# The Effects of Ibotenic Acid Lesions to Auditory and Visual Sectors of the Thalamic Reticular Nucleus During a Visual Discrimination Task with an Auditory Distraction

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It is believed that the main function of the thalamic reticular nucleus (TRN) is to dampen neural activity stemming from the thalamus and the cortex. Inhibition, in this context, has since been linked to attention and distraction, namely the reduction of neural activity caused by the presence of irrelevant stimuli. Recently, there have been a number of studies which have examined more closely the mechanisms of action which take place among the cortex, the thalamus, and the TRN. These studies suggest that cross-modality occurs during activation of the TRN, such that defined sectors of the TRN are active during other distinct sensory processes controlled by the TRN. The current study aimed to observe the effects of lesions to the visual TRN on a visual discrimination task with auditory distractions. Utilizing an entirely new testing paradigm, we tested rats on their ability to distinguish between two visual stimuli during the presence of an auditory stimulus, both before and after perfusions. Though no significant differences were found between pre-lesion and post-lesion performance, we observed that number of correct responses during the distraction task were significantly lower than those during the discrimination task. This evidence suggests that our testing paradigm could prove to be useful to others in measuring cross-modality among distinct sectors of the TRN.

## INTRODUCTION

Much research has been devoted to the thalamic reticular nucleus (TRN), the brain region located between the thalamus and the cortex. All connections between the thalamus and the cortex must first pass through the TRN, whose inhibitory outputs project only to the thalamus. It was presumed that since the TRN projects only GABAergic outputs, and that the thalamocortical and corticothalamic connections are predominantly excitatory, it is these excitatory connections which give rise to activity in the TRN (Crick, 1984). The position and the interconnectedness of the TRN to the thalamus reveal that it is an influential factor in regulating the flow of signals which project sensory information; indeed, there is research which suggests that this processing in an important factor in the concept of attention and distraction. Francis Crick was one of the first researchers to suggest that the TRN plays a role in attention. Based mainly on the structure, location, and nature of the neurons in the TRN, Crick (1984) proposed its role as an attentional searchlight whose function is to focus attention on certain stimuli. Further research has done well to support the searchlight hypothesis, revealing that the TRN is responsible for gating certain stimuli by suppressing extraneous sensory information (Pinault & Deschenes, 1998).

Because TRN outputs are entirely inhibitory, the notion of attention by means of suppressing surrounding sensory input has been the focus of many studies. Acting as a gateway through which sensory information from thalamocortical and corticothalamic pathways is regulated, the TRN is divided into sectors whose function is to act as separate gates for distinct sensory stimuli (Guillery *et al.*, 1998). Named as such because of their influence on corresponding sensory outputs from the thalamus, areas such as the somatosensory, auditory, and visual sectors of the TRN have been targeted in research on sensory perception. Yu *et al.* (2009, 2011) found that neurons located in the auditory sector responded to neural stimulation of cells in the medial geniculate body, and that auditory discrimination tasks activated neurons in both of these areas. McAlonan *et al.* (2008) studied the effects of a visual discrimination task on changes in activation of the visual TRN and the lateral geniculate body and found activation in both sectors during the task. This evidence reveals the existence of neural networks between the thalamus and the TRN which activate during tasks involving auditory and visual stimuli, networks which show the comprehensive nature of sensory processing from the thalamus to the cortex and which can explain the role of the TRN in this processing.

Sakata *et al.* (2002) found that following an auditory-visual discrimination task, rats showed Fos enhancement in the auditory cortex and the visual cortex which corresponded with the learning of auditory and visual tasks, respectively. This finding seems to fit the hypothesis that the TRN acts to inhibit task-irrelevant stimuli—if the stimulus was task-relevant, the TRN would not have inhibited certain thalamic projections to the auditory or visual cortices, and thus we would expect to see higher Fos expression in these corresponding cortical areas.

McAlonan *et al.* (2000) found that following an auditory-visual discrimination task, rats that were previously conditioned in either an auditory task or a visual task showed higher Fos protein expression in the auditory and the visual sectors of the TRN, respectively. Because all corticothalamic and thalamocortical connections must pass through, and thus activate, the TRN, it is not surprising to find higher neuronal content in a sector constantly activated during these tasks. These results could also correspond with Sakata *et al.*'s (2002) findings, assuming some form of precise lateral inhibition occurred within the TRN—that is, if an area of the cortex associated with one modality sends projections to the TRN to suppress thalamic sensory input in that same modality, then both the cortical and the TRN sectors of this modality would be expected to show higher levels of Fos protein expression. TRN and thalamic activity during similar tasks may lend us insight into the nature of thalamoreticular and thalamic activation.

Neuronal activation that occurs as spikes in frequency has been examined in at least two conditions: following the activation of areas of the thalamus which are connected directly to the TRN neurons (Kimura *et al.*, 2007; Lam & Sherman, 2011; Yu *et al.*, 2011), and during tasks designed to manipulate attention and distraction (McAlonan *et al.*, 2000; McAlonan *et al.*, 2006; Yu *et al.*, 2009). Since both conditions activate the thalamus in a similar manner, it is possible that the two are indivisible. That is, activation of the thalamus upon the presence of a new sensory stimulus will project to the TRN, in line with an attentional shift, at which point the TRN could quickly inhibit the thalamus and then promptly resume resting potential. In support of this theory are the short latency and duration of visual TRN activation observed by McAlonan *et al.* (2006) during the presentation of a stimulus, followed by a quick (200-400 ms) decrease to similar levels of pre-stimulus activity. Furthermore, the networks of open-loop and closed-loop circuits among the cortex, TRN, and thalamus could explain this brief activation during the onset of a new stimulus: one schematic proposed by Zikopoulos and Barbas (2007) suggests that a single TRN neuron could project to two different areas of the thalamus during an attentional task. In the context of sensory gating, the idea that individual neurons in the TRN have the ability to send inhibitory projections to multiple regions in the thalamus suggests that widespread activation is not necessary, even in the presence of multiple stimuli. If this is true, we would expect to see brief, high activation—brief because these neurons are essentially killing two birds with one stone, and high because performing two jobs requires more energy.

Indeed, brief spikes of activation in the TRN have been observed during tasks in which the stimuli presented were relevant, but also during tasks in which the stimuli presented were irrelevant (McAlonan *et al.*, 2006; McAlonan *et al.*, 2008; Yu *et al.*, 2009). Rapid onset of activation in the auditory TRN occurred during an auditory task in which a deviant stimulus, a tone which differed from the conditioned tone, was presented (Yu *et al.*, 2009). This brief activation was also observed in visual TRN neurons during a task in which a visual stimulus existing outside of the targeted neuron's receptive field was displayed (McAlonan *et al.*, 2008). What each of these studies reveals is that activation in modality-specific areas of the TRN showed higher spikes during the presentation of irrelevant stimuli than of relevant stimuli. In addition to these findings, McAlonan *et al.* (2006) observed spikes in visual TRN activation during an attentional task in which an auditory stimulus was to be attended. This finding reveals that attentional tasks related to a certain modality can still evoke similar, albeit weaker, activation in a differing modality-specific sector. Each of these studies found activation in a modality-specific sector of the TRN during an attentional task which presented a deviant or non-relevant stimulus. This effect has been observed in other studies while the fact that the TRN responds to deviant stimuli, at all, presents an interesting task to researchers who use behavioral measures to collect data. If this area of the brain is active during both relevant and irrelevant stimulus presentation, but not solely sensory input, then its function may prove to be more than just an open gateway from the thalamus to the cortex.

By recording neurons in the visual sectors of the TRN and of the thalamus of macaque monkeys, McAlonan *et al.* (2008) discovered high visual thalamus activation when the monkeys attended to a stimulus and high TRN activation when that stimulus was located outside of the receptive field of the targeted neuron. When the stimulus was located within the receptive field of the TRN neuron, then, reduced activation in the visual thalamus was observed. This finding illustrates the reciprocal relationship between the visual sectors of the TRN and of the thalamus: when one shows increased activation, the other shows decreased activation. The nature of this activation may be similar in other modality-specific sectors, such as auditory or somatosensory areas. Indeed, Yu *et al.* (2009) tested the responses of the TRN and the thalamus during an auditory task and discovered that inactivation of the auditory TRN led to increased responses in the auditory thalamus. Although there is evidence that this reciprocity exists within individual sensory modalities, there has increasingly been research conducted which suggests that there may be reciprocity among different sectors of the TRN and the thalamus due to communication among differing sensory systems.

There is evidence to support that the distinct sensory sectors are interlinked, and that the networks between the TRN and the thalamus contain neurons with cross-modal relationships (Guillery *et al.*, 1998; Crabtree & Isaac, 2002; Kimura *et al.*, 2007; Kimura *et al.*, 2011). In their study on the organization of thalamic input to the TRN, Lam and Sherman (2011) used photostimulation to reveal the physical makeup of the TRN; what they found was that although organization among different sensory inputs existed via distinct clusters of neurons, about one-quarter of these neurons also received input from thalamic regions which relayed different sensory information to the cortex. Other research has revealed similar structure and functionality: within the TRN, there are distinct sectors whose activation correlates with each sensory process, but that certain neurons within these sectors can become activated by different sensory areas in the thalamus and/or cortex (Crabtree & Isaac, 2002; Kimura *et al.*, 2007; Kimura *et al.*, 2011). We are currently aware of neurons in the auditory sector of the TRN which directly influence visual, somatosensory, and visceral areas in the thalamus; of neurons that project from the auditory TRN to the lateral geniculate nucleus (LGN) and vice-versa; and of neurons in the auditory and visual sector of the TRN which are influenced by the visual and auditory cortices, respectively (Yu *et al.*, 2009; Kimura *et al.*, 2011; Yu *et al.*, 2011). Cross-modal connections among these sectors reveal another level of influence in these pathways—it is possible that the activation of certain neurons in one modality-specific sector of the TRN can affect the sensory processing in a different modality. This possibility furthers the evidence in support of the searchlight hypothesis, and it also augments the research conducted on the TRN's involvement in attention and distraction.

Stemming from Crick's research in 1984, many studies have been devoted to understanding the TRN's involvement in attention and distraction. Weese *et al.* (1999) conducted an experiment in which ablation of the visual TRN resulted in lowered reaction times in a visual orienting task which followed a cue in the same location as the stimulus, despite the fact that there was no significant lowering of correct responses post-lesion. This study notes that the abolition of the validity effect—when reaction time to a visual target is lower when a preceding cue is in the same location as the target than when it is in a different location than the target—is evidence in support of the TRN's role in attention. Indeed, TRN activation in rats spikes upon the presentation of a sensory stimulus, but it decreases quickly thereafter (McAlonan *et al.*, 2006; McAlonan *et al.*, 2008), suggesting that the TRN is especially sensitive to the sudden onset of a sensory input. This notion is supported by the consistent firing patterns observed in both auditory and visual modalities (McAlonan *et al.*, 2006; Yu *et al.*, 2009). The nature of this activation fits into the argument for the TRN's role in attention and distraction—brief TRN activity following stimulus presentation suggests a rapid direction of attention, while the quick decrease in activation would suggest a transfer of sensory processing to a different system following the attentional shift. Since the TRN inhibits sensory input from the thalamus to the cortex, and its neurons prefer deviant stimuli over standard stimuli (Yu *et al.*, 2009), we can infer that TRN modulation of these specific thalamic sectors can reduce activation in the presence of a distraction.

The known interconnectedness of these sensory pathways suggests strongly that there are many other connections that exist among the different sectors. Given the information available to us, we have come to a few conclusions. The first is that the TRN works to inhibit the thalamus, thereby reducing its activation, and that this process weakens the signal to the cortex. The second is that sectors in the TRN devoted to certain sensory processes will act on sectors in the thalamus devoted to these same sensory processes. The third is that many of these sectors are known to be cross-modal: they can affect more than just their devoted sensory output. Based on these conclusions, we can speculate about the nature of other sectors whose connections have yet to be recorded. The visual sector of the TRN is one of these areas—although we are aware that certain neurons in the visual TRN are influenced by the auditory cortex (Yu *et al.*, 2011), there is no current research that reveals any cross-modal connections emanating from this sector. The proposed study hopes to discover further interactions which could explain role of the visual TRN in visual and auditory processing. Furthermore, we hope to find a cross-modal interaction between the auditory and the visual sectors of the TRN which could play a role in discrimination tasks in rats. Kimura *et al.* (2011) developed a schematic based on a comprehensive review of the literature regarding cross-modality among neurons in the TRN and the thalamus, which we have used as a guideline for the current study (See Figure 1). In addition to this diagram, we have devised another schematic based on our review of the literature, which outlines the connections that exist among the TRN and the thalamus.

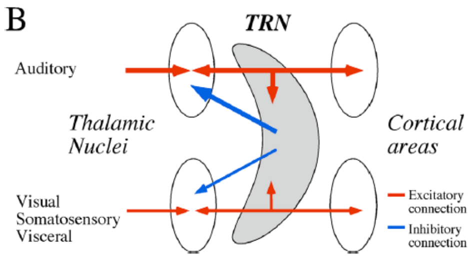

Figure 1. Model proposed by Kimura *et al.* (2011). The Auditory TRN receives multiple inputs from thalamic nuclei, and it has inhibitory connections within the TRN and among the different sectors of the thalamus.

We suspect that there exists a relationship between the auditory sector of the TRN and the visual sector of the TRN or the thalamus which allows inhibition of auditory sensory processing by the visual TRN. Additionally, we believe that the auditory TRN may have an effect on visual processing during a visual discrimination task. The present study expects to find that ablating either the visual sector or the auditory sector of the rat TRN following behavioral training in a visual discrimination task with an auditory distraction will have multiple effects. Variables such as percentage of correct and incorrect responses, reaction time, movement time, and percentage of premature responses, will be recorded during pre-lesion and post-lesion testing; these will be analyzed to determine whether there is a significant difference between the two groups. We will also compare these data to the post-lesion data of both the auditory TRN lesion and visual TRN lesion groups.

## METHODS

*Animals:* 6 Long Evans hooded rats were housed in groups of two in plastic boxes on a 12 hour light/dark cycle. Testing was conducted in the dark phase. The rats were fed daily, but available water was restricted to testing periods and the half hour which follows. All IACUC guidelines were followed thoroughly.

*Apparatus:* The testing apparatus was a 22 cm long, 13 cm wide box. Along one wall of this box were 3 poke holes whose diameters measure 1 cm each and two light bulbs, one located near the bottom of the wall and one located near the top of the wall. On opposite wall were a water dispenser and a speaker. The box was ventilated.

*Testing paradigm:* The tasks were based on the audio and visual discrimination tasks used by Sakata *et al.* (2002). The behavior training took place in the testing apparatus before trials began. The rats were habituated to the chamber for one hour per session, during which administration of water will was with the turning on of a light. Following these sessions were sessions in which the rats' exploration of the poke holes activated activate the light and made available the water dispenser. Once a strong association was made between the activation of the lights and poking the nose into the holes, the rats were reinforced with water only when they poked their nose into the hole that corresponded to either the top or the bottom light.

A visual stimulus, illumination of either the top light or the bottom light, was randomly presented for 1 second in each of the trials. Following stimulus presentation, the response panel was available for 3 seconds. The rat was required to poke its nose in the left hole if the top light was illuminated and poke its head in the right hole if the bottom light was illuminated. A correct response resulted in the availability of the water dispenser. An incorrect response resulted in a "time-out" period of 1 second, during which the all lights were extinguished and no water was available. Testing sessions were completed after 128 correct trials. After the rats had reached at least 80% correct trials, auditory stimulus trials was interspersed among visual discrimination trials. These trials followed the same procedure as the visual discrimination trials, but the visual stimulus was presented in tandem with an auditory stimulus, a 2 kHz tone. Upon reaching at least 80% correct trials for six consecutive training days, the rats were be prepared for surgery.

*Surgery:* The rats received bilateral infusions of the neurotoxin ibotenic acid into the TRN. Anesthesia was induced with an intraperitoneal injection of ketamine (.95 mg/kg body weight) and xylazine (.4 mg/kg). The rats were then be placed in a stereotaxic frame with atraumatic ear bars, with the nose bar set so that the skull was level. A midline incision was made along the scalp, and the skin and fascia were retracted to expose the skull. A hole was then drilled on either side of the skull, at the coordinates of 3.3 mm posterior, +/-3.5 mm lateral to bregma. The needle of a 1 ml syringe containing 0.3 ml of 0.09 M ibotenic acid was then be lowered to 5.6 mm below the skull surface, and the ibotenic acid was infused manually at a rate of 0.15 ml every 3 min. The syringe was left in place for 3 min before being withdrawn slowly. The incision in the scalp was then be closed using sterile metal needle and nylon thread. Finally, the animal was placed in a warm box to recover before transfer back to its home box. Testing with both visual discrimination and visual discrimination with auditory distraction resumed after a 3 day waiting period; this testing will last 6 days.

_Histology_: At the conclusion of postsurgical testing, the rats were killed by intraperitoneal administration of Ketamine (1.5 mg/kg) and xylaxine (.3 mg/kg). The rats were perfused transcardially with buffered saline for 5 min at a rate of 10 ml/min, followed by a 4% paraformaldehyde in phosphate buffer for 5 min at the same rate. Their brains were then removed and placed into a 20% sucrose–4% paraformaldehyde phosphate buffer solution until processed. Serial coronal sections 40 microns thick will be cut using a freezing microtome, and one slice per third section of 400 mm will be taken for staining with cresyl violet.

_Data Analysis:_ Lesion location, reaction times, number of correct and incorrect responses, and other similar variables such as premature responses, intertrial responses, and movement time from pre-lesion and post-lesion trials will be compared. Repeated-measures ANOVA will be used with two within-subject factors: surgery (preoperative and postoperative) and lesion location (visual TRN or auditory TRN).

## RESULTS AND DISCUSSION

We developed the visual discrimination task with auditory distractions in order to measure rats' performance before and after lesions in the visual TRN. Though there was no main effect for lesion on left or right correct responses (F 2,68=.198, p=.658; F 2,68=.012, p=.912, respectively) , there was a main effect for trial type on number of correct responses, such that distraction trials yielded significantly lower correct left and right responses (F 2,68=19.45, p=.000; F 2,68=21.728, p=.000, respectively). There was no interaction effect for trial and presence of lesion on left and right correct responses (F 2,68=.249, p=.619, F 2,68=1.27, p=.264, respectively). It is likely that the lesion did not affect the thalamic reticular nucleus due to missing its location on one or both of the infusions. It is also possible that, given time constraints, the rats were unable to learn fully the tasks—indeed, many of the rats would perform well on some days and poorly on others for no apparent reason. Though histology will be able to confirm whether the infusions were made accurately, we do not currently have these data.

Although we cannot conclude whether the infusions were accurate, the current data drawn from this research suggests two main findings. The first is that when presented with a distracting stimulus, such as an auditory tone or white noise burst, in tandem with a discrimination task, rats perform more poorly on the task (see also Sakata *et al.* 2002). This phenomenon is likely due, in part, to the role of the TRN in inhibiting the effects of irrelevant stimuli (Guillery *et al.*, 1998; McAlonan *et al.*, 2006; McAlonan *et al.*, 2008). While our findings may support this hypothesis, they may also negate it, depending upon the location of the lesions—that is, if the visual TRN were accurately infused, our rats' performance post-lesion would suggest that this area of the brain may not be as involved attentional gating as we had originally believed. Despite this possibility, our research is beneficial in that we were able to add one more testing paradigm which can be used to study the effects of visual and auditory discrimination tasks. We are currently unaware of any other research which utilizes an adaptation of Weese *et al.*'s (1999) reaction time task with visual cues and nose poke holes. This paradigm can be used to measure movement and reaction time, as well as premature and late errors, revealing even more measures by which to observe behavior during attention and distraction tasks. Limited time or resources could lead others in this field to use a similar method when conducting research. Concerning cross-modality among the different sectors of the TRN, our study will be able to provide more information when a complete histology is performed.

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