole operant chambers. Only the 3 central of the available 9 holes were used.

5.3.3. Training

Training and testing followed the protocol described in Weese *et al*. (1999). After being introduced to the operant boxes (see *3.3.3.* for more details) animals were trained to maintain sustained nose-pokes in the illuminated central hole of the 3-hole array for a variable foreperiod delay (0.2, 0.3, 0.4 or 0.5 seconds). Successful nose-pokes resulted in the delivery of sucrose pellets in the food magazine. *Early withdrawals* resulted in 2-second dark timeouts without the delivery of food. When every individual animal's performance consisted of approximately 80% successful trials per 30 minute session, training proceeded to its next and final stage (see Figure 5.1). During that stage, a successful central nose poke (again of a variable delay) resulted in the presentation of a bright light (duration: 150ms) in one of the two side holes. The animals' task was to nose-poke in the hole where the target appeared. Preceding the target presentation, and 50ms after the onset of a nosepoke, a cue, in the form of a 100ms-long dim light would appear in one of the two holes. On 50% of trials the cue was in the same location as the subsequent target (valid cue), and for the other 50% the cue was presented in the alternate location with relation to the target (invalid cue). Cues were expected to attract attention involuntarily to their location, thus biasing response latencies to subsequent target stimuli. More specifically, valid cues

were expected to speed response latencies by attracting attention to the location where targets would subsequently appear. On the other hand, invalid cues were expected to mislead attention away from the subsequent target location, slowing down target detection and thus response latency. Nose-pokes in the hole where the stimulus appeared resulted in the delivery of a sucrose pellet in the food magazine. Nose-pokes in the hole opposite that of the target (incorrect errors) and failures to produce a response towards one of the two holes 2 seconds after the presentation of a target stimulus (late errors) resulted in a 2-second dark timeout and in the delivery of no food for that trial. Reaction times were recorded as the time between the presentation of the target stimulus and the withdrawal from the central nosepoke hole.

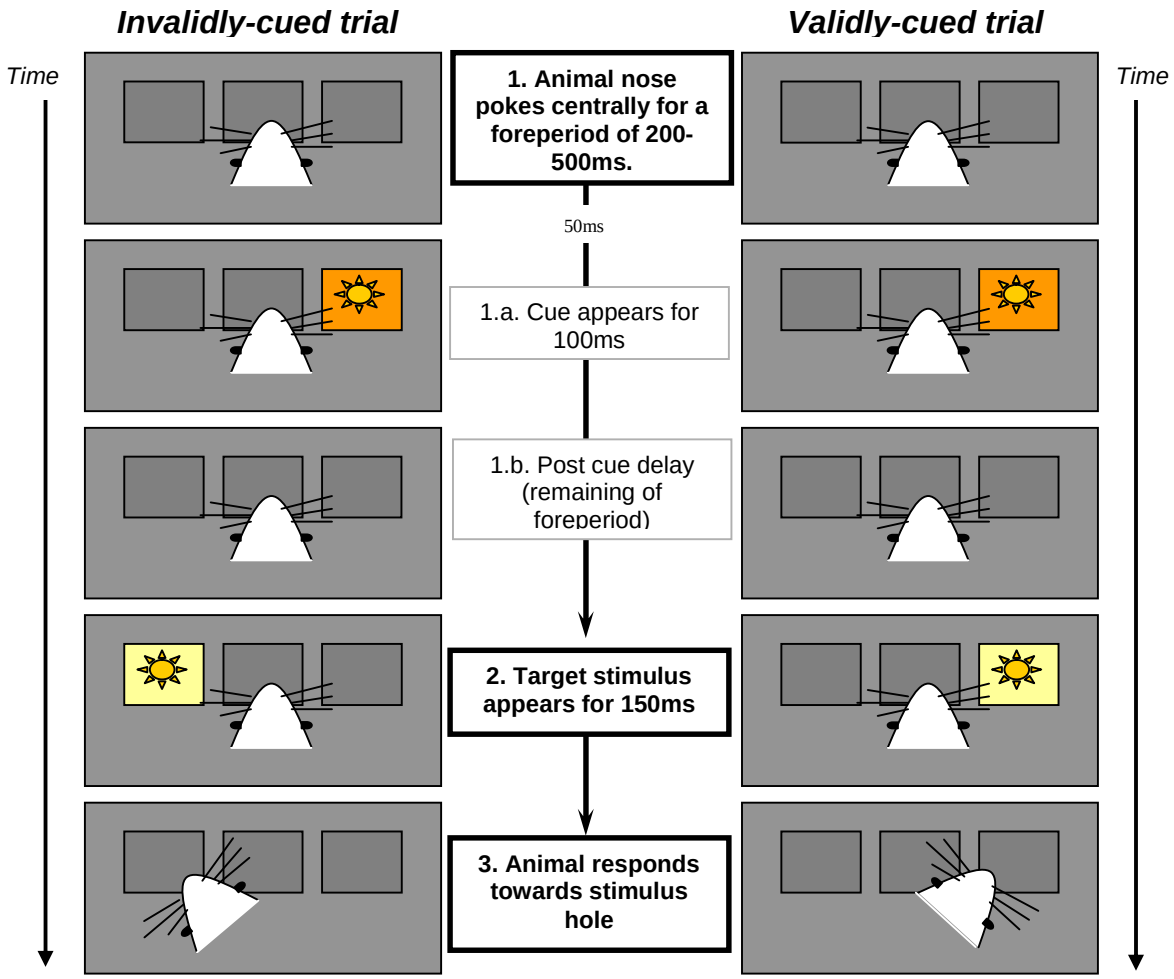

Figure 5.1. Schematic representation of the Posner task, illustrating the event sequences for both validly and invalidly cued trials.

5.3.4. Pre-operative data collection

Two criteria had to be satisfied before pre-operative data could be collected and animals proceed to surgery. Firstly, animals had to respond faster to validly-cued than to invalidly-cued targets (i.e. illustrate a validity effect) and, secondly, animals had to reach a stable baseline level of approximately 70% correct responses per session. The latter criterion was fulfilled within 6 weeks of training on the Posner task. After that point, the former criterion was inspected bi-weekly (using data from 14 days) by a repeated measures ANOVA on all animals' modal reaction times with *cue validity* (valid; invalid)

and *foreperiod* (0.2; 0.3; 0.4; 0.5 sec) as within-subjects variables. The analysis carried out in week 10 of training, revealed that animals responded faster to validly cued targets compared to invalidly cued ones (main effect of cue validity: $F(1,22)=59.05$, $p<.001$), especially for early foreperiods (cue validity by forepriod interaction: $F(3,66)=6.19$, $p=.001$). Inspection of individual animals' performance revealed that three animals did not demonstrate a validity effect and as a consequence these were excluded from the experiment at this point. Following the satisfaction of the two criteria, pre-operative data were collected over the subsequent 14 days.

5.3.5. Surgery

Animals received unilateral excitotoxic lesions of TRNvis, with the side of lesion (left or right) pseudo-randomly determined. Animals were anaesthetised by inhalation of 5% Isoflurane in an anaesthetic induction chamber. They were then placed in a stereoscopic frame, while Isoflurane levels were maintained at 2.5-3% through a facemask fitted to the nosepiece of the frame. While in the frame, animals were given 0.05ml of Carprofen (Rimadyl™) sub-cutaneously. An incision at the surface of the scalp exposed the skull so that both bregma and lambda were clearly visible. A 0.3 x 0.5cm craniotomy was made on one side of the mid-sagittal suture between approximately –1.5 and –4.5mm from bregma, to expose the brain underneath. Anterior-posterior (AP) and mediolateral (ML) measurements were taken from bregma and dorsoventral (DV) measures were taken from the brain dura. Bilateral injections of 0.2µl of 0.04M ibotenic acid were made using glass micropipettes (external diameter of tip: ~30µm) at coordinates AP:

-3.4, ML:+/- 3.7, DV: -5.2mm. Pipettes were left *in situ* for 4 minutes following the injection to allow the absorption of the injected liquid and minimise its upward suction during their retraction. The wound was sealed using 4 metal suture clips (B.Braun, Sheffield, UK). Following surgery animals were given 0.3ml of diazepam, intraperitoneally. Animals were allowed to recover for 7 days before they could be tested.

5.3.6. Post-operative data collection

Following the 7-day recovery period, animals were re-introduced to the task for 3 to 5 days in order to establish responding (data from these sessions was not used in the analyses). Post-operative data were collected over 14 days.

5.3.7. Behavioural data analysis

Behavioural data were analysed using a repeated measures analysis of variance (SPSS, v.12) with *stimulus side* relative to lesion (ipsilateral; contralateral), *cue validity* (valid; invalid), *foreperiod* (0.2; 0.3; 0.4; 0.5 sec) and *surgery* (pre-operative data; post-operative data) as within-subjects variables. Separate analyses were carried out for modal reaction times, incorrect errors, late errors and early withdrawal (anticipatory) errors. Subsequently, post-hoc tests were performed where necessary.

5.3.8. Post-operative ASP training and analyses

Following the post-operative collection of data for the Posner task, animals were introduced to, and trained in, the ASP task (see *4.3.2.*). Given the similar behavioural requirements of the Posner and ASP tasks (i.e. “sustain a central

nose-poke and then nose-poke in the illuminated side hole") the animals were directly introduced to the ASP task without any intermediate training stages. Training in the ASP task lasted for a total of 6 weeks. Data from the last 14 days were used in the analyses to inspect whether the animals had learned the spatiotemporal probabilities of the task. Data were analysed using a repeated measures analysis of variance (SPSS, v.12) with *target side* relative to lesion (ipsilateral; contralateral) and *foreperiod* (0.2; 0.3; 0.4; 0.5; 0.6) as within-subjects variables and *lesion side* (left; right) as a between-subjects variable.

5.3.9. Histological procedures

Following the completion of the behavioural testing, all animals were transcardially perfused with 4% paraformalyhyde in 0.1M Phosphate Buffered Saline (PBS), their brains removed and postfixed overnight in 20% sucrose in PBS. 1 in 4 coronal sections through the thalamus were cut using a freezing microtome, and free-floating slices were processed separately for parvalbumin and NeuN to examine the extent of the lesions in TRNvis and dorsal thalamus. Brain sections were then mounted onto gelatinised slides and stained with cresyl violet to enhance the delineation of brain structures. Sections were examined under the microscope and the extent of the lesions was recorded.

5.4. Results

5.4.1. Extent of lesions

TRNvis (dorsal end of cTRN, between approximately –2.7 and –4.0 from bregma) was completely removed in 18 of the 20 animals that received surgery. In most animals, the lesion extended ventrally to also include the dorsal end of TRNaud. Dorsal thalamic damage was limited to a restricted zone within the dorsal and ventral ventroposterolateral nucleus (VPL). A total of six animals were excluded from the analyses; two sustained incomplete lesions of TRNvis and four sustained lesions that extended considerably beyond cTRN, into dorsal thalamus. Figure 5.2 shows the minimum and maximum extent of the lesions of the 14 lesioned animals used in the behavioural analyses.

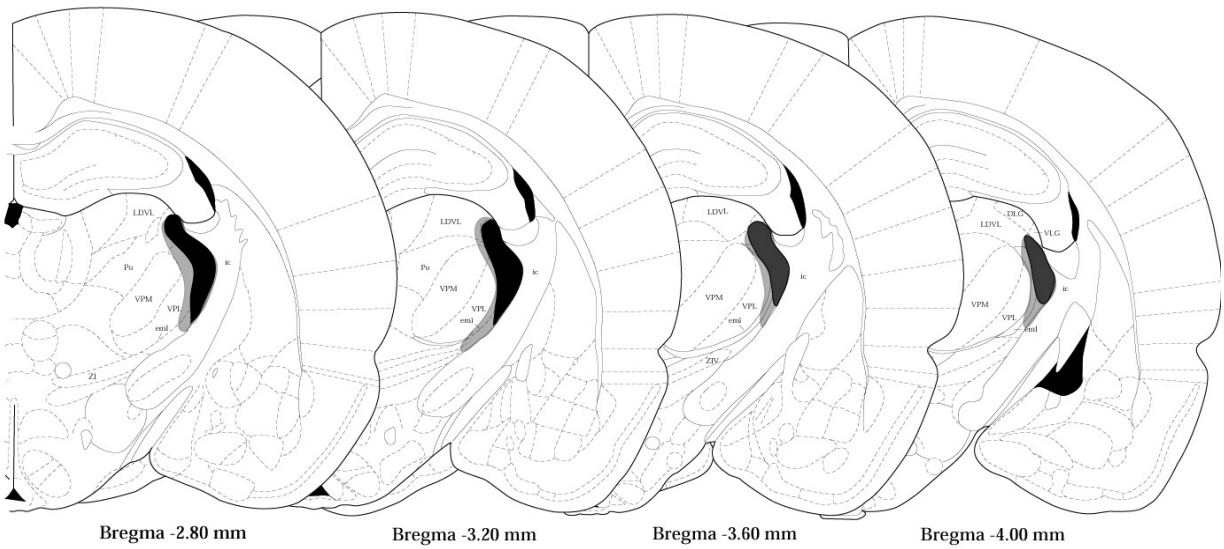

Figure 5.2: Minimum (black) and maximum (grey) extent of lesions. Numbers represent distance from bragma. ic: internal capsule, eml: external medullary lamina, ldvl: ventrolateral laterodorsal nucleus, Po: Posterior nucleus, vpl: ventroposteriolateral nucleus, vpm: ventroposteromedial nucleus, zi: zona inserta, ziv: ventral zona inserta.

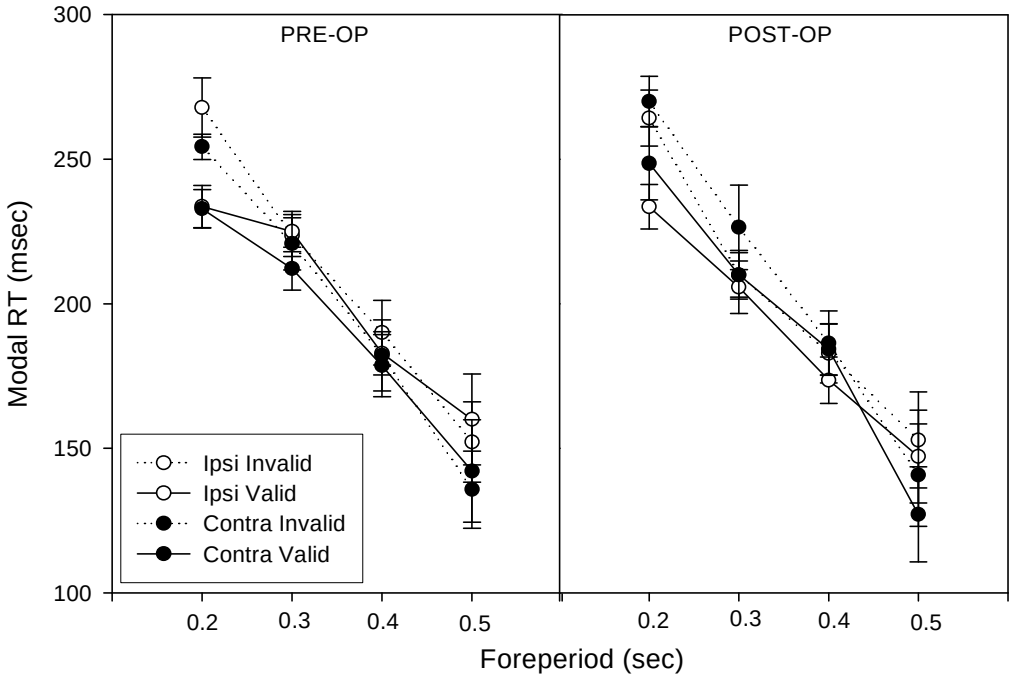

Figure 5.3: Modal response latencies across foreperiods, stimulus sides and cue validities, before and after the infliction of the TRNvis lesions

5.4.2. Behavioural results: Posner task

Fourteen animals in total (seven with left TRNvis lesions and seven with right TRNvis lesions) were used for the behavioural analyses of the Posner task.

5.4.2.1. Response Latency

Response latency became increasingly faster with increasing foreperiod (main effect of foreperiod: $F(3,39)=79.9$, $p<.001$). At early foreperiods, validly cued targets were responded faster than invalidly cued targets, illustrating a validity effect (validity by foreperiod interaction: $F(3,39)=4.09$, $p=.013$, and main effect of validity: $F(1,13)=14.71$, $p=.002$). A pair-samples t-test confirmed that at the earliest foreperiod (0.2 sec) response latencies for invalid trials were

significantly slower compared to those for valid trials for both the ipsilateral (t(13)=3.17, p<.012, new adjusted alpha using the Bonferroni correction method) and contralateral sides (t(13)=2.98, p<.012) pre-operatively. The lesion did not change the effect of cue validity: responses to validly cued targets were faster than invalidly cued targets after surgery for both sides (no surgery by cue validity effect: F(1,13)=1.14, p>.05, and no surgery by cue validity by target side interaction: F(1,13)=.20, p>.05, see figures 5.3 and 5.4). A pair-samples t-test showed that at the earliest foreperiod (0.2 sec) response latencies for invalid trials were significantly slower compared to those for valid trials for both the ipsilateral (t(13)=3.5, p<.012) and contralateral sides (t(13)=3, p<.012) post-operatively too. Modal reaction times to contralateral targets were somewhat slowed post-operatively, however this only approached significance (no surgery by stimulus side interaction: F(1,13)= 4.25, p=.060). A repeated measures analysis of variance (SPSS, v.12) carried out on post-operative data only, with target side (ipsilateral; contralateral), cue validity (valid; invalid) and foreperiod (0.2;0.3;0.4;0.5) as within-subject variables, revealed that cue validity effect on reaction times remained significant (cue validity main effect: F(1,13)=9.39, p=.009)

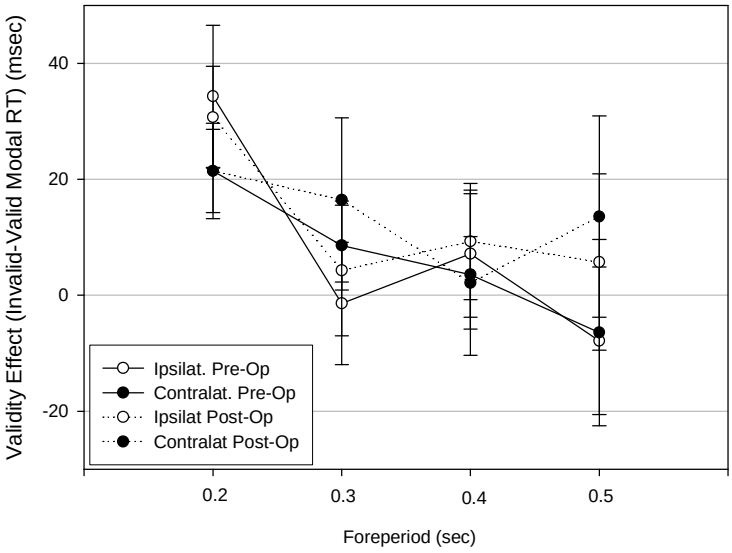

Figure 5.4: Magnitude of validity effect for each side, pre- and post-operatively

5.4.2.2. Response accuracy

The number of anticipatory responses increased with increased foreperiod latency (main effect of foreperiod: $F(3,39)=88.77$, $p<.001$), a pattern that was unaffected by the lesions (all surgery interactions: *NS*). The frequency of incorrect errors varied within the four foreperiods with the more errors produced at the earliest foreperiod (0.2sec) (main effect of foreperiod: $F(3,39)=19.15$, $p<.001$, see Figure 5.5). Furthermore, more incorrect errors were produced for invalidly cued targets than validly cued ones (main effect of cue validity: $F(1,13)=42.09$, $p<.001$, see Figure 5.5). Surgery did not affect this pattern of incorrect responding (all surgery interactions: *NS*). No significant effects were observed with regard to late responding.

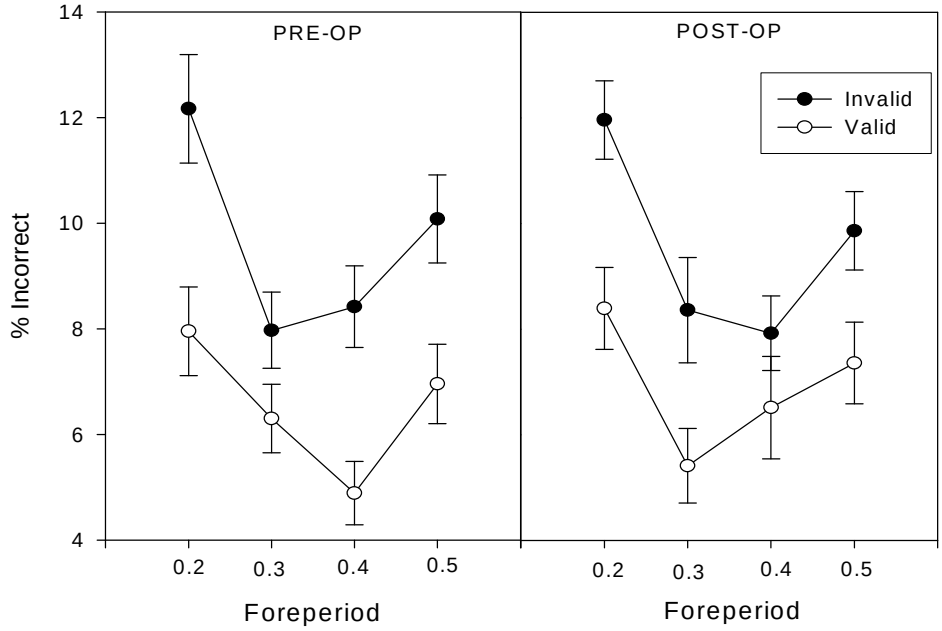

Figure 5.5: Pre-operative and post-operative percentages of incorrect responses across foreperiods, sides and cue validities

5.4.3. Behavioural Results: ASP task

Of the 14 animals used in the Posner task analyses, 11 were used for the final analyses of the ASP task. Three animals developed ulcerative pododermatitis and could not be trained in the operant boxes due to their condition. Of these 11 animals, 6 sustained left TRNvis lesions and 5 sustained right TRNvis lesions.

5.4.3.1. Response latency

Response latency was faster for late foreperiods compared to early ones (main effect of foreperiod: $F(4,36)=134.34$, $p<.001$). Furthermore, the pattern of responding reflected the spatiotemporal probabilities of the ASP task, as left TRNvis lesioned animals responded faster to ipsilateral to the lesion (left) targets at early foreperiods and faster to contralateral to the lesion (right) at late foreperiods; on the other hand, right TRNvis lesioned animals responded

faster to contralateral to the lesion (left) targets at early foreperiods and faster to ipsilateral to the lesion (right) at late foreperiods (target side by foreperiod by lesion side interaction: $F(4,36)=9.12$, $p<.001$, see figures 5.6 and 5.7). Finally, there was no difference in response latency between ipsilateral and contralateral to the lesion targets (no side main effect: $F(1,9)=.8$, $p>.05$).

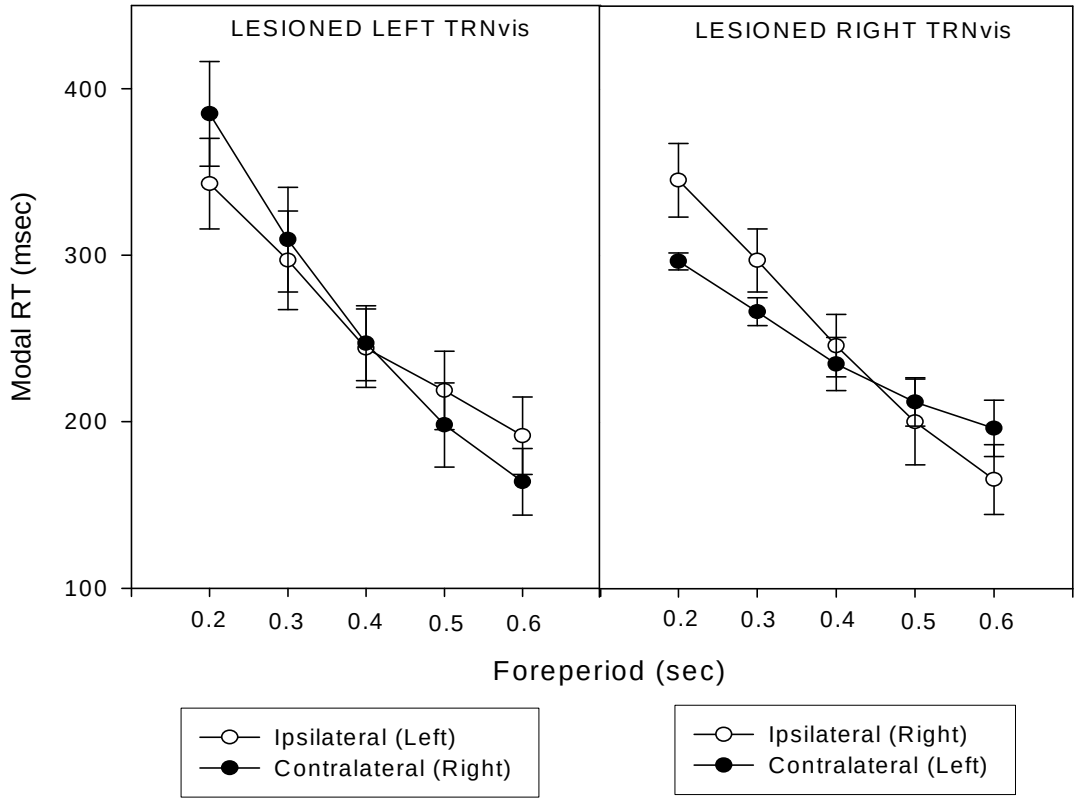

Figure 5.6: Modal reaction times for left and right targets across all foreperiods for animals sustaining left and right TRNvis lesions

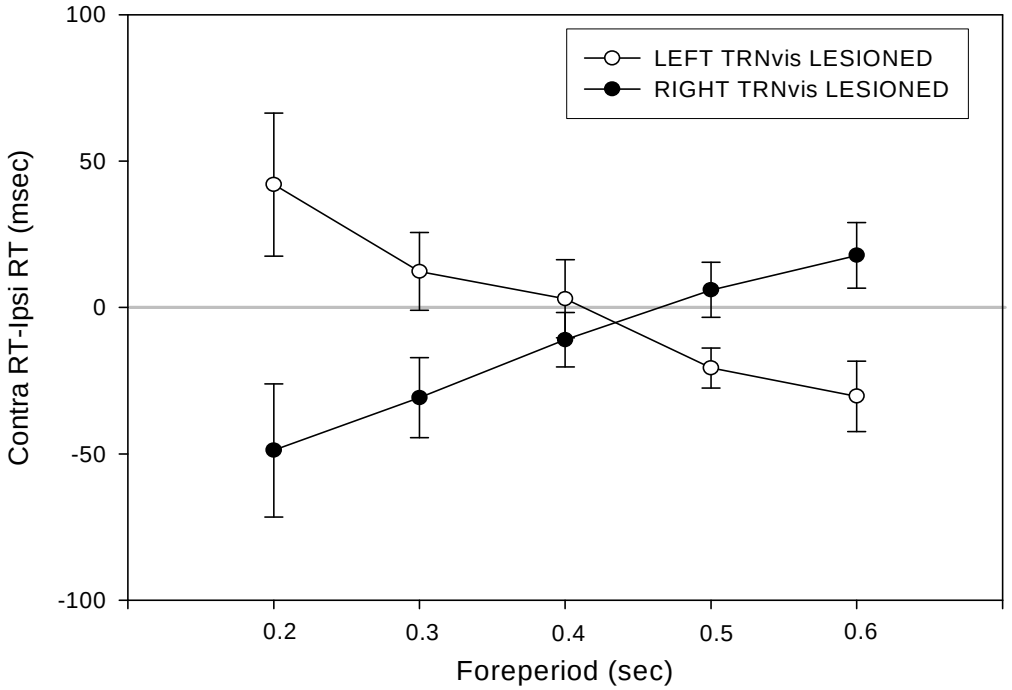

Figure 5.7: Subtracted contralateral from ipsilateral modal reaction times for each foreperiod for left- and right-TRNvis lesioned animals.

5.4.3.2. Response accuracy

The frequency of incorrect responses varied across foreperiods (main effect of foreperiod: F(4,36)=5.93, *p*=.001). However, the frequency of errors for contralateral and ipsilateral to the lesion targets differed for animals sustaining lesions to the left or right TRNvis (target side by foreperiod by lesion side interaction: F(4,36)=3.57, *p*=.015). More specifically, similarly to the incorrect error pattern seen and explained in chapter *IV* (see *4.4.3.*), animals responded according to an instantaneous conditional probability. That is, at early foreperiods animals were equally likely to make a left or right erroneous response, but as foreperiod progressed animals became more likely to respond to the (increasingly probable) right side at the expense of the (increasingly improbable) left side, thus increasing the frequency of left errors.

Given that in our analyses target side was coded not as actual spatial side (left vs right) but according to its relation to the lesion side (ipsilateral vs contralateral) the left and right TRNvis lesioned groups demonstrated reversed patterns of the above interaction (see Figure 5.8).

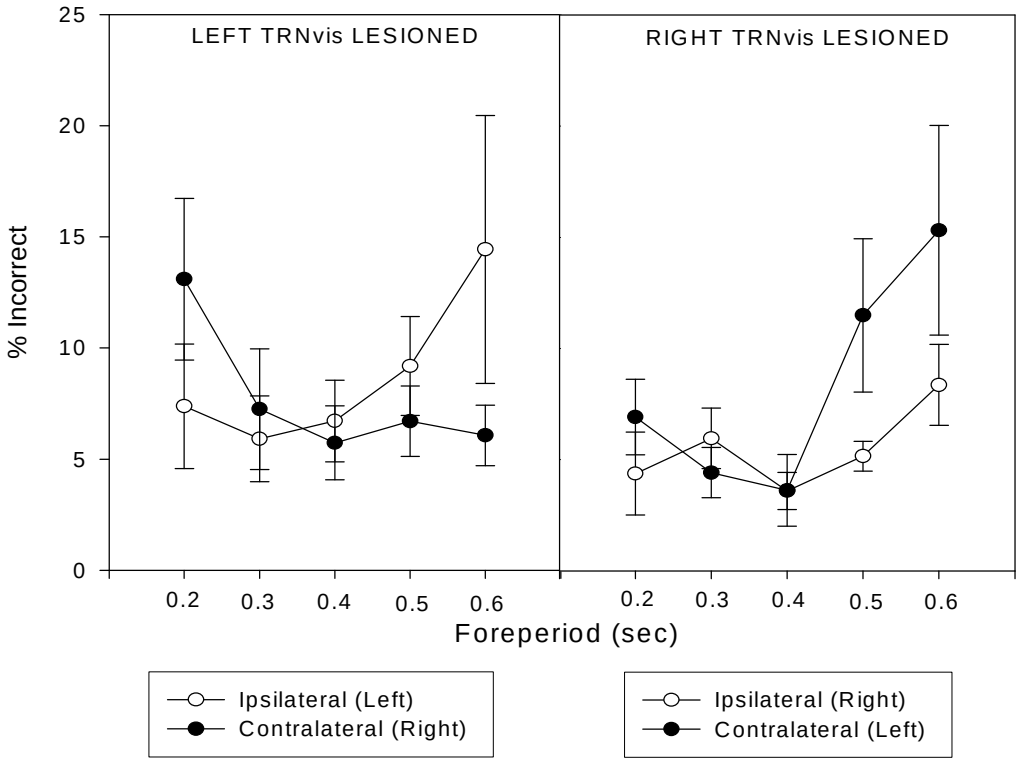

Figure. 5.8: Frequency of incorrect errors for all foreperiods for both left- and right-TRNvis lesioned animals.

5.5. Discussion

5.5.1. Posner Task

The Posner task measures covert orienting through the use of cue stimuli that attract attention towards the location of subsequent target stimuli (i.e. *valid cues*) or away from it (i.e. *invalid cues*), thus creating a "validity effect" by respectively speeding up and slowing down target response latencies. Indeed, we found that our animals responded faster to validly cued targets than to invalidly cued ones for the earliest foreperiod (0.2 seconds), which is the foreperiod with the maximal temporal proximity between the cue and the target stimuli. As foreperiod latency progressed, the temporal gap between the cue and the target increased, thus providing more time for the re-adjustment of covert orienting following its initial capture by the cue. As a consequence, no validity effect was observed for late foreperiods. In addition to the manipulation of response latencies to subsequent targets, cues also affected the accuracy of responding. More specifically, target stimuli that were preceded by invalid cues were responded to incorrectly more frequently than validly cued ones, a further reflection of the cues' attraction of attention that often resulted in responses to be made towards their side. The fact that this was the case across all foreperiods probably reflects the triggering of motor preparation processes (for a response towards the location of the cue) that could not be inhibited within the time window defined by the cue presentation and the latest foreperiod (0.5 seconds). On the basis of previous findings (Weese *et al*., 1999), we predicted that, following unilateral TRNvis lesions, the cue validity effect would be abolished for target stimuli appearing at the contralateral to the lesion side, the result of an inability to "move" attention

covertly (covert orienting) within the visual hemifield corresponding to the lesioned TRNvis. What we found instead, however, was that the infliction of the lesions did not alter the above pattern of responding as the validity effect for the earliest foreperiod was preserved for both sides (ipsilateral and contralateral). This evidence suggests that the ability to covertly orient attention within visual space is independent of normal TRNvis functioning.

This lack of a contralateral deficit (with regard to covert orienting) could not be explained by contralateral visual information being processed by the intact ipsilateral TRNvis (and thus the “healthy” ipsilateral LGN). This is because in the rodent LGN, which constitutes the only sensory input source for the TRNvis and, conversely, the major output target of TRNvis (see *1.2.6.1.*), 92-97% of the visual information arrives from the contralateral retina (Polyak, 1957; Jeffrey, 1984, as cited in Grieve, 2005), thus making it effectively a monocular structure. It can be assumed, therefore, that the intact TRNvis-LGN pair had access to insufficient amount of visual information from the contralateral eye to be able to compensate for the lesioned TRNvis. In addition, the area of binocular vision in the rodent is limited to less than 70° degrees (Block, 1969; Heffner and Heffner, 1992), occupying the visual space directly in front of the animal’s nose, which in our task corresponded to the area occupied by the central hole. As a consequence, the signal carried by each eye contained visual information from one visual hemifield only, within which stimulation from one stimulus hole was available.

On the whole, therefore, despite the use of the same training equipment and behavioural regime, our findings do not match those of Weese *et al*. This leads us to assume that the nature (extent and/or severity) of the lesions inflicted by the two studies may have differed.

5.5.1.1. Lesion specificity and comparison

In the present study (and similarly to the previous chapter) the inflicted lesions covered the whole of dorsocaudal TRN (i.e. TRNvis) and, moreover, were verifiably selective to that area, with no or very little damage extending into dorsal thalamus. Although circumstantial, it would be reasonable to assume that the lesions inflicted by Weese *et al*. were greater in extent. This is not only because of the use of injection needles of wider tip than the ones used here, but also due to the greater concentration of the neurotoxic agent used. It is known that as the toxin travels away from the injection site, it weakens in molarity (toxicity), resulting in less neuronal damage (Brace, Latimer and Winn, 1997). As a consequence, the neuronal damage caused by a 0.04M ibotenic acid injection (as the ones used in the present investigation) will be limited to considerably closer to the injection site compared to a 0.09M injection of the same volume (used by Weese *et al*.). Considering that our lesions were practically selective to TRNvis, any potential extension of the neurotoxin's effect would have resulted in dorsal thalamic damage. Furthermore, it is known that the greater the level of ibotenic acid toxicity, the greater the degree of demyelination of thalamocortical and corticothalamic cells traversing the lesioned area (Coffey, Perry, Allen, Sinden and Rawlins, 1988; Stellar, Hall and Waraczynski, 1991). Therefore, our lesions can be

assumed to have interfered less with the direct thalamo-cortico-thalamic communication, compared to the ones inflicted by Weese *et al*.

As a consequence of the presumed different lesions implemented, a direct comparison of the behavioural effects of TRNvis lesions between Weese *et al*. and our study in chapter *IV*, with regard to the nature of covert orienting (exogenous and endogenous covert orienting), cannot be carried out. A more appropriate comparison in this regard would be between the effects of TRNvis lesions on the Posner task in the present study and the effect of such lesions on the ASP task, as examined here and also in chapter *IV*.

5.5.1.2.TRNvis lesioning, validity effect and stimulus detection ability

As suggested in chapter *IV* (see *4.5.1.*), except from an inability to move covert orienting in visual space, a reduction or abolition in the validity effect following the TRNvis lesion could have also resulted from a reduced ability to detect contralateral cue stimuli. This would have resulted in a failure of these cues in attracting attention to their location, thus speeding up subsequent response latencies to (invalidly cued) ipsilateral targets and slowing down response latencies to (validly cued) contralateral targets, eradicating the time discrepancy that defines the validity effect. This suggestion came about considering the involvement of cTRN in the generation of burst firing in thalamocortical relay cells (Crick, 1984; French, Sefton and Mackay-Sim, 1985); a firing mode that could facilitate, specifically, the detection of brief/non-salient stimuli (such as the cue stimuli used in the Posner task). This, however, was not reflected by our data, as contralateral cue detection

(measured indirectly through the cues' effect on response latencies to target stimuli) was unchanged post-operatively.

We had hypothesised that the reason behind the lack of stimulus detection detriments in the ASP task in chapter *IV* (and also in the second part of the present investigation) and in the divided attention task in chapter *III*, after the infliction of the TRNvis lesions, was the relatively long duration of the target stimuli used in these studies (0.5 and 1.5 seconds respectively). Due to the shorter duration of the target stimuli used here (0.15 sec) a contralateral target detection deficit following the lesion would not have been altogether surprising. However, we found that, here too, the lesion did not impair, albeit marginally, the detection of contralateral target stimuli. Combined, the above observations suggest that stimulus duration may not necessarily be the determining factor that recruits TRNvis's involvement. However, the proximity to statistical significance, with regard to the lesion effect on the speed of detection of contralateral targets and, also, the fact that the cues' detection was only measured indirectly, do not allow us to completely dismiss the idea that TRNvis' function could be related to detection enhancement mechanisms for brief stimulation. This may worth looking at in the future. Factors such as the (un)predictability or novelty of a stimulus have also been suggested as possessing great importance with regard to TRNvis recruitment, due to the fact that they appear to generate burst firing in the thalamocortical cells that convey their signal to cortex (Sherman and Guillery, 2001; Weyand, Boudreaux and Guido, 2001). How much these factors determine the involvement of TRNvis in these processes, however, could only be answered

though a direct investigation of the effects of TRNvis lesions on the detection of stimuli of variable degrees of novelty/predictability and duration or, even better, through the electrophysiological characterisation of activities in interconnected thalamocortical and TRNvis cells *in vivo,* during the presentation of such stimuli.

5.5.2. ASP task

As predicted, animals that sustained unilateral TRNvis lesions learned the spatiotemporal probabilities of the ASP task and responded to stimuli according to these. The side of the lesion (left of right) had no effect on their ability to learn the task. More specifically, regardless of the side of the lesion, they responded faster to left targets at early foreperiods and faster to right targets at late foreperiods. Moreover, the pattern of incorrect responses reflected the instantaneous spatiotemporal probabilities, as the animals produced more errors for the improbable left targets at late foreperiods. This evidence adds to that of the previous chapter, which suggested that TRNvis lesions do not interfere with the ability to perform the ASP task, and thus it can be assumed not to impair the ability to move endogenous attention within visual space. Furthermore, as initially suspected, these results suggest that lesion laterality (unilateral vs bilateral) is unrelated to the maintenance of this ability.

5.5.3. Concluding remarks

Taken together, the findings of the two parts of the present study (and also of the study in chapter *IV*) suggest that TRNvis may not participate with an

active role in the neuronal network underlying the generation of covert orienting behaviours, both in an endogenous and an exogenous context. Despite its role in burst generation (with all the benefits that this firing mode carries with regard to attention, see Crick, 1984; Sherman, 2005) and the topographic signal of its inhibitory output on thalamocortical cells, which could allow the enhancement of the relay of sensory information for selective foci within visual space (corresponding to the areas of attentional orienting), the evidence we present here does not support such a role for the TRNvis.

Chapter VI. General Discussion

6.1.Theoretical background and experimental summary

Due to the relatively late onset of awareness regarding the potential role of TRN/cTRN in attentional processes, combined with the difficulty of employing most experimental techniques on it (see *1.4.*), the available evidence for the plausibility of such a functional attribution is somewhat limited. This is particularly the case when it comes to behavioural evidence, which is limited to the findings of only a handful of studies (see *1.4.1.-1.4.3.*). The evidence from these studies, however, indicate that the functional role of cTRN goes beyond sensation and that it possibly extends to processes directly or indirectly related to attention. For example, early functional Fos immunocytochemistry investigations suggested that the modality-specific sectors of cTRN do not activate when sensory stimulation is simply delivered through their associated modalities but only when this stimulation is actively attended (Montero, 1997, 1999; McAlonan, Bowman, and Brown, 2000). In addition, lesions of TRNvis have been found not to render animals blind but to specifically affect their ability to covertly orient in visual space (Weese, Philips and Brown, 1999). However, methodological and technical limitations in the above investigations bring to question the validity of their findings (see *2.2.* and *4.5.3.* for details). In the four experimental chapters of this thesis we attempted to tackle some of these technical issues and, in addition, examine the potential functional role of cTRN in a variety of attentional behaviours.

In chapter *II* we demonstrated that selective TRNvis activation is elicited when attention is directed to visual stimulation, but not when visual stimulation is presented and not attended. What differentiated these results from the ones of previous similar investigations (see above) is that no differential sensory stimulation or differential sensory pre-exposure factors were in effect. These results, therefore, make a stronger case for a TRNvis (and by extension cTRN) involvement in attention. Having demonstrated that TRNvis activity is linked with attentive behaviours (visual selective attention in particular), we then attempted to examine the potential involvement of this area in other forms of attention, in order to generate a more complete profile of its functional role in attentional mechanisms. We decided to do so by means of lesioning of the cTRN and observing potential changes in attentional behaviours. In this attempt, we were helped by the ability to inflict satisfactorily selective lesions within cTRN, through the use of glass micropipettes (as injecting medium) and the choice of ibotenic acid of medium toxicity (0.04M). Initially (chapter *III*), we sought to examine whether combined TRNvis/TRNaud lesions would impair performance in a cross modal (visual/auditory) divided attention task. Subsequently (chapters *IV* and *V*), we focused on visual (visuospatial in particular) attention only and investigated the effects of TRNvis lesions on the performance of covert orienting behaviours that were generated either by exogenous (spatial cueing) or endogenous (learned spatio-temporal probabilities) means. In line with previous reports (Weese *et al.*, 1999), our lesions did not result in any purely sensory deficits (blindness or deafness), as animals continued to respond to visual and auditory stimulation, an observation that fits with the modulatory

(rather than driving) functional profile of the nucleus (McAlonan and Brown, 2002; Sherman, 2005). However, the destruction of cTRN did not result in observable differences in the animals' ability to perform the examined attentional behaviours.

6.2. Chapter II: cTRN activation by attentional behaviours

The activation of TRNvis observed in chapter *II* following the performance of a visual discrimination task may reflect the involvement of this sector in the active modulation of the signal carried by visual thalamocortical relay cells, which constitute its only output projection targets (Hale and Sefton, 1982; Coleman and Mitrofanis, 1996). Given that TRNvis' projections are exclusively GABAergic (Houser, Vaughn, Barber and Roberts, 1980; DeBiasi, Frassoni and Spreafico, 1986), and thus exert inhibitory post-synaptic effects upon thalamocortical cells, their modulatory function could be carried out in two possible ways. The first possible modulatory function is thought to be one of minimisation of background noise activity in thalamocortical cells, aiming at enhancing their communication of behaviourally relevant visual signals to cortex. This could be achieved by hyperpolarising thalamocortical cells, not enough however to prevent action potentials from being generated, but sufficiently to minimise spontaneous activity within them, thus allowing signals from behaviourally-relevant stimuli to emerge as more salient. Because of the presumed interference of the low luminance levels (under which the visual discrimination task was carried out) with the perception of the visual aspect of interest (colour of digging bowl) a minimisation of noise levels in thalamocortical cells may have been necessary to carry out successfully the

visual identification/discrimination. It has to be said, however, that due to the presence of $GABA_B$ and extrasynaptic $GABA_A$ receptors in thalamocortical cells (e.g. von Krosigk, 1992; Ulrich and Huguenard, 1996a; 1996b; also see Cox, Huguenard and Prince, 1997; Jia, *et al*., 2005), and the resultant long-lasting hyperpolarizing effects of their activation, it is possible that TRNvis' GABAergic output could have also de-inactivated I_T in these cells thus causing a transition of firing mode from tonic (the predominant firing mode during awake states) to burst. Even though burst firing may appear inappropriate for the analysis of stimulation, due to its non-linear signal transmission properties (Sherman and Guillery, 2001), its better signal-to noise ratio of transmission may have made it preferable over the usually "noisy" tonic activity in the communication of visual signals under the unfavourable visual conditions that the visual discrimination was performed. This issue was also raised in chapter *IV,* where it has been postulated that the duration or saliency of a stimulus (in other words, the sensitivity of that stimulus' detection to background noise) may be the determining factor for the generation of burst activity in thalamocortical cells and thus the involvement of TRNvis. Whether TRNvis' activation, seen in visually attentive animals, and its resultant inhibition of visual thalamic cells induced burst firing or not in the latter could only be answered through single-cell electrophysiological recordings over the performance of the task.

The second modulatory function that TRNvis' selective activation in chapter *II* could have reflected is one of lateral inhibition. Lateral inhibition mechanisms can be very effective in "weakening" activity in sensory areas adjacent to the

one(s) of interest, thus minimising the degree by which these could interfere with the latter's processing. In other words, lateral inhibition can eliminate potential attentional competition from other sensory areas, thus enhancing the processing of the signal of interest. Given that in our visual discrimination task attentional competition did not come from within the visual modality but, instead, mainly from somatosensation (i.e. the other sensory modality that the animals had been trained to make discriminations), a lateral inhibition mechanism may have been aiming at the weakening of activities in the somatosensory thalamus. It has been suggested (Crick, 1984; also see Montero, 1997) that in situations where there is competition for attentional resources between different sensory modalities, the long dendritic arbours of TRN cells (see Scheibel and Scheibel, 1966) may be put into use, contributing to a lateral inhibition mechanism *between* cTRN sectors. In such a mechanism, cells of the cTRN sector corresponding to the attended modality would inhibit cells in the cTRN sector(s) corresponding to the non-attended modality/-ies in order to preserve the attentional focus. In our task, for example, cells in TRNvis may have activated in order not only to lower noise levels in visual thalamocortical cells but also in order to inhibit TRNsom cells (i.e. the cTRN cells corresponding to the "competing" modality) and thus prevent them from performing a similar action on somatosensory thalamocortical cells. The spatial proximity of the cTRN sectors corresponding to the three main sensory modalities in which attentional competition is more likely to take place (i.e. vision: TRNvis, audition: TRNaud and somatosensation: TRNsom) is such that a model of triadic, inter-sector, lateral inhibition would be favoured (see Figure 1.3, in *general introduction*).

However, although this seems plausible, it has to be noted that there is no empirical evidence in support of such a mechanism. Furthermore, this scenario does not explain how the activation of cells in one sector results in the inhibition of cells in other sectors without also resulting in the inhibition of cells within the same sector. In addition, as mentioned above, the inter-sector cTRN lateral inhibition hypothesis is based on the assumption that the majority of cTRN cells possess long enough dendrites to cross sectors' borders, an anatomical feature the actuality of which is currently questioned (see Pinault, Smith and Deschênes, 1997).

The contribution of cTRN (as reflected by its modality-specific activation) in attentional behaviours is thought to be dictated by its multiple inputs coming from cortex (Jones, 1975; see *1.2.4.*), basal forebrain (e.g. Jourdain, Semba and Fibiger, 1989; Asanuma, 1989, Semba, 2000, also see *1.2.8.2.*), brain stem (e.g. Jourdain *et al.,* 1989; Asanuma, 1992; Spreafico *et al.*, 1993, see *1.2.8.2.*) and the dorsal thalamus itself (Jones, 1975, see *1.2.4.*). Of these projections, the one with the most influence on cTRN function is believed to be the heavy glutamatergic corticothalamic feedback projection from layer VI of sensory cortices, which collateralises within the nucleus en route to dorsal thalamus (Liu and Jones, 1999). These corticothalamic projections are thought to carry top-down instructions, which directly, but also indirectly through cTRN, modulate activity in selected areas of the dorsal thalamus according to the immediate attentional/behavioural demands (see Montero, 2000). Discontinuation of these projections diminishes attention-related activation within cTRN (Montero, 2000), but it is unknown whether it also

results in attentional deficits. The role of the basal forebrain and the various brainstem projections in the recruitment of cTRN during attention-demanding situations could be of equal importance. Despite the lack of sensory topography in their signal, some of these projections do posses a crude segregation of their signals to the different modality sectors of the cTRN (e.g. see Spreafico *et al.*, 1993; Semba, 2000). This could allow these projections to target selectively only specific sensory cTRN sectors, thus contributing to a sensory-selective manner to any attentional processes taking place there.

The fact that no cellular Fos activation was observed in TRNsom after tactile attentive behaviours in chapter *II* was seen as a potential indication that the involvement of cTRN in attentional behaviours may differ between different forms of attention (the other alternative explanation being the overall low Fos activation in somatosensory thalamic pathways). This is because the visual and tactile attentional tasks were not analogous within their respective modalities and could have, therefore, required different degrees of involvement from their respective cTRN sectors in order to be carried out. Indeed, as was seen in chapters *III-V*, cTRN is not involved in all attentional processes as its destruction did not affect several aspects of some attentional behaviours.

6.3. Chapters III-V: cTRN lesions and attentional behaviours

6.3.1. Chapter III. cTRN lesioning and cross-modal divided attention

In chapters *III-V* we looked at a range of different attentional behaviours and the degree to which their performance was affected by lesions in the cTRN

sector associated with the sensory modality they were carried out in. In chapter *III* we observed that combined TRNvis and TRNaud lesions did not affect the ability to divide attention between the visual and auditory modalities, as there was neither an increase nor a reduction in the behavioural costs associated with the simultaneous monitoring of two informational channels, compared to the monitoring of only one. Nonetheless, the animals' ability to discriminate between the test stimuli (within each modality) was poorer following the lesions, an effect however unrelated to the division of attention. Having in mind the idea that cTRN may act as a "noise filter" in thalamocortical cells (see *6.2.*), the finding that TRNvis/aud lesions resulted in poorer discriminatory ability could be explained by elevated background noise activities in thalamocortical cells due to the absence of TRNvis/aud intervention. In other words, the lack of TRNvis/aud hyperpolarisation on visual and auditory thalamocortical cells may have prevented the clearance of unwanted, interfering, noise, which could have hampered the detailed analysis of the transmitted signal, making the identification of the stimulation more ambiguous. The greater ambiguity of signal identification could have then resulted in more erroneous identifications of the stimuli and the generation of more incorrect responses. However, it has to be noted that high levels of baseline noise activity are not always bad for the analysis of stimulation. More specifically, relatively high levels of noise, when relatively stable across time, can provide more "room" for the better delineation of certain stimulus properties, especially those related to contrasts or movement within sensory space (Sherman and Guillery, 2001). However, cTRN inhibition

may be specifically targeting irregular noise that is likely to interfere with the perception of the signal of interest and not baseline noise in general.

In a very similar scenario to the above, the greater ambiguity in stimulus identification could have resulted from the expansion of receptive fields of thalamocortical cells, the result of the cessation of TRNvis/aud inhibition upon them (Lee, Friedberg and Ebner, 1994a). The expansion of thalamocortical cell receptive fields, however, could be simply an alternative way of defining increased noise transmission in these cells as it could represent a reduction in the specificity of the sensory signal that activates them.

6.3.2. Chapters IV and V: cTRN lesioning and visual covert orienting.

In the last two chapters we investigated the role of TRNvis in the animals' ability to move covert orienting within visual space. We looked at two different expressions of covert orienting triggered by either exogenous means (Posner task, chapter *V*) or endogenous ones (ASP task, chapters *IV* and *V*).

We found that TRNvis lesions (bilateral and unilateral alike) did not prevent the covert movement of attention, as the behavioural benefits (and costs) stemming from covert orienting (regardless of its endogenous or exogenous nature) were preserved following surgery. This suggested that despite the topography of its signal, TRNvis may not be responsible for the generation and movement of the attentional spotlight as once suggested (see Crick, 1984), at least to the degree that this was assessed by the two abovementioned behavioural tasks. Our results, therefore, contrast with those

of Weese *et al.* (1999), who reported impaired contralateral covert orienting in the Posner task following TRNvis lesions. The lack of covert orienting deficits in chapter *IV* was initially attributed to three possible reasons (or any combination of these): *a)* the fact that, in contrast to the Posner task used by Weese *et al*., covert orienting in the ASP task was generated by endogenous means and could therefore depend on a different neuronal substrate that required little or no involvement by TRNvis, *b)* the comparatively longer duration of the stimuli used in the ASP task that may have not compromised detection and thus prevented possible post-lesion stimulus acquisition deficits from being expressed, and *c)* the different nature of the lesions in the two studies. However, after observing that similar lesions to the ones in chapter *IV* did not result in covert orienting deficits in the Posner task too (chapter *V*), we dismissed the first two assumptions and we concluded that the different behavioural results reported here and in Weese *et al*. were more likely to be due to differences in the lesions. Due to the verifiable selectivity of the lesions in our investigations, we believe that our results reflect more reliably the role of TRNvis in covert orienting.

6.4. Stimulus detection and cTRN involvement

Due to the hypothesised implication of thalamocortical burst activity (and consequently of cTRN involvement) in processes of stimulus detection (Guido, Lu, Vaughan, Godwin and Sherman, 1995; Guido and Weyand, 1995) we had postulated that the lesioning of cTRN would result in stimulus detection detriments. The lack of such effects following the lesions in the divided attention and ASP tasks was attributed to the relatively long duration

of the stimuli used in these tasks, which may have resulted in a floor-effect regarding the difficulty of these stimuli at being detected. In the Posner task, where targets were considerably shorter in duration (thus a greater challenge to detect), we observed that TRNvis lesions somewhat slowed response latencies to contralateral target stimuli. Even though this effect was not statistically significant, it approached significance and therefore some caution must be exercised in accepting this negative result. If it is replicated, such an effect would imply that TRNvis's involvement in stimulus detection processes may be specifically required for stimuli of short duration (i.e. detection challenging stimuli). This would have to be looked in more detail, by investigating the involvement of TRNvis in the detection of stimuli of variable duration and saliency under identical attentional/behavioural conditions (unlike here, where the stimuli of variable duration were presented in different contexts (tasks)).

In addition to being optimal for the detection of brief and sub-threshold stimuli, burst firing has also been suggested to represent the ideal "stimulus acquisition" means for novel or unexpected stimuli, regardless of their duration and saliency (Sherman and Guillery, 2001). Indeed some evidence suggests that novel or surprising stimuli generate burst firing in thalamocortical cells (see Weyand, Boudreaux and Guido, 2001). Due to the lack of paired-pulse depression (see Swadlow and Gusev, 2001; Sherman, 2001) burst firing in thalamocortical cells could maximally activate post-synaptic cortical cells, acting as a wake-up call also for the presence of unforeseeable and potentially behaviourally-relevant stimulation. Such an

optimisation of detection of unpredictable stimuli could be behaviourally invaluable especially to prey species but also, in general, to any species with natural enemies. Possessing the ability to detect such stimuli, especially when brief or near threshold, can enhance significantly one's chances of surviving a predator attack. One may consider the ASP task as an example of a situation where unpredictable stimulation takes place (i.e. when a target appears at a temporally improbable location). In the ASP task, however, the degree of "surprise" caused by a spatiotemporaly improbable target was minimal, as it represented the only alternative to a probable target and moreover it could only appear at a fixed location. If novelty/unpredictability is indeed one of the factors that determines the generation of burst firing in thalamocortical cells, and thus the degree of involvement by cTRN, then it is possible that the repetitive training/testing regime of the behavioural tests we used, took away this element from the test stimuli and thus minimised the requirement for both the generation of burst firing in dorsal thalamus and consequently for cTRN involvement, thus explaining the lack of detection detriments following its lesioning.

6.5. rTRN and the cortical cholinergic input attentional system

As described above, the ability to carry out attentional behaviours such as covert orienting within visual space or the ability to process simultaneously two separate modality informational channels for the detection and identification of stimuli, was unaffected by the lesioning of cTRN. However, these, or highly related, attentional behaviours have been found to deteriorate following interference with the normal cholinergic activity of the brain. For

instance, in tests of divided attention, the administration of the muscarinic receptor antagonist scopolamine is known to increase response latencies in conditions where attention has to be cross-modally divided but not in conditions where attention is directed to a single modality (McGaughy, Turchi and Sarter, 1994), thus suggesting a role for acetylcholine in processes of division of attention. Similar behavioural findings have been reported after 192-IgG Saporin lesions targeting cholinergic corticopetal cells of basal forebrain, thus localising the source of the deficit to the cortical cholinergic input system (Turchi and Sarter, 1997). Moreover, similar lesions have been found to abolish the validity effect in the ASP task by removing the response latency acceleration and deceleration elements from spatiotemporaly probable and improbable stimuli, respectively (Farovik and Brown, 2006). The cortical cholinergic input system, therefore, appears to posses a robust, broadly-tuned, role in the generation of the above attentional processes.

Despite receiving collaterals of the corticofugal cholinergic projections of basal forebrain (Jourdain, Semba and Fibiger, 1989), cTRN does not appear to participate in the above processes that the discontinuation of the corticofugal part of this projection affects. The two branches of the cholinergic basal forebrain output projections (to cortex and TRN respectively), therefore, may represent branches of separate functional networks, with the functions of the latter remaining by and large unknown. This applies not only to cTRN but also to the rostral part of the TRN (rTRN), which receives the majority of the basal forebrain cholinergic projections (Jourdain *et al*., 1989). Like its caudal counterpart, rTRN has been linked to processes of information filtering and

selection. More specifically, rTRN's functions have been proposed by Wilton, Baird, Muir and Aggleton, (2001) to be concerned with the *"focusing of attention with regards to the information processed (in its afferent and efferent areas)"(p.186)*(i.e. the prefrontal and retrosplenial cortices and the mediodorsal and anterior thalamic nuclei, see *1.2.7.2.*), which are associated with a multitude of executive functions. However, similarly to cTRN, the difficulty of inducing selective lesions in rTRN (M'Harzi, Collery and Delacour, 1991; Collery, M'Harzi and Delacour, 1993; Tait and Brown, *unpublished observations*; McAlonan and Brown, *unpublished observations*[*]) has not allowed the thorough investigation of rTRN's functional role. Due to the lack of sensory connections, rTRN has not been considered as a suitable candidate for participation in sensory aspects of attention.

6.6. A requirement for the further delineation of cTRN's functional anatomy

The ideal way of investigating the role of cTRN in attentional processes would be through the simultaneous recording of electrophysiological activity in interconnected cTRN and thalamocortical relay cells, during the performance of various attentional behaviours. This would enable us to identify, and compare, the pattern of activity in cTRN (and its effect on the activity of thalamocortical cells) in situations where the stimulation is attended against situations where sensory stimulation is delivered but not attended. However, the required technology for something like that to be carried out is not

[*]Despite the relatively compact shape of the nucleus at rostral co-ordinates (see *1.2.1.* and Figure *1.2.*), selective lesions of rTRN appear to be equally, if not harder, than cTRN ones. This has been speculated to be due to the reduced thickness of the external medullary lamina (separating TRN from dorsal thalamus) at rostral co-ordinates, which results in the easier penetration of neurotoxins from within rTRN to dorsal thalamus following their injection

currently available. The closest we have come to the above has been, recently, the electrophysiological recordings from the TRNvis of behaving animals (McAlonan, Cavanaugh and Wurtz, 2006). The enhancement of cellular activity observed within TRNvis during the presentation of attended (as opposed to presented but not attended) visual stimulation is in line with the increase of immunocytochemical activation in cells of this area during visual discrimination behaviours (chapter *II*) and further confirms its involvement in processes of visual attention. However, the evidence acquired through this investigation did not reveal much about the effect of the increased TRNvis activation on visual thalamocortical cells. This remains still a subject of speculation.

As it is clear from the general introduction (and the introduction sections of all four experimental chapters), most of the, arguably limited, behavioural work conducted with the aim of investigating the role of cTRN in attentional behaviours has been based on partial information regarding many aspects of its functional anatomy. In order to be able to formulate more specific hypotheses with regard to cTRN function, especially when it comes to attention, more needs to be known about the patterns of thalamo-reticular, reticulo-thalamic, cortico-reticular and reticulo-reticular communication. Given that cTRN activation appears to be mainly dependent on cortical input from layer *VI* (Liu and Jones, 1999; Montero, 2000), it is of vital importance to identify the exact architectural patterns of projections of single corticothalamic fibres on cTRN and dorsal thalamic cells. More specifically, it is necessary to determine whether cortical projections target interconnected pairs of cTRN-

thalamocortical cells or not. Similarly, we need to examine the pattern of projections between single reticular and dorsal thalamic cells, in order to identify the relative proportions of open and closed cortico-reticulo-thalamic loops, as the existing evidence regarding this is unclear (see Desilets-Roy, Varga, Lavallee and Deschênes, 2002). This would enable us to appreciate better the relative contribution of lateral and feedforward inhibition in the modulatory mechanisms exerted by cTRN. If open-loop connections predominate between cTRN and thalamocortical cells, it would be also useful to know whether these circuits are formed between reticular and thalamocortical cells with overlapping or completely different receptive fields. This would allow us to assess whether cTRN is more likely to act as a thalamocortical cell receptive field modulator or as a mediator of a classic lateral inhibition mechanism (i.e. one that aims at “shutting down” attentionally competitive stimulation). This should be examined not only for the topographically precise first-order sensory pathways, but also for the higher order ones, the functions of which (and by extension, the functions of cTRN upon them) are poorly understood. Recent evidence suggests that despite the overall diffuse projection patterns between higher order dorsal thalamic nuclei and cTRN, there are subsets of projections that contain topographic features (Lam and Sherman, 2006) and could, therefore, represent a secondary pathway where attentional modulation of activity could be applied.

Another question that would need to be answered concerns the effects of the burst and tonic firing modes in cTRN cells on the activity of their post-synaptic thalamocortical cells. More specifically, it needs to be determined which of the

two firing modes in cTRN cells is more likely to activate postsynaptic $GABA_A$ vs $GABA_B$ (or extrasynaptic $GABA_A$) receptors and, therefore, which one is more likely to de-inactivate I_T in thalamocortical cells and promote burst firing in them. In the general introduction (see *1.3.5.1.*) it was speculated that the latter may be more likely to occur if cTRN fires in burst mode, but evidence to back this suggestion up is not available. Regardless of whether this holds true or not, we would also have to look in some more detail at the mechanisms that underlie firing mode control, and burst firing generation in particular, in cTRN cells. One such candidate mechanism postulates the involvement of group *II* metabotropic glutamate receptors (mGluR-II). Unlike all other glutamate receptors, mGluR-II are known to hyperpolarise cTRN cells (Cox and Sherman, 1999). Despite being identified almost a decade ago, the functional role of mGluR-II in cTRN remains, by and large, uninvestigated. Only recently, Govindaiah and Cox (2006) reported that mGluR-II are more likely to activate by brief, high frequency, input (i.e. input in the form of burst activity) than by single spike-like activity. The authors also suggested that the activation of mGluR-II on TRN (and also on local interneurons, where present) creates the ideal grounds for the membrane disinhibition of thalamocortical cells. However, it is unclear whether this disinhibition mechanism results in signal transmission augmentation in thalamocortical cells (due to the reduced influence of inhibitory input) or to a signal transmission weakening (due to the inability to de-inactivate the hyperpolarisation-dependent I_T and thus elicit burst firing). There are thoughts that cortical input is the most likely activator of mGluR-II (Sherman, S.M., *personal communication*), but no direct evidence is available yet in support of this. It would be interesting to examine what

inputs activate these receptors, how common their activation is and whether their activation could induce IPSPs in reticular cells. Similarly, it needs to be examined whether the activation of mGluRII can contribute to the de-inactivation of I_{TS} and the change of firing mode in cTRN cells from tonic to burst. Finally, as recent anatomical evidence from primates suggests, cTRN receives direct input from prefrontal cortices (Zikopoulos and Barbas, 2006). Given that this projection represents a direct top-down influence on cTRN activity, it would be very interesting to examine the strength of this projection and its post-synaptic effects on cTRN cells and their responsiveness to sensory input. These and other projects are currently under way aiming at enriching our comprehension of the mechanisms within cTRN and thus help us appreciate the functional capabilities of this area (Sherman, S.M., *personal communication*). In turn, this would also allow us to interpret new behavioural data with less speculation.

6.7. Conclusion

Combined, the results of our four investigations suggest that despite being implicated in attentional processes (e.g. chapter *II*) cTRN may not be involved in the generation of certain attentional behaviours such as the cross-modal division of attention (chapter *III*) and the endogenous and exogenous movement of visuospatial attention (chapters *IV* and *V*). Instead, our findings suggest that cTRN's role may be more specifically concerned with how certain forms of stimulation are dealt with, *within* these forms of attentional behaviour (e.g. detection or analysis enhancement). Overall, therefore, and despite the absence of major attentional deficits following its destruction, our results do

not rule out the involvement of cTRN in what are broadly labelled “attentional processes”. Given the multiple facets of attention, cTRN’s role may be limited to specific aspects of only some of these processes. A more detailed delineation of its functional anatomy and the electrophysiological relationship with its afferents and efferents would aid the identification of these attentional aspects and guide the search for the exact attentional mechanisms, if any, for which cTRN is responsible.

7.1. References

Alderson, H. L., Latimer, M. P., & Winn, P. (2005). Involvement of the laterodorsal tegmental nucleus in the locomotor response to repeated nicotine administration. *Neurosci Lett, 380*(3), 335-339.

Alderson, H. L., Parkinson, J. A., Robbins, T. W., & Everitt, B. J. (2001). The effects of excitotoxic lesions of the nucleus accumbens core or shell regions on intravenous heroin self-administration in rats. *Psychopharmacology (Berl), 153*(4), 455-463.

Alexander, G. M., & Godwin, D. W. (2006). Unique presynaptic and postsynaptic roles of Group II metabotropic glutamate receptors in the modulation of thalamic network activity. *Neuroscience, 141*(1), 501-513.

Arcelli, P., Frassoni, C., Regondi, M. C., De Biasi, S., & Spreafico, R. (1997). GABAergic neurons in mammalian thalamus: a marker of thalamic complexity? *Brain Res Bull, 42*(1), 27-37.

Arnold, H. M., Burk, J. A., Hodgson, E. M., Sarter, M., & Bruno, J. P. (2002). Differential cortical acetylcholine release in rats performing a sustained attention task versus behavioral control tasks that do not explicitly tax attention. *Neuroscience, 114*(2), 451-460.

Asanuma, C. (1989). Axonal arborizations of a magnocellular basal nucleus input and their relation to the neurons in the thalamic reticular nucleus of rats. *Proc Natl Acad Sci U S A, 86*(12), 4746-4750.

Asanuma, C. (1992). Noradrenergic innervation of the thalamic reticular nucleus: a light and electron microscopic immunohistochemical study in rats. *J Comp Neurol, 319*(2), 299-311.

Asanuma, C., & Porter, L. L. (1990). Light and electron microscopic evidence for a GABAergic projection from the caudal basal forebrain to the thalamic reticular nucleus in rats. *J Comp Neurol, 302*(1), 159-172.

Avanzini, G., de Curtis, M., Panzica, F., & Spreafico, R. (1989). Intrinsic properties of nucleus reticularis thalami neurones of the rat studied in vitro. *J Physiol, 416*, 111-122.

Belelli, D., Peden, D. R., Rosahl, T. W., Wafford, K. A., & Lambert, J. J. (2005). Extrasynaptic GABAA receptors of thalamocortical neurons: a molecular target for hypnotics. *J Neurosci, 25*(50), 11513-11520.

Birrell, J. M., & Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. *J Neurosci, 20*(11), 4320-4324.

Block, M. T. (1969). A note on the refraction and image formation of the rat's eye. *Vision Res, 9*(6), 705-711.

Block, R.A. (2007). Intending to Remember: Rapid Mobilization of Attention Enhances Memory. BBCS/SCCC Annual meeting. Victoria, British Columbia.

Bonnel, A. M., & Hafter, E. R. (1998). Divided attention between simultaneous auditory and visual signals. *Percept Psychophys, 60*(2), 179-190.

Bourassa, J., & Deschenes, M. (1995). Corticothalamic projections from the primary visual cortex in rats: a single fiber study using biocytin as an anterograde tracer. *Neuroscience, 66*(2), 253-263.

Brace, H., Latimer, M., & Winn, P. (1997). Neurotoxicity, blood-brain barrier breakdown, demyelination and remyelination associated with NMDA-induced lesions of the rat lateral hypothalamus. *Brain Res Bull, 43*(5), 447-455.

Braun, J. (1998). Divided attention: narrowing the gap between brain and behaviour. IN *The Attentive Brain,* edited by Parasuraman, R., Cambridge: MIT Press.

Bullitt, E. (1990). Expression of c-fos-like protein as a marker for neuronal activity following noxious stimulation in the rat. *J Comp Neurol, 296*(4), 517-530.

Burk, J. A., Herzog, C. D., Porter, M. C., & Sarter, M. (2002). Interactions between aging and cortical cholinergic deafferentation on attention. *Neurobiol Aging, 23*(3), 467-477.

Burk, J. A., & Sarter, M. (2001). Dissociation between the attentional functions mediated via basal forebrain cholinergic and GABAergic neurons. *Neuroscience, 105*(4), 899-909.

Campeau, S., Hayward, M. D., Hope, B. T., Rosen, J. B., Nestler, E. J., & Davis, M. (1991). Induction of the c-fos proto-oncogene in rat amygdala during unconditioned and conditioned fear. *Brain Res, 565*(2), 349-352.

Carden, W. B., & Bickford, M. E. (1999). Location of muscarinic type 2 receptors within the synaptic circuitry of the cat visual thalamus. *J Comp Neurol, 410*(3), 431-443.

Carrasco, M., Penpeci-Talgar, C., & Eckstein, M. (2000). Spatial covert attention increases contrast sensitivity across the CSF: support for signal enhancement. *Vision Res, 40*(10-12), 1203-1215.

Castro-Alamancos, M. A., & Oldford, E. (2002). Cortical sensory suppression during arousal is due to the activity-dependent depression of thalamocortical synapses. *J Physiol, 541*(Pt 1), 319-331.

Cavdar, S., Onat, F. Y., Yananli, H. R., Sehirli, U. S., Tulay, C., Saka, E., et al. (2002). Cerebellar connections to the rostral reticular nucleus of the thalamus in the rat. *J Anat, 201*(6), 485-491.

Chapman, M. A., & Zahm, D. S. (1996). Altered Fos-like immunoreactivity in terminal regions of the mesotelencephalic dopamine system is associated with reappearance of tyrosine hydroxylase immunoreactivity at the sites of focal 6-hydroxydopamine lesions in the nucleus accumbens. *Brain Res, 736*(1-2), 270-279.

Celio, M. R. (1986). Parvalbumin in most gamma-aminobutyric acid-containing neurons of the rat cerebral cortex. *Science, 231*(4741), 995-997.

Chun, M. M., & Turk-Browne, N. B. (2007). Interactions between attention and memory. *Curr Opin Neurobiol, 17*(2), 177-184.

Chung, S., Li, X., & Nelson, S. B. (2002). Short-term depression at thalamocortical synapses contributes to rapid adaptation of cortical sensory responses in vivo. *Neuron, 34*(3), 437-446.

Cicirata, F., Angaut, P., Serapide, M. F., & Panto, M. R. (1990). Functional organization of the direct and indirect projection via the reticularis thalami nuclear complex from the motor cortex to the thalamic nucleus ventralis lateralis. *Exp Brain Res, 79*(2), 325-337.

Coffey, P. J., Perry, V. H., Allen, Y., Sinden, J., & Rawlins, J. N. (1988). Ibotenic acid induced demyelination in the central nervous system: a consequence of a local inflammatory response. *Neurosci Lett, 84*(2), 178-184.

Colby, C. L. (1991). The neuroanatomy and neurophysiology of attention. *J Child Neurol, 6 Suppl*, S90-118.

Coleman, K. A., & Mitrofanis, J. (1996). Organization of the visual reticular thalamic nucleus of the rat. *Eur J Neurosci, 8*(2), 388-404.

Collery, M., M'Harzi, M., & Delacour, J. (1993). Lesions of reticularis thalamic nucleus impair spatial working memory in rats. *Neurosci. Res. Commun*. 12: 41-49.

Conn, P. J., & Pin, J. P. (1997). Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol, 37*, 205-237.

Conley, M., & Diamond, I. T. (1990). Organization of the Visual Sector of the Thalamic Reticular Nucleus in Galago. *Eur J Neurosci, 2*(3), 211-226.

Conley, M., Kupersmith, A. C., & Diamond, I. T. (1991). The Organization of Projections from Subdivisions of the Auditory Cortex and Thalamus to the Auditory Sector of the Thalamic Reticular Nucleus in Galago. *Eur J Neurosci, 3*(11), 1089-1103.

Contreras, D., Curro Dossi, R., & Steriade, M. (1993). Electrophysiological properties of cat reticular thalamic neurones in vivo. *J Physiol, 470*, 273-294.

Cope, D. W., Hughes, S. W., & Crunelli, V. (2005). GABAA receptor-mediated tonic inhibition in thalamic neurons. *J Neurosci, 25*(50), 11553-11563.

Cornwall, J., & Phillipson, O. T. (1988a). Afferent projections to the dorsal thalamus of the rat as shown by retrograde lectin transport. II. The midline nuclei. *Brain Res Bull, 21*(2), 147-161.

Cornwall, J., & Phillipson, O. T. (1988b). Afferent projections to the dorsal thalamus of the rat as shown by retrograde lectin transport--I. The mediodorsal nucleus. *Neuroscience, 24*(3), 1035-1049.

Cornwall, J., & Phillipson, O. T. (1988c). Mediodorsal and reticular thalamic nuclei receive collateral axons from prefrontal cortex and laterodorsal tegmental nucleus in the rat. *Neurosci Lett, 88*(2), 121-126.

Coull, J. T., & Nobre, A. C. (1998). Where and when to pay attention: the neural systems for directing attention to spatial locations and to time intervals as revealed by both PET and fMRI. *J Neurosci, 18*(18), 7426-7435.

Coulter, D. A., Huguenard, J. R., & Prince, D. A. (1989). Calcium currents in rat thalamocortical relay neurones: kinetic properties of the transient, low-threshold current. *J Physiol, 414*, 587-604.

Cox, C. L., Huguenard, J. R., & Prince, D. A. (1997). Nucleus reticularis neurons mediate diverse inhibitory effects in thalamus. *Proc Natl Acad Sci U S A, 94*(16), 8854-8859.

Cox, C. L., & Sherman, S. M. (1999). Glutamate inhibits thalamic reticular neurons. *J Neurosci, 19*(15), 6694-6699.

Crabtree, J. W. (1992a). The Somatotopic Organization Within the Cat's Thalamic Reticular Nucleus. *Eur J Neurosci, 4*(12), 1352-1361.

Crabtree, J. W. (1992b). The Somatotopic Organization Within the Rabbit's Thalamic Reticular Nucleus. *Eur J Neurosci, 4*(12), 1343-1351.

Crabtree, J. W. (1996). Organization in the somatosensory sector of the cat's thalamic reticular nucleus. *J Comp Neurol, 366*(2), 207-222.

Crabtree, J. W. (1998). Organization in the auditory sector of the cat's thalamic reticular nucleus. *J Comp Neurol, 390*(2), 167-182.

Crabtree, J. W., & Killackey, H. P. (1989). The Topographic Organization and Axis of Projection within the Visual Sector of the Rabbit's Thalamic Reticular Nucleus. *Eur J Neurosci, 1*(1), 94-109.

Crick, F. (1984). Function of the thalamic reticular complex: the searchlight hypothesis. *Proc Natl Acad Sci U S A, 81*(14), 4586-4590.

Cropper, E. C., Eisenman, J. S., & Azmitia, E. C. (1984). An immunocytochemical study of the serotonergic innervation of the thalamus of the rat. *J Comp Neurol, 224*(1), 38-50.

de Biasi, S., Frassoni, C., & Spreafico, R. (1986). GABA immunoreactivity in the thalamic reticular nucleus of the rat. A light and electron microscopical study. *Brain Res, 399*(1), 143-147.

de Curtis, M., Spreafico, R., & Avanzini, G. (1989). Excitatory amino acids mediate responses elicited in vitro by stimulation of cortical afferents to reticularis thalami neurons of the rat. *Neuroscience, 33*(2), 275-283.

De Lima, A. D., & Singer, W. (1987). The brainstem projection to the lateral geniculate nucleus in the cat: identification of cholinergic and monoaminergic elements. *J Comp Neurol, 259*(1), 92-121.

Deleuze, C., & Huguenard, J. R. (2006). Distinct electrical and chemical connectivity maps in the thalamic reticular nucleus: potential roles in synchronization and sensation. *J Neurosci, 26*(33), 8633-8645.

Deschenes, M., Madariaga-Domich, A., & Steriade, M. (1985). Dendrodendritic synapses in the cat reticularis thalami nucleus: a structural basis for thalamic spindle synchronization. *Brain Res, 334*(1), 165-168.

Desilets-Roy, B., Varga, C., Lavallee, P., & Deschenes, M. (2002). Substrate for cross-talk inhibition between thalamic barreloids. *J Neurosci, 22*(9), RC218.

Destexhe, A., Contreras, D., Steriade, M., Sejnowski, T. J., & Huguenard, J. R. (1996). In vivo, in vitro, and computational analysis of dendritic calcium currents in thalamic reticular neurons. *J Neurosci, 16*(1), 169-185.

Deutsch, J. A., & Deutsch, D. (1963). Some theoretical considerations. *Psychol Rev, 70*, 80-90.

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacol Rev, 51*(1), 7-61.

Domich, L., Oakson, G., & Steriade, M. (1986). Thalamic burst patterns in the naturally sleeping cat: a comparison between cortically projecting and reticularis neurones. *J Physiol, 379*, 429-449.

Dragunow, M., & Robertson, H. A. (1987). Kindling stimulation induces c-fos protein(s) in granule cells of the rat dentate gyrus. *Nature, 329*(6138), 441-442.

Duncan, J. (1984). Selective attention and the organization of visual information. *J Exp Psychol Gen, 113*(4), 501-517.

Everitt, B. J., & Robbins, T. W. (1997). Central cholinergic systems and cognition. *Annu Rev Psychol, 48*, 649-684.

Fanselow, E. E., Sameshima, K., Baccala, L. A., & Nicolelis, M. A. (2001). Thalamic bursting in rats during different awake behavioral states. *Proc Natl Acad Sci U S A, 98*(26), 15330-15335.

Farovik, A. and V.J. Brown, (2006). Basal Forebrain cholinergic lesions impair endogenous covert orienting of attention in the rat. Society for Neurosciences abstract, 369.19.

FitzGibbon, T. (1994). Rostral reticular nucleus of the thalamus sends a patchy projection to the pulvinar lateralis-posterior complex of the cat. *Exp Neurol, 129*(2), 266-278.

Floran, B., Floran, L., Erlij, D., & Aceves, J. (2004). Activation of dopamine D4 receptors modulates [3H]GABA release in slices of the rat thalamic reticular nucleus. *Neuropharmacology, 46*(4), 497-503.

Freeman, A., Ciliax, B., Bakay, R., Daley, J., Miller, R. D., Keating, G., et al. (2001). Nigrostriatal collaterals to thalamus degenerate in parkinsonian animal models. *Ann Neurol, 50*(3), 321-329.

French, C. R., Sefton, A. J., & Mackay-Sim, A. (1985). The inhibitory role of the visually responsive region of the thalamic reticular nucleus in the rat. *Exp Brain Res, 57*(3), 471-479.

Funke, K., & Eysel, U. T. (1993). Modulatory effects of acetylcholine, serotonin and noradrenaline on the activity of cat perigeniculate neurons. *Exp Brain Res, 95*(3), 409-420.

Funke, K., & Eysel, U. T. (1998). Inverse correlation of firing patterns of single topographically matched perigeniculate neurons and cat dorsal lateral geniculate relay cells. *Vis Neurosci, 15*(4), 711-729.

Funke, K., Pape, H. C., & Eysel, U. T. (1993). Noradrenergic modulation of retinogeniculate transmission in the cat. *J Physiol, 463*, 169-191.

Gandia, J. A., De Las Heras, S., Garcia, M., & Gimenez-Amaya, J. M. (1993). Afferent projections to the reticular thalamic nucleus from the globus pallidus and the substantia nigra in the rat. *Brain Res Bull, 32*(4), 351-358.

Garcia-Cabezas, M. A., Rico, B., Sanchez-Gonzalez, M. A., & Cavada, C. (2007). Distribution of the dopamine innervation in the macaque and human thalamus. *Neuroimage, 34*(3), 965-984.

Gibbs, J. W., 3rd, Schroder, G. B., & Coulter, D. A. (1996). GABAA receptor function in developing rat thalamic reticular neurons: whole cell recordings of GABA-mediated currents and modulation by clonazepam. *J Neurophysiol, 76*(4), 2568-2579.

Godfraind, J.M. (1978). Acetylcholine and somatically evoked inhibition on perigeniculate neurons in the cat. *British Journal of Pharmacology*. 63: 295-302.

Godwin, D. W., Vaughan, J. W., & Sherman, S. M. (1996). Metabotropic glutamate receptors switch visual response mode of lateral geniculate nucleus cells from burst to tonic. *J Neurophysiol, 76*(3), 1800-1816.

Gonzalez, M. F., & Sharp, F. R. (1985). Reticular nucleus of rat thalamus is metabolically activated during trained forelimb movements. *Brain Res, 332*(2), 380-385.

Gonzalo-Ruiz, A., & Lieberman, A. R. (1995a). GABAergic projections from the thalamic reticular nucleus to the anteroventral and anterodorsal thalamic nuclei of the rat. *J Chem Neuroanat, 9*(3), 165-174.

Gonzalo-Ruiz, A., & Lieberman, A. R. (1995b). Topographic organization of projections from the thalamic reticular nucleus to the anterior thalamic nuclei in the rat. *Brain Res Bull, 37*(1), 17-35.

Gonzalo-Ruiz, A., Lieberman, A. R., & Sanz-Anquela, J. M. (1995). Organization of serotoninergic projections from the raphe nuclei to the anterior thalamic nuclei in the rat: a combined retrograde tracing and 5-HT immunohistochemical study. *J Chem Neuroanat, 8*(2), 103-115.

Govindaiah, G., & Cox, C. L. (2006). Metabotropic glutamate receptors differentially regulate GABAergic inhibition in thalamus. *J Neurosci, 26*(52), 13443-13453.

Grieve, K. L. (2005). Binocular visual responses in cells of the rat dLGN. *J Physiol, 566*(Pt 1), 119-124.

Guido, W., Lu, S. M., & Sherman, S. M. (1992). Relative contributions of burst and tonic responses to the receptive field properties of lateral geniculate neurons in the cat. *J Neurophysiol, 68*(6), 2199-2211.

Guido, W., Lu, S. M., Vaughan, J. W., Godwin, D. W., & Sherman, S. M. (1995). Receiver operating characteristic (ROC) analysis of neurons in the cat's lateral geniculate nucleus during tonic and burst response mode. *Vis Neurosci, 12*(4), 723-741.

Guido, W., & Weyand, T. (1995). Burst responses in thalamic relay cells of the awake behaving cat. *J Neurophysiol, 74*(4), 1782-1786.

Guillery, R. W. (1967). Patterns of fiber degeneration in the dorsal lateral geniculate nucleus of the cat following lesions in the visual cortex. *J Comp Neurol, 130*(3), 197-221.

Guillery, R. W. (1995). Anatomical evidence concerning the role of the thalamus in corticocortical communication: a brief review. *J Anat, 187 (Pt 3)*, 583-592.

Guillery, R. W., Feig, S. L., & Lozsadi, D. A. (1998). Paying attention to the thalamic reticular nucleus. *Trends Neurosci, 21*(1), 28-32.

Guillery, R. W., & Sherman, S. M. (2002). Thalamic relay functions and their role in corticocortical communication: generalizations from the visual system. *Neuron, 33*(2), 163-175.

Hahn, B., Ross, T. J., & Stein, E. A. (2006). Neuroanatomical dissociation between bottom-up and top-down processes of visuospatial selective attention. *Neuroimage, 32*(2), 842-853.

Hale, P. T., Sefton, A. J., Baur, L. A., & Cottee, L. J. (1982). Interrelations of the rat's thalamic reticular and dorsal lateral geniculate nuclei. *Exp Brain Res, 45*(1-2), 217-229.

Hallanger, A. E., Levey, A. I., Lee, H. J., Rye, D. B., & Wainer, B. H. (1987). The origins of cholinergic and other subcortical afferents to the thalamus in the rat. *J Comp Neurol, 262*(1), 105-124.

Harris, R. M. (1987). Axon collaterals in the thalamic reticular nucleus from thalamocortical neurons of the rat ventrobasal thalamus. *J Comp Neurol, 258*(3), 397-406.

Harting, J. K., Van Lieshout, D. P., & Feig, S. (1991). Connectional studies of the primate lateral geniculate nucleus: distribution of axons arising from the thalamic reticular nucleus of Galago crassicaudatus. *J Comp Neurol, 310*(3), 411-427.

Hartings, J. A., Temereanca, S., & Simons, D. J. (2003a). Processing of periodic whisker deflections by neurons in the ventroposterior medial and thalamic reticular nuclei. *J Neurophysiol, 90*(5), 3087-3094.

Hartings, J. A., Temereanca, S., & Simons, D. J. (2003b). State-dependent processing of sensory stimuli by thalamic reticular neurons. *J Neurosci, 23*(12), 5264-5271.

Hayama, T., Hashimoto, K., & Ogawa, H. (1994). Anatomical location of a taste-related region in the thalamic reticular nucleus in rats. *Neurosci Res, 18*(4), 291-299.

Heffner, R. S., & Heffner, H. E. (1992). Visual factors in sound localization in mammals. *J Comp Neurol, 317*(3), 219-232.

Herrera, D. G., & Robertson, H. A. (1996). Activation of c-fos in the brain. *Prog Neurobiol, 50*(2-3), 83-107.

Hillyard, S. A., Vogel, E. K., & Luck, S. J. (1998). Sensory gain control (amplification) as a mechanism of selective attention: electrophysiological and neuroimaging evidence. *Philos Trans R Soc Lond B Biol Sci, 353*(1373), 1257-1270.

Holdefer, R. N., & Jacobs, B. L. (1994). Phasic stimulation of the locus coeruleus: effects on activity in the lateral geniculate nucleus. *Exp Brain Res, 100*(3), 444-452.

Houser, C. R., Vaughn, J. E., Barber, R. P., & Roberts, E. (1980). GABA neurons are the major cell type of the nucleus reticularis thalami. *Brain Res, 200*(2), 341-354.

Hu, B., Steriade, M., & Deschenes, M. (1989a). The effects of brainstem peribrachial stimulation on neurons of the lateral geniculate nucleus. *Neuroscience, 31*(1), 13-24.

Hu, B., Steriade, M., & Deschenes, M. (1989b). The effects of brainstem peribrachial stimulation on perigeniculate neurons: the blockage of spindle waves. *Neuroscience, 31*(1), 1-12.

Huang, Q., Zhou, D., Chase, K., Gusella, J. F., Aronin, N., & DiFiglia, M. (1992). Immunohistochemical localization of the D1 dopamine receptor in rat brain reveals its axonal transport, pre- and postsynaptic localization, and prevalence in the basal ganglia, limbic system, and thalamic reticular nucleus. *Proc Natl Acad Sci U S A, 89*(24), 11988-11992.

Huguenard, J. R., & Prince, D. A. (1992). A novel T-type current underlies prolonged Ca(2+)-dependent burst firing in GABAergic neurons of rat thalamic reticular nucleus. *J Neurosci, 12*(10), 3804-3817.

Huguenard, J. R., & Prince, D. A. (1994). Clonazepam suppresses GABAB-mediated inhibition in thalamic relay neurons through effects in nucleus reticularis. *J Neurophysiol, 71*(6), 2576-2581.

Imaki, T., Shibasaki, T., Hotta, M., & Demura, H. (1992). Early induction of c-fos precedes increased expression of corticotropin-releasing factor messenger ribonucleic acid in the paraventricular nucleus after immobilization stress. *Endocrinology, 131*(1), 240-246.

Imaki, T., Shibasaki, T., Hotta, M., & Demura, H. (1993). Intracerebroventricular administration of corticotropin-releasing factor induces c-fos mRNA expression in brain regions related to stress responses: comparison with pattern of c-fos mRNA induction after stress. *Brain Res, 616*(1-2), 114-125.

Inglis, W. L., Olmstead, M. C., & Robbins, T. W. (2001). Selective deficits in attentional performance on the 5-choice serial reaction time task following pedunculopontine tegmental nucleus lesions. *Behav Brain Res, 123*(2), 117-131.

Jahnsen, H., & Llinas, R. (1984a). Electrophysiological properties of guinea-pig thalamic neurones: an in vitro study. *J Physiol, 349*, 205-226.

Jahnsen, H., & Llinas, R. (1984b). Voltage-dependent burst-to-tonic switching of thalamic cell activity: an in vitro study. *Arch Ital Biol, 122*(1), 73-82.

Jia, F., Pignataro, L., Schofield, C. M., Yue, M., Harrison, N. L., & Goldstein, P. A. (2005). An extrasynaptic GABAA receptor mediates tonic inhibition in thalamic VB neurons. *J Neurophysiol, 94*(6), 4491-4501.

Jiang, Y., & Chun, M. M. (2001). Selective attention modulates implicit learning. *Q J Exp Psychol A, 54*(4), 1105-1124.

Jones, D., & Gonzalez-Lima, F. (2001). Mapping Pavlovian conditioning effects on the brain: blocking, contiguity, and excitatory effects. *J Neurophysiol, 86*(2), 809-823.

Jones, E.G. (1975). Some aspects of the organization of the thalamic reticular nucleus. *J. Comp. Neurol*. 162: 285-308.

Jones, S. V. (1993). Muscarinic receptor subtypes: modulation of ion channels. *Life Sci, 52*(5-6), 457-464.

Jonides J. (1980) Toward a model of the mind's eye's movement, *Canadian Journal of Psychology* **34** (1980), pp. 103–112.

Jourdain, A., Semba, K., & Fibiger, H. C. (1989). Basal forebrain and mesopontine tegmental projections to the reticular thalamic nucleus: an axonal collateralization and immunohistochemical study in the rat. *Brain Res, 505*(1), 55-65.

Kaas, J. H., Guillery, R. W., & Allman, J. M. (1972). Some principles of organization in the dorsal lateral geniculate nucleus. *Brain Behav Evol, 6*(1), 253-299.

Kamin, L. J. (1969). Predictability, surprise, attention and conditioning. In: Punishment and Aversive Behaviour (Campbell, B.A., and Church, R.M.) 279-296. New York: Appleton-Century-Crofts.

Kay, L. M., & Sherman, S. M. (2007). An argument for an olfactory thalamus. *Trends Neurosci, 30*(2), 47-53.

Kayama, Y., & Koyama, Y. (1998). Brainstem neural mechanisms of sleep and wakefulness. *Eur Urol, 33 Suppl 3*, 12-15.

Kayama, Y., Negi, T., Sugitani, M., & Iwama, K. (1982). Effects of locus coeruleus stimulation on neuronal activities of dorsal lateral geniculate nucleus and perigeniculate reticular nucleus of the rat. *Neuroscience, 7*(3), 655-666.

Kayama, Y., Shimada, S., Hishikawa, Y., & Ogawa, T. (1989). Effects of stimulating the dorsal raphe nucleus of the rat on neuronal activity in the dorsal lateral geniculate nucleus. *Brain Res, 489*(1), 1-11.

Khan, Z. U., Gutierrez, A., Martin, R., Penafiel, A., Rivera, A., & De La Calle, A. (1998). Differential regional and cellular distribution of dopamine D2-like receptors: an immunocytochemical study of subtype-specific antibodies in rat and human brain. *J Comp Neurol, 402*(3), 353-371.

Kim, U., & McCormick, D. A. (1998a). Functional and ionic properties of a slow afterhyperpolarization in ferret perigeniculate neurons in vitro. *J Neurophysiol, 80*(3), 1222-1235.

Kim, U., & McCormick, D. A. (1998b). The functional influence of burst and tonic firing mode on synaptic interactions in the thalamus. *J Neurosci, 18*(22), 9500-9516.

Kim, U., Sanchez-Vives, M. V., & McCormick, D. A. (1997). Functional dynamics of GABAergic inhibition in the thalamus. *Science, 278*(5335), 130-134.

Kolmac, C. I., & Mitrofanis, J. (1997). Organisation of the reticular thalamic projection to the intralaminar and midline nuclei in rats. *J Comp Neurol, 377*(2), 165-178.

Kozak, R., Bowman, E. M., Latimer, M. P., Rostron, C. L., & Winn, P. (2005). Excitotoxic lesions of the pedunculopontine tegmental nucleus in rats impair performance on a test of sustained attention. *Exp Brain Res, 162*(2), 257-264.

Kozak, R., Bruno, J. P., & Sarter, M. (2006). Augmented prefrontal acetylcholine release during challenged attentional performance. *Cereb Cortex, 16*(1), 9-17.

Kultas-Ilinsky, K., Yi, H., & Ilinsky, I. A. (1995). Nucleus reticularis thalami input to the anterior thalamic nuclei in the monkey: a light and electron microscopic study. *Neurosci Lett, 186*(1), 25-28.

Lam, Y., & Sherman, S. M. (2006). Different organization of the TRN inputs to VPL/VPM and PO revealed by laser photostimulation. Society for Neuroscience abstract. 446.5/S4.

Landisman, C. E., Long, M. A., Beierlein, M., Deans, M. R., Paul, D. L., & Connors, B. W. (2002). Electrical synapses in the thalamic reticular nucleus. *J Neurosci, 22*(3), 1002-1009.

Lavallee, P., & Deschenes, M. (2004). Dendroarchitecture and lateral inhibition in thalamic barreloids. *J Neurosci, 24*(27), 6098-6105.

Lavin, A., & Grace, A. A. (1998). Dopamine modulates the responsivity of mediodorsal thalamic cells recorded in vitro. *J Neurosci, 18*(24), 10566-10578.

Lee, S. M., Friedberg, M. H., & Ebner, F. F. (1994a). The role of GABA-mediated inhibition in the rat ventral posterior medial thalamus. I. Assessment of receptive field changes following thalamic reticular nucleus lesions. *J Neurophysiol, 71*(5), 1702-1715.

Lee, S. M., Friedberg, M. H., & Ebner, F. F. (1994b). The role of GABA-mediated inhibition in the rat ventral posterior medial thalamus. II. Differential effects of GABAA and GABAB receptor antagonists on responses of VPM neurons. *J Neurophysiol, 71*(5), 1716-1726.

Le Gros Clarck, W.E. (1932). The structure and connections of the thalamus. *Brain,* 55*, 406-470.*

Le Gros Clark, W.E. and Penman, G.G. (1934). The projection of the retina in the lateral geniculate body. Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character, Vol. 114, No. 788. pp. 291-313.

Léna, C., & Changeux, J. P. (1997). Role of Ca2+ ions in nicotinic facilitation of GABA release in mouse thalamus. *J Neurosci, 17*(2), 576-585.

Levey, A. I., Hallanger, A. E., & Wainer, B. H. (1987a). Choline acetyltransferase immunoreactivity in the rat thalamus. *J Comp Neurol, 257*(3), 317-332.

Levey, A. I., Hallanger, A. E., & Wainer, B. H. (1987b). Cholinergic nucleus basalis neurons may influence the cortex via the thalamus. *Neurosci Lett, 74*(1), 7-13.

Liu, T., Pestilli, F., & Carrasco, M. (2005). Transient attention enhances perceptual performance and FMRI response in human visual cortex. *Neuron, 45*(3), 469-477.

Liu, X. B., & Jones, E. G. (1999). Predominance of corticothalamic synaptic inputs to thalamic reticular nucleus neurons in the rat. *J Comp Neurol, 414*(1), 67-79.

Liu, X. B., Warren, R. A., & Jones, E. G. (1995). Synaptic distribution of afferents from reticular nucleus in ventroposterior nucleus of cat thalamus. *J Comp Neurol, 352*(2), 187-202.

Livingstone, M. S., & Hubel, D. H. (1981). Effects of sleep and arousal on the processing of visual information in the cat. *Nature, 291*(5816), 554-561.

Lo, F. S., Lu, S. M., & Sherman, S. M. (1991). Intracellular and extracellular in vivo recording of different response modes for relay cells of the cat's lateral geniculate nucleus. *Exp Brain Res, 83*(2), 317-328.

Long, M. A., Landisman, C. E., & Connors, B. W. (2004). Small clusters of electrically coupled neurons generate synchronous rhythms in the thalamic reticular nucleus. *J Neurosci, 24*(2), 341-349.

Lourenco Neto, F., Schadrack, J., Berthele, A., Zieglgansberger, W., Tolle, T. R., & Castro-Lopes, J. M. (2000). Differential distribution of metabotropic glutamate receptor subtype mRNAs in the thalamus of the rat. *Brain Res, 854*(1-2), 93-105.

Lozsadi, D. A. (1994). Organization of cortical afferents to the rostral, limbic sector of the rat thalamic reticular nucleus. *J Comp Neurol, 341*(4), 520-533.

Lozsadi, D. A., Gonzalez-Soriano, J., & Guillery, R. W. (1996). The course and termination of corticothalamic fibres arising in the visual cortex of the rat. *Eur J Neurosci, 8*(11), 2416-2427.

Lu, S. M., Guido, W., & Sherman, S. M. (1993). The brain-stem parabrachial region controls mode of response to visual stimulation of neurons in the cat's lateral geniculate nucleus. *Vis Neurosci, 10*(4), 631-642.

Lu, Z. L., & Dosher, B. A. (1998). External noise distinguishes attention mechanisms. *Vision Res, 38*(9), 1183-1198.

Lübke, J. (1993). Morphology of neurons in the thalamic reticular nucleus (TRN) of mammals as revealed by intracellular injections into fixed brain slices. *J Comp Neurol, 329*(4), 458-471.

MacKay, D.G., & Ahmetzanov, M, V. (2005). Emotion, memory, and attention in the taboo Stroop paradigm: An experimental analog of flashbulb memories. *Psychological Science*, 16, 25-32.

Marks, G. A., & Roffwarg, H. P. (1991). Cholinergic modulation of responses to glutamate in the thalamic reticular nucleus of the anesthetized rat. *Brain Res, 557*(1-2), 48-56.

Marks, G. A., & Roffwarg, H. P. (1993). Spontaneous activity in the thalamic reticular nucleus during the sleep/wake cycle of the freely-moving rat. *Brain Res, 623*(2), 241-248.

McAlonan, K., & Brown, V. J. (2002). The thalamic reticular nucleus: more than a sensory nucleus? *Neuroscientist, 8*(4), 302-305.

McAlonan, K., Brown, V. J., & Bowman, E. M. (2000). Thalamic reticular nucleus activation reflects attentional gating during classical conditioning. *J Neurosci, 20*(23), 8897-8901.

McAlonan, K., Cavanaugh, J., & Wurtz, R. H. (2006). Attentional modulation of thalamic reticular neurons. *J Neurosci, 26*(16), 4444-4450.

McCormick, D. A. (1992). Neurotransmitter actions in the thalamus and cerebral cortex. *J Clin Neurophysiol, 9*(2), 212-223.

McCormick, D. A., & Pape, H. C. (1990). Noradrenergic and serotonergic modulation of a hyperpolarization-activated cation current in thalamic relay neurones. *J Physiol, 431*, 319-342.

McCormick, D. A., & Prince, D. A. (1986). Acetylcholine induces burst firing in thalamic reticular neurones by activating a potassium conductance. *Nature, 319*(6052), 402-405.

McCormick, D. A., & Prince, D. A. (1988). Noradrenergic modulation of firing pattern in guinea pig and cat thalamic neurons, in vitro. *J Neurophysiol, 59*(3), 978-996.

McCormick, D. A., & von Krosigk, M. (1992). Corticothalamic activation modulates thalamic firing through glutamate "metabotropic" receptors. *Proc Natl Acad Sci U S A, 89*(7), 2774-2778.

McCormick, D. A., & Wang, Z. (1991). Serotonin and noradrenaline excite GABAergic neurones of the guinea-pig and cat nucleus reticularis thalami. *J Physiol, 442*, 235-255.

McCune, S. K., Voigt, M. M., & Hill, J. M. (1993). Expression of multiple alpha adrenergic receptor subtype messenger RNAs in the adult rat brain. *Neuroscience, 57*(1), 143-151.

McGaughy, J., Everitt, B. J., Robbins, T. W., & Sarter, M. (2000). The role of cortical cholinergic afferent projections in cognition: impact of new selective immunotoxins. *Behav Brain Res, 115*(2), 251-263.

McGaughy, J., Kaiser, T., & Sarter, M. (1996). Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. *Behav Neurosci, 110*(2), 247-265.

McGaughy, J., Turchi, J., & Sarter, M. (1994). Crossmodal divided attention in rats: effects of chlordiazepoxide and scopolamine. *Psychopharmacology (Berl), 115*(1-2), 213-220.

Melchitzky, D. S., & Lewis, D. A. (2001). Dopamine transporter-immunoreactive axons in the mediodorsal thalamic nucleus of the macaque monkey. *Neuroscience, 103*(4), 1033-1042.

M'Harzi, M., Collery, M., and Deleacour, J. (1991). First evidence for a possible role of the reticular thalamic nucleus in working memory in rats. *Neurosci. Res. Comm*. 8: 167-174.

Montero, V. M. (1997). c-fos induction in sensory pathways of rats exploring a novel complex environment: shifts of active thalamic reticular sectors by predominant sensory cues. *Neuroscience, 76*(4), 1069-1081.

Montero, V. M. (1999). Amblyopia decreases activation of the corticogeniculate pathway and visual thalamic reticularis in attentive rats: a 'focal attention' hypothesis. *Neuroscience, 91*(3), 805-817.

Montero, V. M. (2000). Attentional activation of the visual thalamic reticular nucleus depends on 'top-down' inputs from the primary visual cortex via corticogeniculate pathways. *Brain Res, 864*(1), 95-104.

Montero, V. M., Guillery, R. W., & Woolsey, C. N. (1977). Retinotopic organization within the thalamic reticular nucleus demonstrated by a double label autoradiographic technique. *Brain Res, 138*(3), 407-421.

Morrison, J. H., & Foote, S. L. (1986). Noradrenergic and serotoninergic innervation of cortical, thalamic, and tectal visual structures in Old and New World monkeys. *J Comp Neurol, 243*(1), 117-138.

Motter, B. C. (1993). Focal attention produces spatially selective processing in visual cortical areas V1, V2, and V4 in the presence of competing stimuli. *J Neurophysiol, 70*(3), 909-919.

Mrzljak, L., Bergson, C., Pappy, M., Huff, R., Levenson, R., & Goldman-Rakic, P. S. (1996). Localization of dopamine D4 receptors in GABAergic neurons of the primate brain. *Nature, 381*(6579), 245-248.

Muir, J. L., Everitt, B. J., & Robbins, T. W. (1994). AMPA-induced excitotoxic lesions of the basal forebrain: a significant role for the cortical cholinergic system in attentional function. *J Neurosci, 14*(4), 2313-2326.

Mukherjee, P., & Kaplan, E. (1995). Dynamics of neurons in the cat lateral geniculate nucleus: in vivo electrophysiology and computational modeling. *J Neurophysiol, 74*(3), 1222-1243.

Mulle, C., Madariaga, A., & Deschenes, M. (1986). Morphology and electrophysiological properties of reticularis thalami neurons in cat: in vivo study of a thalamic pacemaker. *J Neurosci, 6*(8), 2134-2145.

Mullen, R. J., Buck, C. R., & Smith, A. M. (1992). NeuN, a neuronal specific nuclear protein in vertebrates. *Development, 116*(1), 201-211.

Nakanishi, S. (1992). Molecular diversity of glutamate receptors and implications for brain function. *Science, 258*(5082), 597-603.

Nicolelis, M. A. (2002). Depression at thalamocortical synapses: the key for cortical neuronal adaptation? *Neuron, 34*(3), 331-332.

Nicolelis, M. A., Baccala, L. A., Lin, R. C., & Chapin, J. K. (1995). Sensorimotor encoding by synchronous neural ensemble activity at multiple levels of the somatosensory system. *Science, 268*(5215), 1353-1358.

Nikolaev, E., Werka, T., & Kaczmarek, L. (1992). C-fos protooncogene expression in rat brain after long-term training of two-way active avoidance reaction. *Behav Brain Res, 48*(1), 91-94.

Ohara, P. T., & Havton, L. A. (1996). Dendritic arbors of neurons from different regions of the rat thalamic reticular nucleus share a similar orientation. *Brain Res, 731*(1-2), 236-240.

Ohara, P. T., & Lieberman, A. R. (1985). The thalamic reticular nucleus of the adult rat: experimental anatomical studies. *J Neurocytol, 14*(3), 365-411.

Pape, H. C., & McCormick, D. A. (1989). Noradrenaline and serotonin selectively modulate thalamic burst firing by enhancing a hyperpolarization-activated cation current. *Nature, 340*(6236), 715-718.

Parasuraman, R. (1998). "The attentive brain". Cambridge, MA: MIT Press.

Pare, D., Hazrati, L. N., Parent, A., & Steriade, M. (1990). Substantia nigra pars reticulata projects to the reticular thalamic nucleus of the cat: a morphological and electrophysiological study. *Brain Res, 535*(1), 139-146.

Pare, D., Smith, Y., Parent, A., & Steriade, M. (1988). Projections of brainstem core cholinergic and non-cholinergic neurons of cat to intralaminar and reticular thalamic nuclei. *Neuroscience, 25*(1), 69-86.

Paxinos, G., and Watson, C. (1998). "The rat brain in stereoscopic coordinates". 4rth Edition. Sydney: Academic.

Pertovaara, A., Kemppainen, P., & Leppanen, H. (1992). Lowered cutaneous sensitivity to nonpainful electrical stimulation during isometric exercise in humans. *Exp Brain Res, 89*(2), 447-452.

Peschanski, M., & Besson, J. M. (1984). Diencephalic connections of the raphe nuclei of the rat brainstem: an anatomical study with reference to the somatosensory system. *J Comp Neurol, 224*(4), 509-534.

Pinault, D. (2004). The thalamic reticular nucleus: structure, function and concept. *Brain Res Brain Res Rev, 46*(1), 1-31.

Pinault, D., Bourassa, J., & Deschenes, M. (1995a). The axonal arborization of single thalamic reticular neurons in the somatosensory thalamus of the rat. *Eur J Neurosci, 7*(1), 31-40.

Pinault, D., Bourassa, J., & Deschenes, M. (1995b). Thalamic reticular input to the rat visual thalamus: a single fiber study using biocytin as an anterograde tracer. *Brain Res, 670*(1), 147-152.

Pinault, D., & Deschenes, M. (1992). Muscarinic inhibition of reticular thalamic cells by basal forebrain neurones. *Neuroreport, 3*(12), 1101-1104.

Pinault, D., & Deschenes, M. (1998a). Anatomical evidence for a mechanism of lateral inhibition in the rat thalamus. *Eur J Neurosci, 10*(11), 3462-3469.

Pinault, D., & Deschenes, M. (1998b). Projection and innervation patterns of individual thalamic reticular axons in the thalamus of the adult rat: a three-dimensional, graphic, and morphometric analysis. *J Comp Neurol, 391*(2), 180-203.

Pinault, D., Smith, Y., & Deschenes, M. (1997). Dendrodendritic and axoaxonic synapses in the thalamic reticular nucleus of the adult rat. *J Neurosci, 17*(9), 3215-3233.

Pirker, S., Schwarzer, C., Wieselthaler, A., Sieghart, W., & Sperk, G. (2000). GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience, 101*(4), 815-850.

Plummer, K. L., Manning, K. A., Levey, A. I., Rees, H. D., & Uhlrich, D. J. (1999). Muscarinic receptor subtypes in the lateral geniculate nucleus: a light and electron microscopic analysis. *J Comp Neurol, 404*(3), 408-425.

Pollin, B., & Rokyta, R. (1982). Somatotopic organization of nucleus reticularis thalami in chronic awake cats and monkeys. *Brain Res, 250*(2), 211-221.

Posner, M. I. (1980). Orienting of attention. *Q J Exp Psychol, 32*(1), 3-25.

Posner, M. I., Walker, J. A., Friedrich, F. J., & Rafal, R. D. (1984). Effects of parietal injury on covert orienting of attention. *J Neurosci, 4*(7), 1863-1874.

Posner, M. I., & Gilbert, C. D. (1999). Attention and primary visual cortex. *Proc Natl Acad Sci U S A, 96*(6), 2585-2587.

Raczkowski, D., & Fitzpatrick, D. (1989). Organization of cholinergic synapses in the cat's dorsal lateral geniculate and perigeniculate nuclei. *J Comp Neurol, 288*(4), 676-690.

Ramcharan, E. J., Gnadt, J. W., & Sherman, S. M. (2000). Burst and tonic firing in thalamic cells of unanesthetized, behaving monkeys. *Vis Neurosci, 17*(1), 55-62.

Reinagel, P., Godwin, D., Sherman, S. M., & Koch, C. (1999). Encoding of visual information by LGN bursts. *J Neurophysiol, 81*(5), 2558-2569.

Risbrough, V., Bontempi, B., & Menzaghi, F. (2002). Selective immunolesioning of the basal forebrain cholinergic neurons in rats: effect on attention using the 5-choice serial reaction time task. *Psychopharmacology (Berl), 164*(1), 71-81.

Roelfsema, P. R., Lamme, V. A., & Spekreijse, H. (1998). Object-based attention in the primary visual cortex of the macaque monkey. *Nature, 395*(6700), 376-381.

Rogawski, M. A., & Aghajanian, G. K. (1980a). Activation of lateral geniculate neurons by norepinephrine: mediation by an alpha-adrenergic receptor. *Brain Res, 182*(2), 345-359.

Rogawski, M. A., & Aghajanian, G. K. (1980b). Modulation of lateral geniculate neurone excitability by noradrenaline microiontophoresis or locus coeruleus stimulation. *Nature, 287*(5784), 731-734.

Rouiller, E. M., Colomb, E., Capt, M., & De Ribaupierre, F. (1985). Projections of the reticular complex of the thalamus onto physiologically characterized regions of the medial geniculate body. *Neurosci Lett, 53*(2), 227-232.

Rowland, L. A., & Shanks, D. R. (2006). Attention modulates the learning of multiple contingencies. *Psychon Bull Rev, 13*(4), 643-648.

Salt, T. E., & Eaton, S. A. (1996). Functions of ionotropic and metabotropic glutamate receptors in sensory transmission in the mammalian thalamus. *Prog Neurobiol, 48*(1), 55-72.

Sanchez-Vives, M. V., Bal, T., & McCormick, D. A. (1997). Inhibitory interactions between perigeniculate GABAergic neurons. *J Neurosci, 17*(22), 8894-8908.

Sanderson, K. J. (1971). Visual field projection columns and magnification factors in the lateral geniculate nucleus of the cat. *Exp Brain Res, 13*(2), 159-177.

Sarter, M., & Bruno, J. P. (1997). Trans-synaptic stimulation of cortical acetylcholine and enhancement of attentional functions: a rational approach for the development of cognition enhancers. *Behav Brain Res, 83*(1-2), 7-14.

Sarter, M., Bruno, J. P., & Turchi, J. (1999). Basal forebrain afferent projections modulating cortical acetylcholine, attention, and implications for neuropsychiatric disorders. *Ann N Y Acad Sci, 877*, 368-382.

Sarter, M., Hasselmo, M. E., Bruno, J. P., & Givens, B. (2005). Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res Brain Res Rev, 48*(1), 98-111.

Scheibel, M. E., & Scheibel, A. B. (1966). The organization of the nucleus reticularis thalami: a Golgi study. *Brain Res, 1*(1), 43-62.

Scheibel, M. E., & Scheibel, A. B. (1972). Specialized organizational patterns within the nucleus reticularis thalami of the cat. *Exp Neurol, 34*(2), 316-322.

Semba, K. (2000). Multiple output pathways of the basal forebrain: organization, chemical heterogeneity, and roles in vigilance. *Behav Brain Res, 115*(2), 117-141.

Sherman, S. M. (1996). Dual response modes in lateral geniculate neurons: mechanisms and functions. *Vis Neurosci, 13*(2), 205-213.

Sherman, S. M. (2001). Tonic and burst firing: dual modes of thalamocortical relay. *Trends Neurosci, 24*(2), 122-126.

Sherman, S. M. (2005). Thalamic relays and cortical functioning. *Prog Brain Res, 149*, 107-126.

Sherman, S. M. (2006). The role of thalamus in cortical function: Not just a simple relay. Journal of Vision, 6 (13), pp28, 28a.

Sherman, S. M., & Guillery, R. W. (1996). Functional organization of thalamocortical relays. *J Neurophysiol, 76*(3), 1367-1395.

Sherman, S. M., & Guillery, R. W. (1998). On the actions that one nerve cell can have on another: distinguishing "drivers" from "modulators". *Proc Natl Acad Sci U S A, 95*(12), 7121-7126.

Sherman, S.M., and R.W. Guillery (2001). "Exploring the Thalamus". New York: Academic Press.

Shosaku, A., Kayama, Y., & Sumitomo, I. (1984). Somatotopic organization in the rat thalamic reticular nucleus. *Brain Res, 311*(1), 57-63.

Shosaku, A., & Sumitomo, I. (1983). Auditory neurons in the rat thalamic reticular nucleus. *Exp Brain Res, 49*(3), 432-442.

Siegel, J. M. (1979). Behavioral functions of the reticular formation. *Brain Res, 180*(1), 69-105.

Sillito, A. M., Kemp, J. A., & Berardi, N. (1983). The cholinergic influence on the function of the cat dorsal lateral geniculate nucleus (dLGN). *Brain Res, 280*(2), 299-307.

Sprague, J. M. (1967). The effects of chronic brainstem lesions on wakefulness, sleep and behavior. *Res Publ Assoc Res Nerv Ment Dis, 45*, 148-194.

Spreafico, R., Amadeo, A., Angoscini, P., Panzica, F., & Battaglia, G. (1993). Branching projections from mesopontine nuclei to the nucleus reticularis and related thalamic nuclei: a double labelling study in the rat. *J Comp Neurol, 336*(4), 481-492.

Stehberg, J., Acuna-Goycolea, C., Ceric, F., & Torrealba, F. (2001). The visceral sector of the thalamic reticular nucleus in the rat. *Neuroscience, 106*(4), 745-755.

Stellar, J. R., Hall, F. S., & Waraczynski, M. (1991). The effects of excitotoxin lesions of the lateral hypothalamus on self-stimulation reward. *Brain Res, 541*(1), 29-40.

Steriade, M., Contreras, D., Curro Dossi, R., & Nunez, A. (1993). The slow (< 1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *J Neurosci, 13*(8), 3284-3299.

Steriade, M., Domich, L., & Oakson, G. (1986). Reticularis thalami neurons revisited: activity changes during shifts in states of vigilance. *J Neurosci, 6*(1), 68-81.

Steriade, M., and McCarley, R.W. (1990). "Brainstem control of wakefulness and sleep". New York. Plenum.

Steriade, M., McCormick, D. A., & Sejnowski, T. J. (1993). Thalamocortical oscillations in the sleeping and aroused brain. *Science, 262*(5134), 679-685.

Sugitani, M. (1979). Electrophysiological and sensory properties of the thalamic reticular neurones related to somatic sensation in rats. *J Physiol, 290*(2), 79-95.

Sumitomo, I., Nakamura, M., & Iwama, K. (1976). Location and function of the so-called interneurons of rat lateral geniculate body. *Exp Neurol, 51*(1), 110-123.

Swadlow, H. A., & Gusev, A. G. (2001). The impact of 'bursting' thalamic impulses at a neocortical synapse. *Nat Neurosci, 4*(4), 402-408.

Tai, Y., Yi, H., Ilinsky, I. A., & Kultas-Ilinsky, K. (1995). Nucleus reticularis thalami connections with the mediodorsal thalamic nucleus: a light and electron microscopic study in the monkey. *Brain Res Bull, 38*(5), 475-488.

Thomson, A. M. (1988). Inhibitory postsynaptic potentials evoked in thalamic neurons by stimulation of the reticularis nucleus evoke slow spikes in isolated rat brain slices--I. *Neuroscience, 25*(2), 491-502.

Thomson, D. M. and V.J. Brown (2006). Failure of response inhibition under conditions of high prior-probability of a target following lesions of the subthalamic nucleus in the rat. Experimental Psychology Society symposium talk, Birmingham, UK.

Treisman, M. (1960). Stimulus-response theory and expectancy. *Br J Psychol, 51*, 49-60.

Treue, S. (2001). Neural correlates of attention in primate visual cortex. *Trends Neurosci, 24*(5), 295-300.

Tsumoto, T., Creutzfeldt, O. D., & Legendy, C. R. (1978). Functional organization of the corticofugal system from visual cortex to lateral geniculate nucleus in the cat (with an appendix on geniculo-cortical mono-synaptic connections). *Exp Brain Res, 32*(3), 345-364.

Turchi, J., & Sarter, M. (1997). Cortical acetylcholine and processing capacity: effects of cortical cholinergic deafferentation on crossmodal divided attention in rats. *Brain Res Cogn Brain Res, 6*(2), 147-158.

Turner, J. P., & Salt, T. E. (2000). Synaptic activation of the group I metabotropic glutamate receptor mGlu1 on the thalamocortical neurons of the rat dorsal lateral geniculate nucleus in vitro. *Neuroscience, 100*(3), 493-505.

Turner, J. P., & Salt, T. E. (2003). Group II and III metabotropic glutamate receptors and the control of the nucleus reticularis thalami input to rat thalamocortical neurones in vitro. *Neuroscience, 122*(2), 459-469.

Uhlrich, D. J., Cucchiaro, J. B., Humphrey, A. L., & Sherman, S. M. (1991). Morphology and axonal projection patterns of individual neurons in the cat perigeniculate nucleus. *J Neurophysiol, 65*(6), 1528-1541.

Uhlrich, D. J., Cucchiaro, J. B., & Sherman, S. M. (1988). The projection of individual axons from the parabrachial region of the brain stem to the dorsal lateral geniculate nucleus in the cat. *J Neurosci, 8*(12), 4565-4575.

Ulrich, D., & Huguenard, J. R. (1996a). GABAB receptor-mediated responses in GABAergic projection neurones of rat nucleus reticularis thalami in vitro. *J Physiol, 493 (Pt 3)*, 845-854.

Ulrich, D., & Huguenard, J. R. (1996b). Gamma-aminobutyric acid type B receptor-dependent burst-firing in thalamic neurons: a dynamic clamp study. *Proc Natl Acad Sci U S A, 93*(23), 13245-13249.

Vertes, R. P. (1984). Brainstem control of the events of REM sleep. *Prog Neurobiol, 22*(3), 241-288.

Wang, S., Bickford, M. E., Van Horn, S. C., Erisir, A., Godwin, D. W., & Sherman, S. M. (2001). Synaptic targets of thalamic reticular nucleus terminals in the visual thalamus of the cat. *J Comp Neurol, 440*(4), 321-341.

Ward, N. M., & Brown, V. J. (1996). Covert orienting of attention in the rat and the role of striatal dopamine. *J Neurosci, 16*(9), 3082-3088.

Weese, G. D., Phillips, J. M., & Brown, V. J. (1999). Attentional orienting is impaired by unilateral lesions of the thalamic reticular nucleus in the rat. *J Neurosci, 19*(22), 10135-10139.

Weyand, T. G., Boudreaux, M., & Guido, W. (2001). Burst and tonic response modes in thalamic neurons during sleep and wakefulness. *J Neurophysiol, 85*(3), 1107-1118.

Williamson, A. M., Ohara, P. T., Ralston, D. D., Milroy, A. M., & Ralston, H. J., 3rd. (1994). Analysis of gamma-aminobutyric acidergic synaptic contacts in the thalamic reticular nucleus of the monkey. *J Comp Neurol, 349*(2), 182-192.

Wilton, L. A., Baird, A. L., Muir, J. L., & Aggleton, J. P. (2001). Excitotoxic lesions of the rostral thalamic reticular nucleus do not affect the performance of spatial learning and memory tasks in the rat. *Behav Brain Res, 120*(2), 177-187.

Yen, C. T., Conley, M., Hendry, S. H., & Jones, E. G. (1985). The morphology of physiologically identified GABAergic neurons in the somatic sensory part of the thalamic reticular nucleus in the cat. *J Neurosci, 5*(8), 2254-2268.

Yantis, S. (2000). *Visual Perception: Key Readings*. Philadelphia: Psychology Press

Yingling, C. D., & Skinner, J. E. (1976). Selective regulation of thalamic sensory relay nuclei by nucleus reticularis thalami. *Electroencephalogr Clin Neurophysiol, 41*(5), 476-482.

Yoshida, M., Sasa, M., & Takaori, S. (1984). Serotonin-mediated inhibition from dorsal raphe nucleus of neurons in dorsal lateral geniculate and thalamic reticular nuclei. *Brain Res, 290*(1), 95-105.

Zahm, D. S., Jensen, S. L., Williams, E. S., & Martin, J. R., 3rd. (1999). Direct comparison of projections from the central amygdaloid region and nucleus accumbens shell. *Eur J Neurosci, 11*(4), 1119-1126.

Zahm, D. S., Williams, E. A., Latimer, M. P., & Winn, P. (2001). Ventral mesopontine projections of the caudomedial shell of the nucleus accumbens and extended amygdala in the rat: double dissociation by organization and development. *J Comp Neurol, 436*(1), 111-125.

Zentall, T. R. (2005). Selective and divided attention in animals. *Behav Processes, 69*(1), 1-15.

Zhang, L., & Jones, E. G. (2004). Corticothalamic inhibition in the thalamic reticular nucleus. *J Neurophysiol, 91*(2), 759-766.

Zhu, J. J., & Uhlrich, D. J. (1998). Cellular mechanisms underlying two muscarinic receptor-mediated depolarizing responses in relay cells of the rat lateral geniculate nucleus. *Neuroscience, 87*(4), 767-781.

Zikopoulos, B., & Barbas, H. (2006). Prefrontal projections to the thalamic reticular nucleus form a unique circuit for attentional mechanisms. *J Neurosci, 26*(28), 7348-7361.