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Roles of sub-regions of the Ventromedial Nucleus of the Thalamus (VMT) in an Attentional task

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An abstract of
A thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

The ventromedial nucleus of the thalamus (VMT) is a longitudinally elongated nucleus of mostly medium to large, multipolar and rounded closely packed cells. Autoradiography studies examining thalamocortical outputs have shown that the medial VMT projects to medial prefrontal cortex, while the lateral VMT projects to lateral prefrontal cortex, sensorimotor. Unpublished work in the Neill lab has investigated the effects of transient VMT manipulation on the performance of rats in the 5-choice serial reaction time task (5-CSRTT). The study showed that injections of the GABA agonist muscimol in the center-to-medial VMT produced an increase in premature responding in the 5-CSRTT. However, inadvertently misplaced cannulae, in which muscimol was injected into the medial VMT of one hemisphere and the lateral VMT of the other, showed large increases in errors of omission. Additionally, the results of a few rats with injections into the more medial VMT, at volume of 0.5 µl, showed an increase in premature responding, while the same amount of drug, at the same site, increased errors of omission when administered in a volume of 1.0 µl. Based on the unpublished results, two hypotheses were formed: (1) injections of higher volumes of muscimol into the medial VMT will result in increased errors of omission, presumably due to diffusion to a nearby tissue, and (2) that the lateral VMT is this tissue. To investigate these claims, sub-regions of the VMT were transiently deactivated by the GABA agonist muscimol and activated by GABA antagonist picrotoxin. The results of this study are consistent with the proposed hypotheses and suggest that the medial VMT function is one of "behavioral inhibition," while the lateral VMT is related to motoric slowing or loss of motivational attention.

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INTRODUCTION

Psychologist and philosopher William James (1890) wrote that attention is "taking possession of the mind of one out of what may seem several simultaneously possible objects or trains of thoughts. It implies withdrawal from some things in order to deal effectively with others." More recently, Wickens and McCarley (2008) have metaphorically described attention as both a mental filter (*sensory attention*) and mental fuel (*motivational attention*). They proposed that "sensory attention" allows for the selecting of certain stimuli to be processed and the filtering of less relevant events. By "motivational attention," they meant that mental resources or fuel limit the information processed, controlling the level of processing that can be carried out at once.

Continuing along the line of sensory and motivational attention, Davies and Parasuraman (1982) expanded the concept further, dividing it into discrete sub-groups. Explained within the context of highway driving, Davies and Parasuraman state that initially, the driver will want to concentrate on the task of driving (*focused attention*). Rarely, however does the driver engage in only one task, but often will select between alternatives, for example checking the map or his/her blind-spot (*selective attention*). Sometimes, however, the driver will succeed at multitasking, or in other words processing tasks in parallel (*divided attention*). These three components seem related to Wickens and McCarley's (2008) idea of "sensory attention." Ultimately, the driver will devote his/her efforts towards performing a prolonged task, whether that activity be as complex as completing an 11 hour road trip, or as simple as maintaining the speed limit between interstates (*sustained attention*). This component seems related to Wickens and McCarley's "motivational attention."

Supporting the idea of components of attentions, research from human positron emission tomography has shown that selective attention conditions activate the lateral orbitofrontal and insular premotor cortices, divided attention conditions activate the anterior cingulate and dorsolateral prefrontal cortices **(**Corbetta et al. 1991; Muir et al., 1996a**),** and sustained attention conditions activate the right frontal and parietal lobes **(**Pardo et al., 1991; Sarter et al., 2001**).** These cortical areas receive input from the thalamus. Studies in humans (e.g., Kinomura et al., 1996**;** Coull et al., 1998; Shipp, 2004**)** have identified the thalamus in attentional functions related to selective and sustained attention, as part of the fronto-parietal-thalamic attention network. Summarizing all of the above, the human data indicate that both thalamus and cortex are involved in aspects of attention.

Lesion experiments in rats have shown the importance of the medial prefrontal (mPFC), anterior dorsolateral, cingulate and parietal cortices in attentional processes **(**Muir et al. 1994**,** 1995; Christakou et al., 2001, 2004; Chudasama and Muir, 2001). A number of test paradigms have been used in these studies, including the 5-choice serial reaction time task (5-CSRTT), signal discrimination task, and attentional set-shifting procedures. The 5-CSRTT is a widely used procedure to assess sustained, selective attention, and divided attention in rats (Muir et al., 1996a; Chudasama and Muir, 2001; Maddux and Holland, 2010). The task requires rats to nose poke into one of five briefly illuminated stimulus-response apertures per trial to earn food as a reward (selective attention). Good performance requires that the rat continuously scan the apertures, because trials are presented every 5 sec over a 30 min or a 100 trials session (sustained attention).

The 5-CSRTT procedure measures a number of parameters relevant to attention, including response accuracy, speed of responding, errors of omission, and premature responding. Studies using the 5-CSRTT with rat subjects have reported a decrease in accuracy, increased correct response latencies, and increased perseverative responding following medial prefrontal (mPFC) lesions (Muir et al., 1996b). Additionally, a reduction in accuracy with an increase in perservative responding has been reported following ventral mPFC lesions (Passetti et al., 2002).

Scheibel (1997) showed the close relationship between the cortex and the thalamus. Specifically, the thalamus provides the major cortical afferents, acting as a filter between the cortex and rest of brain. Defects in filtering could lead to input overload (Andreasen, 1997). Most notably, studies have shown that pathologies concerning attentional abnormalities may stem from a deficit in sensory processing **(**Mather et al., 1983; Dunn et al., 2003).

Thalamocortical projections can be crudely divided into two types, called "specific" and "nonspecific." The terminology derives from electrophysiological data suggesting the thalamocortical mechanism is capable of altering neuronal activity in widespread regions of the cerebral cortex (Morison and Dempsey, 1942).

Although both systems project to the cortex, they receive different inputs. Neurons of the specific system are thought to carry, via intricate firing patterns, information representing attended stimuli, whether from sensory organs or elsewhere in brain. In contrast, nonspecific neurons are not thought to carry information via intricate firing patterns. Rather, their firing rates "modulate" or "bias" the responsivity of the specific system neurons to their inputs.

Anatomically, the nucleus ventralis medialis thalami or ventromedial nucleus of the thalamus (VMT) is a constituent of the 'nonspecific' thalamus. The VMT is a longitudinally elongated nucleus of mostly medium to large, multipolar and rounded closely packed cells (Herkenham, 1979). It receives GABAergic afferents from the substantia nigra pars reticulata, the globus pallidus, and the entopedunuclar nucleus **(**Carter and Fibiger, 1978**;** Kha et al., 2001). Most notably, the axonal output of the VMT projects to layer I of the frontal cortex (leonard, 1969), and more particularly, the superficial portion of layer I of the cortex (Herkenham, 1979). This projection to layer 1 has been described as "massive and highly convergent**"** (Rubio-Garrido et al., 2009). The VMT-cortex projection uses glutamate as its transmitter, and is therefore excitatory on the cortical cells. Layer 1 is relatively poor in neuronal cell bodies; the input from VMT terminates on the apical dendrites of (specific) cortical pyramidal cells whose cell bodies are in deeper layers of cortex (layers 3 and 5) (Cauller et al. 1998, Rubio-Garrido et al., 2009).

The above anatomy and physiology can be used to suggest that the VMT, by modulating cortical responsivity, should be crucial in attentional processes. However, most research pertaining to the VMT has concentrated mainly on gross motor effects, with minimal concern to attentional processes (Alexander et al., 1986; Uylings et al., 2003). Previous studies examining acute and chronic manipulations of the VMT suggest a close association between VMT and the mechanisms controlling posture and locomotion. Starr and Summerhayes (1983a) reported that treating the VMT with a neural excitant or GABA antagonist caused hypermobility, while treating with a GABA agonist rendered the rat less active. In another study, Starr and Summerhayes (1983b)

found that rats with unilateral electrolesions of the VMT exhibited reduced locomotor activity, while those with bilateral lesions showed catalepsy. Therefore, the VMT has a strong role in motor control.

Jeljeli et al. (2003) showed that bilateral electrolytic lesions of the VL-VM thalamic complex impaired the acquisition, but not the performance of a motor skill in cats. These results suggested a more subtle effect of VMT lesions than simply a motoric deficiency. Consistent with this result, Neill (unpublished) has shown electrolesions of the VMT impair rats' ability to learn a variety of operant tasks, while showing minimal effect on preoperatively learned tasks. For both the results of Jeljeli et al. and Neill, if the effect of VMT damage was simply motoric, performance of preoperatively learned tasks should have been affected. On the other hand, a selective effect on learning is compatible with an attentional role of the VMT.

More recently, McGee and Neill (unpublished) have examined the effect of VMT manipulations on the performance of rats in the 5-CSRTT. McGee found that injections of the GABA agonist muscimol, which produces a transient deactivation of neuronal cell bodies in the injection site, increased premature responding in the 5-CSRTT. These injections were in the center-to-medial VMT. However, a few rats with inadvertently misplaced cannulae, in which muscimol was injected into the medial VMT of one hemisphere and the lateral VMT of the other, showed large increases in errors of omission. Additionally, the results of a few rats with injections into the more medial VMT, at her standard volume of 0.5 µl, showed an increase in premature responding, while the same amount of drug, at the same site, increased errors of omission when administered in a volume of 1.0 µl.

Based on McGee's observations from rats with misplaced cannulae or larger injection volumes, two hypotheses were formed: (1) injections of higher volumes of muscimol into the medial VMT will result in increased errors of omission, presumably due to diffusion to a nearby tissue, and (2) that the lateral VMT is this tissue. In the current study, these two hypotheses were tested in two separate experiments in separate groups of rats.

In addition, the effect of injection of the GABA antagonist picrotoxin into the lateral VMT was examined to determine, if the pharmacology is correct, whether the effect would be the opposite of muscimol injections into the same site.

METHODS

Subjects

Eleven male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) weighing 325-445 grams prior to surgery served as subjects. All animals were housed individually, maintained on a 12 hour normal phase light-dark cycle (lights on 0700 hr) and received water *ad libitum*. The rats were food deprived and chronically held at 90% of their free-feeding bodyweights. All behavioral procedures were conducted between 1500 and 1900 hrs. All experimental procedures were carried out in accordance with Emory's Division of Animal Resources (DAR), approved by the Institutional Animal Care and use Committee of Emory University, and were in compliance with National Institutes of Health guidelines for the care and use of laboratory animals. One subject died before completion of behavioral training.

Surgical Procedure

Stereotaxic surgeries were conducted under administration of 3-4% isoflurane. All rats were implanted with a 22-gauge bilateral guide cannulae assembly (Plastics One, Inc., Roanoke, VA) with flush stylets and a center-to-center distance of 3-4 mm (depending on the target site). Guide cannulae tips were aimed to terminate 1 mm above the medial VMT (n=4) for experiment 1 (AP= 6.6, L= 1.5, DV= 4.0) and lateral VMT (n=6) for experiment 2 (AP= 6.6, L= 2.0, DV= 4.0). Stereotaxic coordinates were obtained using the atlas of Paxinos and Watson (1998). The implants were secured to the skull with jeweler's screws and dental cement. The incisions were cleaned with an OTC antibiotic containing polymyxin B, bacitracin, and neomycin (CVS, Woonsocket, RI) and sutured in front and behind the cement skullcap polyethylene sutures. Flush stylets were inserted in the cannulae and a protective dust cap (both Plastics One, Inc., Roanoke, VA) was attached to the top of the assembly to prevent debris from clogging the guide cannulae. Rats were carefully observed for 24 to 48 hours following all surgical procedures and were allowed to recover for a minimum of six days. Food and water were available *ad libitum* during the recovery process.

Apparatus

The test apparatus for this experiment consisted of three 25 X 25-cm aluminum and Plexiglas chambers. The rear wall of each chamber was concave and contained five apertures, each 2.5 cm square, 4 cm deep, and 2 cm above floor level. Illumination of each aperture was provided by a LED located at the rear of the aperture; with an infrared photocell beam located at the entrance of each aperture to monitor the rat's nose poke responding. Located in the opposite wall was a trough type pellet receptacle (2" x 2"

square) with a LED in the rear and an infrared beam at the entrance. This was where nutritionally balanced 45 mg food pellets (BioServe, Frenchtown, NJ) were delivered to a hopper positioned 0.5 cm above the bottom of the chamber.

The chambers were individually placed within sound-attenuating cabinets and were ventilated by low-level noise fans, which also served to mask extraneous background noise. A 3 W incandescent overhead lamp was illuminated to serve as a ready signal to indicate the upcoming aperture cue trial.

Behavioral Procedure

Rats were trained to attend to a brief visual stimulus presented randomly in one of five spatial locations. The task used in the present study has obvious analogies with the Continuous Performance test of attention by Mirsky and Rosvold (1960). Thus, it contains elements not only of a sustained attention paradigm, with animals being required to monitor the apertures for brief presentations of the visual target during the 30 min session, but also requires the animal to divide attention across five spatial locations.

During the training period, rats were familiarized with the apparatus in two sessions. During these sessions, any nose poke into any aperture, breaking the light beam, resulted in a food pellet being delivered to the pellet receptacle. After the two nose-poke training sessions, 5-CSRTT training began. Each trial, rats were required to nose poke into transiently illuminated apertures for a food reward. The requirements of each trial were dependent on the rats training stage, with subsequent stages having shorter durations of test parameters. Each rat moved through stages 1 through 6 as successive criteria were met as described in Table 1, taken from Bari et al. (2008). We found most of our rats would perform at the 1.25 sec duration stimuli of Stage 6, but could not meet the

criteria of Bari et al.; we lowered the criteria to >60% accuracy and <25% omission, and all drug testing occurred using the 1.25 sec stimuli of Stage 6.

Training stage	Stimulus duration (s)	Intertrial interval (ITI) (s)	Limited hold (LH) (s)	Criterion to move to next stage
1	30	2	30	≥30 correct trials
2	20	2	20	≥30 correct trials
3	10	5	10	≥50 correct trials
4	5	5	5	≥50 correct trials >80% accuracy
5	2.5	5	5	≥50 correct trials >80% accuracy <20% omission
6	1.25	5	5	≥50 correct trials >80% accuracy <20% omission

Table 1: 5-CSRTT training schedule (modified from Bari et al., 2008).

At the beginning of each test session, the house light was illuminated and a single food pellet was delivered to the magazine. The breaking of the pellet receptacle light beam to collect this pellet initiated the first trial. After a fixed 5-s inter-trial interval (ITI), one of the five apertures was illuminated for 1.25 sec. Responses in this aperture during illumination and for 5 s afterward (the limited hold period) were rewarded with the delivery of a food pellet, and a correct response was recorded. Responses in a non-illuminated hole during the signal/limited hold period (incorrect response), failure to respond within the signal/ limited hold period (omission), and responses in an aperture during the ITI (premature response) were all punished with a 5-s period of darkness (time out). Responses in any aperture after a correct response were recorded as perseverative errors, but had no consequence. Responses in any aperture after an incorrect response, a premature, or an omission during the 5-s time out, which followed all of these conditions were recorded as time out responses, but had no consequences.

During any one session, the light stimulus was presented an equal number of times in each of the five holes in a random order. A daily session terminated after 100 trials or after 30 min of testing.

After an animal stably performed on Stage 6 for a number of days, the rats were removed from food deprivation for a few days and cannulae were implanted into the medial or lateral VMT as previously described.

Following at least one-week postoperative recovery, rats were placed again on food deprivation. When body weights were at the 90% free feeding level, postoperative behavioral testing began. The first two post-operative sessions were at Stage 5, as described in table 1. All subsequent sessions were at Stage 6.

Performance measures

Accuracy: The proportion of responses that were correct (number of correct responses/total number of responses), expressed as a percentage. This measured errors of commission (incorrect responses) without including errors of omission.

Errors of omission: The number of trials in which no response was made during the limited hold period was recorded. This measure reflects possible failures of detection as well as motivational/motor deficits, depending on the overall pattern of effects.

Premature responding: The number of responses in the apertures during the ITI was recorded. This measure reflects deficits in inhibitory mechanisms of response preparation.

Time out responding: The number of responses in the apertures during a 5-s time out period (period of darkness) after an incorrect response, an omission or a premature response. This measure reflects the efficacy of inhibitory processes of response control.

Speed: Three measures of response speed were recorded. The first was the latency to respond correctly: the time between the onset of the light stimulus and the point at which the rat's nose broke the infrared beam of the illuminated hole. The second was the latency to respond incorrectly: the time between the onset of the light stimulus and the point at which the rat's nose broke the infrared beam of a non-illuminated hole, or incorrect hole. The third was the latency to collect reward: the time between performance of a correct response and the retrieval of the food pellet from the food receptacle.

Intracerebral microinjections

Bilateral microinjections into the medial VMT and lateral VMT were performed at a flow rate of 6 µl/min by means of a stainless steel infusion cannula (30 gauge; Plastics One, Inc., Roanoke, VA) cut to protrude 1 mm beyond the tip of the guide cannulae. The plastic stopper on the infusion cannula was permanently affixed using superglue to prevent injector shortening or lengthening over time. The infusion cannula was securely attached to PE-10 standard wall cannula tubing (Clay-Adams, Parsipanny, NJ) by first relaxing the tubing with friction. The other end of the PE-10 tubing was connected to a 10 µl Hamilton syringe (Hamilton Company, Reno, NV) and mounted on a mechanical infusion pump (Sage Instruments, Cambridge, MA). A single bilateral sham injection (no fluid) was administered to all subjects following surgical recovery to induce the initial tissue trauma from injector insertion during a non-drug trial. During drug trials, the flush stylets were removed from the guide cannulae and the injector was lowered into each guide cannula. During the infusion procedure, rats were allowed a small range of mobility in a non-bedded home cage replica.

Experiment 1: A 20 ng dose of muscimol HBr in a volume of either 0.5 µl/side or 1.0 µl/side was injected into the medial VMT of all rats with medial VMT cannulae to test the hypothesis that the larger volume would be more likely to cause omission errors by diffusion to the lateral VMT. Control injections of both 0.5 µl/side and 1.0 µl/side of the isotonic saline vehicle were done in the same animals on different test sessions.

Experiment 2: Varying doses of muscimol (10, 20, and 40 ng) in a constant volume of 0.5 µl/side and a 50ng dose of picrotoxin in a constant volume of 0.5 µl/side were injected into the lateral VMT of all rats with lateral VMT cannulae. A control vehicle injection of 0.5 µl/side isotonic saline was also done in all rats.

Injection cannulae were maintained in place for 30 seconds after injection completion to allow for diffusion of the drug/vehicle into the brain tissue. Upon completion of drug injection, the flush stylets were reinserted into the guide cannulae to prevent drugs from reentering the guide cannulae.

Drugs

This study utilized the $GABA_A$ agonist muscimol HBr and the $GABA_A$ antagonist picrotoxin (Sigma-Aldrich Co., St. Louis, MO) dissolved in 0.9% w/v NaCI (saline).

Analysis of Results

After completion of all behavioral testing, the rats were humanely killed by CO_2 exposure in the Emory DAR facility in the Rollins Research Building. They were then intracardially perfused with isotonic saline followed by 10% formol-saline. After a few days of fixation, the brains were removed, 50 micron-thick frozen sections taken through the area of the guide cannulae, and the sections mounted on slides. The sections were subsequently stained with thionine and examined to confirm placement of cannula.

Analyses of variance for repeated measures were performed on percent correct, percent omission, premature responses, and time-out responses over the saline and three muscimol doses for the lateral VMT experimentation. When significant effects of muscimol were found, Newman-Keuls multiple-comparisons tests were performed to compare individual group means. Responses to injection of 0.5 µl of picrotoxin 50ng were compared to vehicle injections using paired student's t-tests.

Responses to 0.5 µl and 1.0 µl of muscimol 20ng into the medial VMT were compared by paired student's t-tests.

RESULTS

Experiment 1: Injections into the Medial VMT

Histology

Fig. 1 confirms that cannulae were found in the posterior and anterior regions of the medial VMT. The anterior-posterior (AP) placement of cannulae did not seem to correlate to any differences in drug effects between animals.

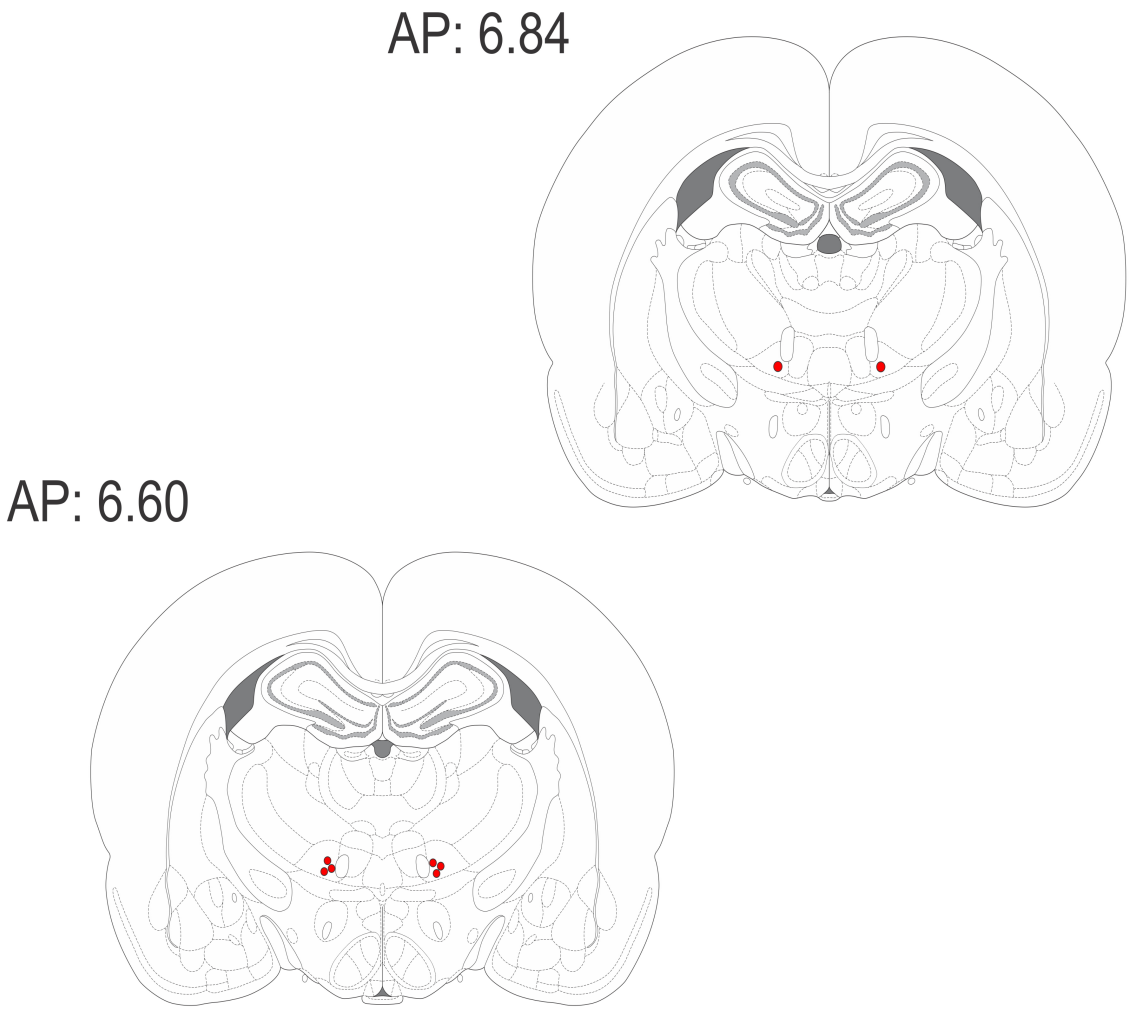

Figure 1: Location of cannulae placement in medial VMT.

5-CSRTT Performance

As shown in Figs 2A and 2B, there was no significant difference in the accuracy of responding for volume 0.5 µl vs. 1.0 µl ($t=1.44$, $p> 0.05$). Nevertheless, there was a trend for rats at volume 1.0 µl to show an increase in errors of omission, though this effect was not statistically significant ($t=2.32$, $p> 0.05$). Fig. 2C, shows a significant effect of infusion volume on premature responding ($t=3.35$, $p=0.04$), with time-out responding (Fig. 2D) showing a similar effect. The level of responding at volume 0.5 µl was significantly greater than responding at volume 1.0 µl ($t=5.72$, $p=0.01$). Furthermore, no effect for perseverative responding was found for infusion of muscimol at volume 0.5 µl vs. 1.0 µl ($t=2.08$, $p> 0.05$) (Fig. 2E).

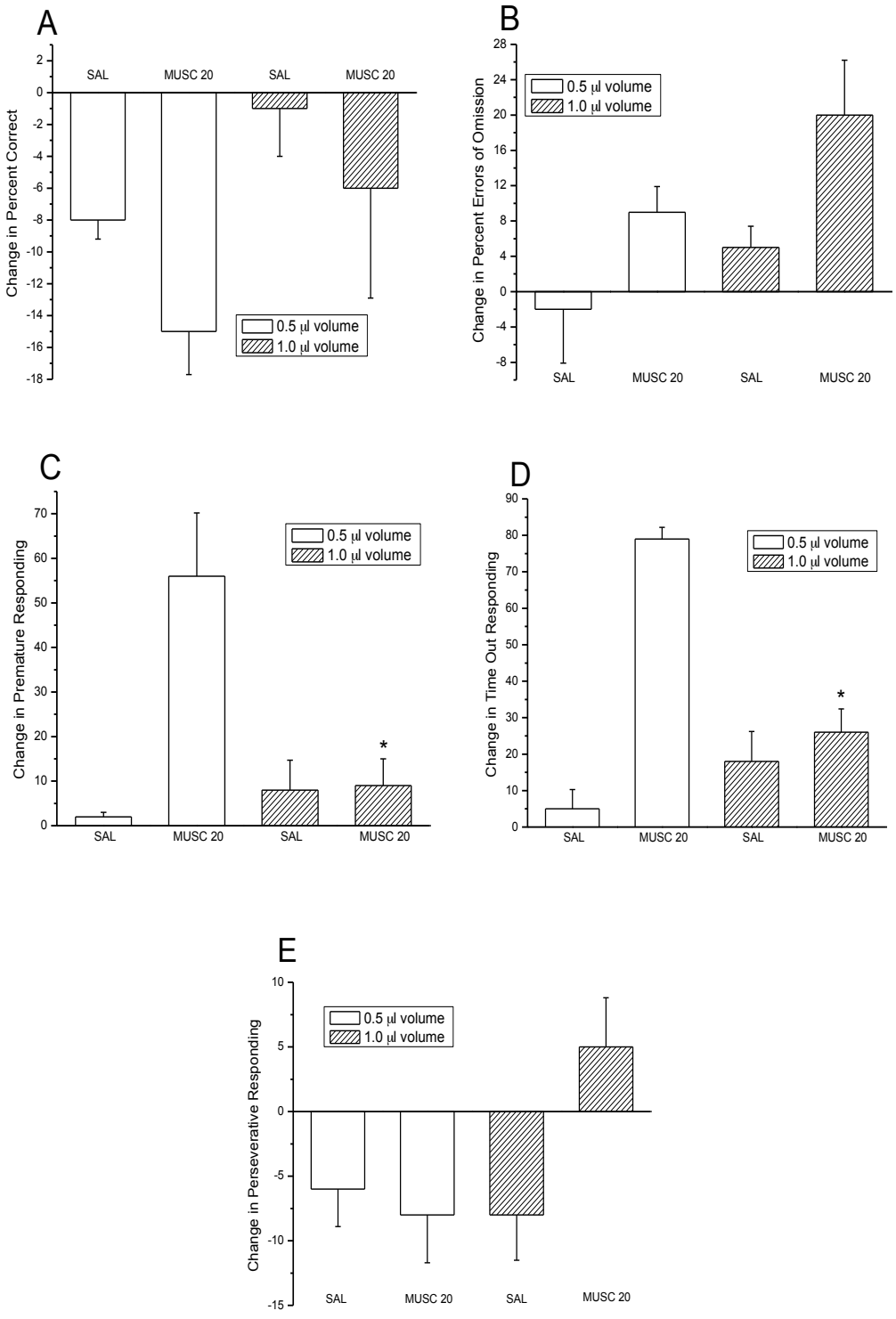

*Figure 2: Performance of rats after injections of muscimol into the medial VMT. A: Choice accuracy, B: Errors of omission, C: Premature responding, D: Time out responding, E: Perseverative responding. Error bars indicate SEM. * represents difference between 0.5 µl vs. 1.0 µl volume of muscimol, p< 0.05.*

Response Latencies

Latency to correct response (t=1.20, p=0.32) (Fig. 3A), latency to collect reward (t=0.88, p=0.45) (Fig 3B), and latency to incorrect response (t=1.71, p=0.19) (Fig.3C) all had no effect across volume 0.5 µl vs. 1.0 µl.

Latencies of Responding

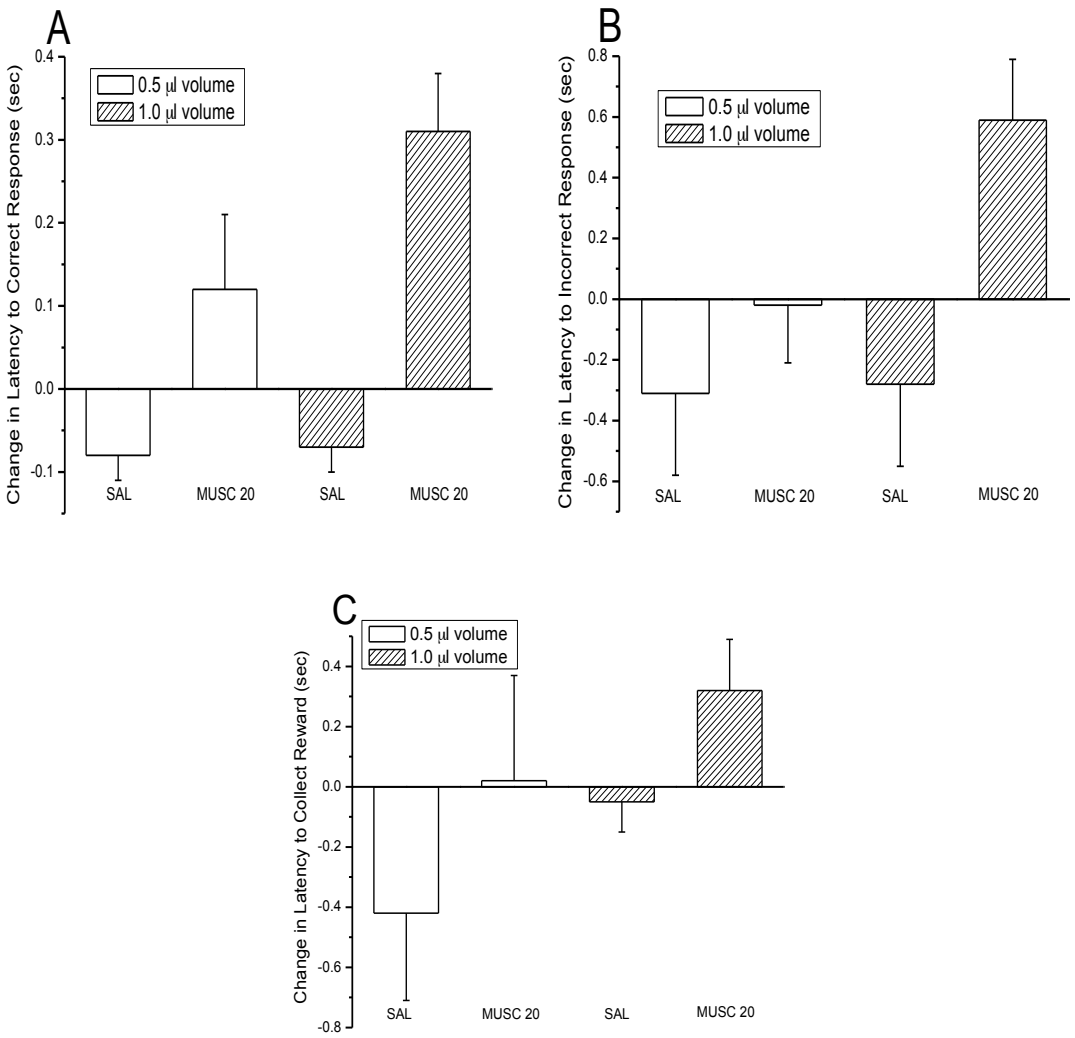

Figure 3: Latency to respond after injection of muscimol in the medial VMT. A: Latency to correct response, B: Latency to incorrect response, C: Latency to collect reward. Error bars indicate SEM.

Experiment 2: Injections into the Lateral VMT

Histology

Fig. 4 confirms that cannulae were found in the posterior and anterior regions of the lateral VMT. The anterior-posterior (AP) placement of cannulae did not seem to correlate to any differences in drug effects between animals.

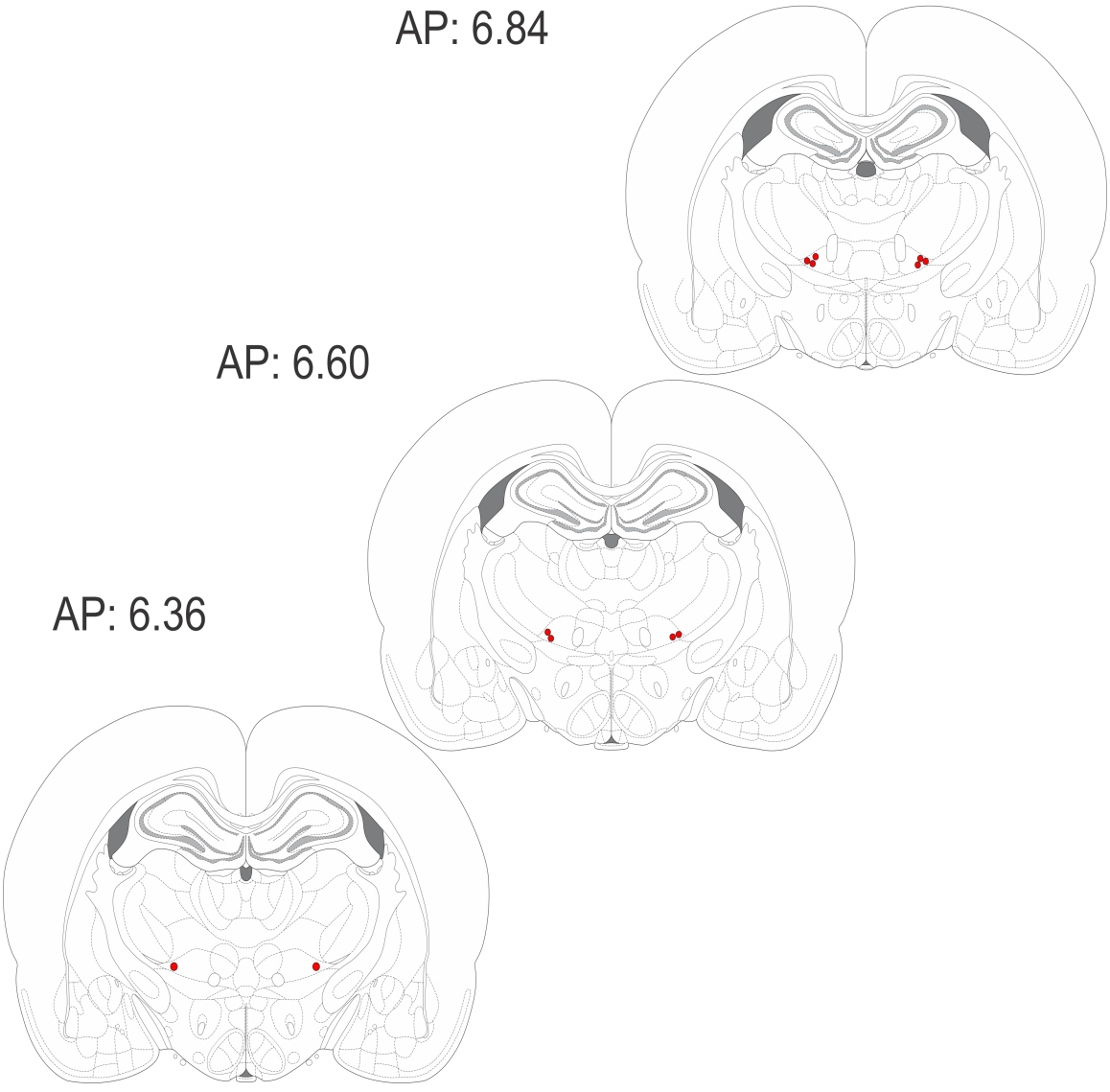

Figure 4: Location of cannulae placement in lateral VMT.

Accuracy

As shown in Fig. 5, injections of the GABA agonist (10, 20 and 40 ng of muscimol 0.5 µl/side) into the lateral VMT did not have an effect on the accuracy of responding ($F(3, 15) = 0.45$, $p=0.72$), and the data were highly variable.

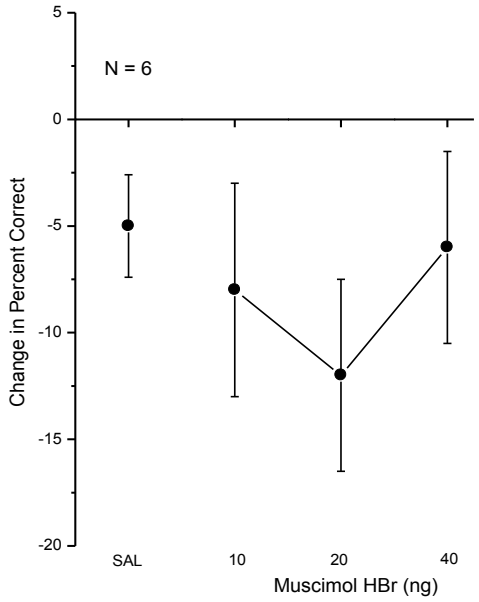

Figure 5: Percent accuracy of responding after injections in the lateral VMT.

Errors of Omission

As shown in Fig. 6, all rats showed an increase in the percent errors of omission across muscimol doses. There was a significant main effect of treatment ($F(3, 15) = 23.64$, $p< 0.001$), with omissions at doses muscimol 20 and 40ng being greater than with vehicle ($p=0.05$).

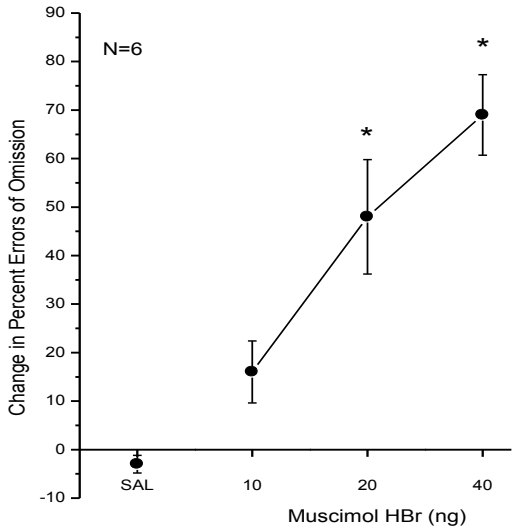

Figure 6: Percent Errors of Omission for responding. $p < 0.05$.*

Premature Responding

As shown in Fig. 7, muscimol injections into the lateral VMT tended to decrease premature responding, but the effect was not statistically significant (F (3, 15) = 2.92, 0.07).

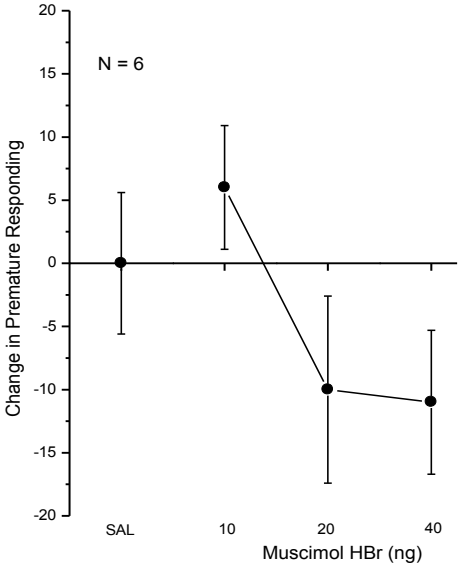

Figure 7: Premature Responding after injections into the lateral VMT.

Time-out Responding

For a rat to record a time-out response, the subject must nose poke in an aperture during the 5-s period of darkness following an incorrect response, premature response or error of omission. Muscimol effects on time out responses are shown in Fig. 8. Consistent with the trend toward decrease premature responding (Fig. 7), however, there was a significant main effect of treatment ($F(3, 15) = 4.33$, $p=0.02$). There was a decrease in time-out responding across the doses of muscimol (Fig. 8).

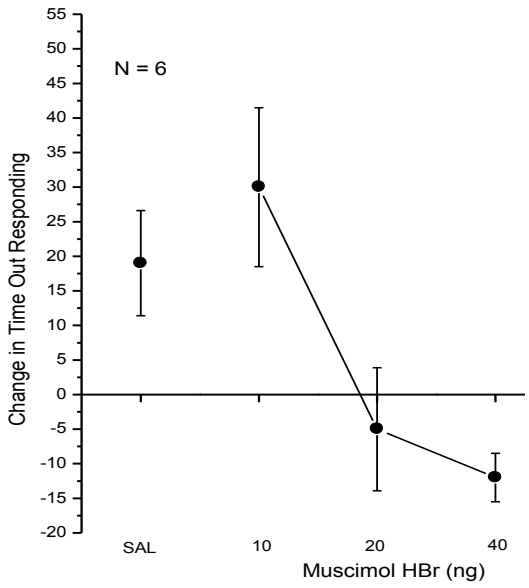

Figure 8: Time-out Responding after injections into the lateral VMT.

Perseverative Responding

As shown in Fig. 9, there was no effect in perseverative responding following treatment with drug ($F(3, 15) = 1.05$, $p=0.39$).

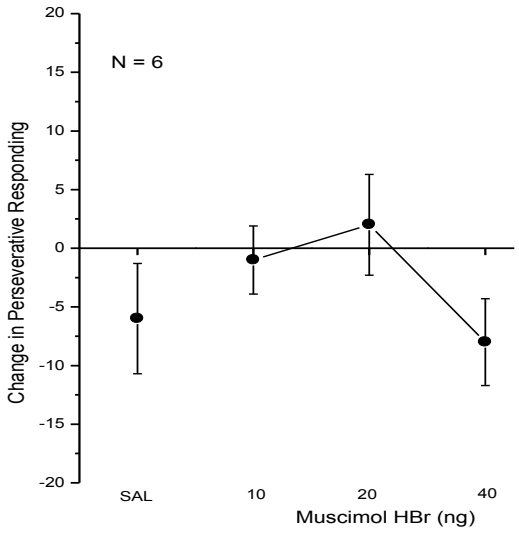

Figure 9: Perseverative Responding after injections in the lateral VMT.

Response Latencies

Latencies for Correct Responding

As shown in Fig. 10, there was a significant main effect of treatment on latency to correct responses ($F(3, 15) = 7.92$, $p < 0.05$), with latencies at muscimol (20 and 40 ng) significantly greater than latencies with vehicle injection ($p = 0.05$).

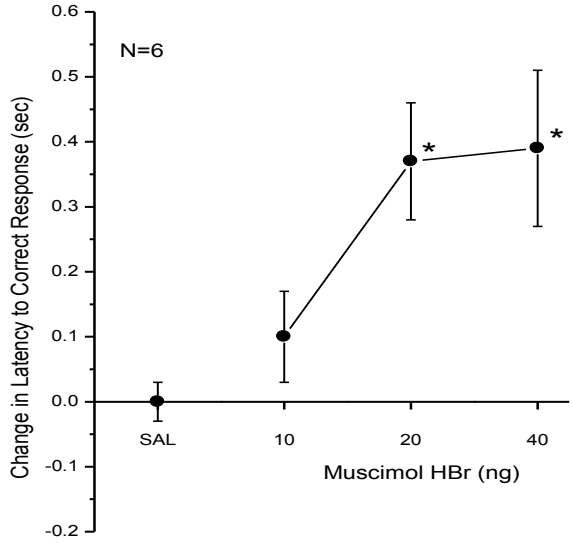

*Figure 10: Latency to Correct Response after injections into lateral VMT. * p< 0.05.*

There was a significant main effect of treatment on incorrect response latencies ($F(3, 15) = 4.51$, $p < 0.05$) (Fig. 11), although subsequent Newman-Keuls tests did not show significant dose differences.

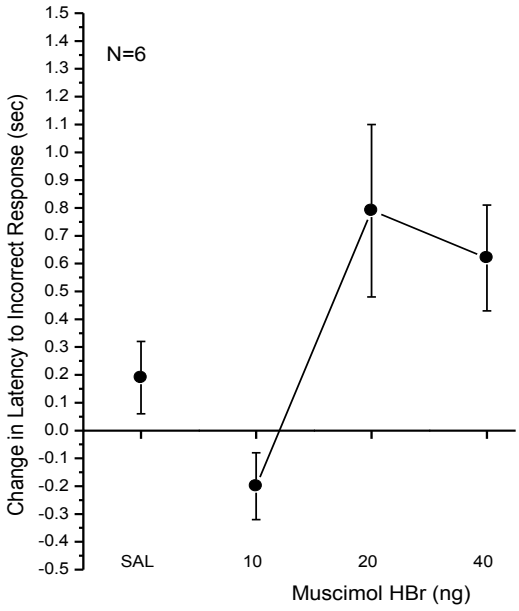

Figure 11: Latency to Incorrect Response after injections into lateral VMT.

In Fig. 12, there was a significant main effect of treatments on latency to collect reward ($F(3, 15) = 3.86$, $p<0.05$), with latencies after muscimol (10 and 40 ng) greater than after vehicle ($p=0.05$). However, the response after vehicle was highly variable perhaps associated with the rats' handling during procedural testing.

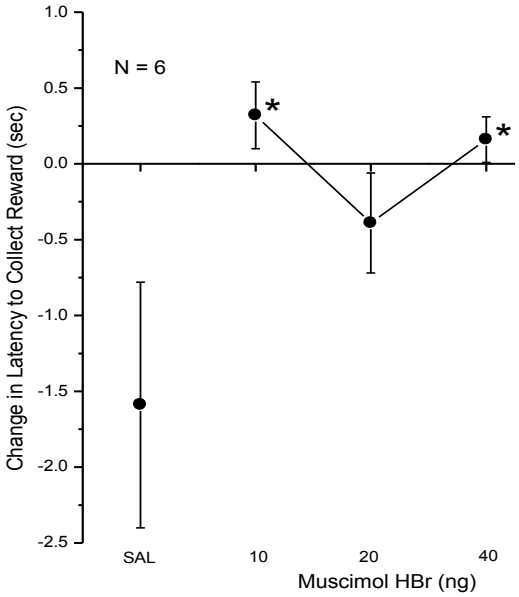

Figure 12: Latency to Collect Reward after injections into lateral VMT.

Picrotoxin injections in the lateral VMT

5-CSRTT Performance

As shown in Fig. 13A, picrotoxin injections into the lateral VMT had no effect on the accuracy of responding (t= 1.62, p= 0.18). Similarly, picrotoxin injected into the lateral VMT had no effect on errors of omission (t= 1.69, p=0.17) (Fig. 13B). However, premature responding (t= 3.03, p=0.04) was significantly greater after drug injection than after vehicle (Fig. 13C). Time out responding following picrotoxin, though tending to be greater than following vehicle injection, was not significantly so (t= 2.47, p= 0.07) (Fig. 13D). Perseverative responding was not significantly affected by picrotoxin (t=0.05, p=0.96) (Fig. 13E).

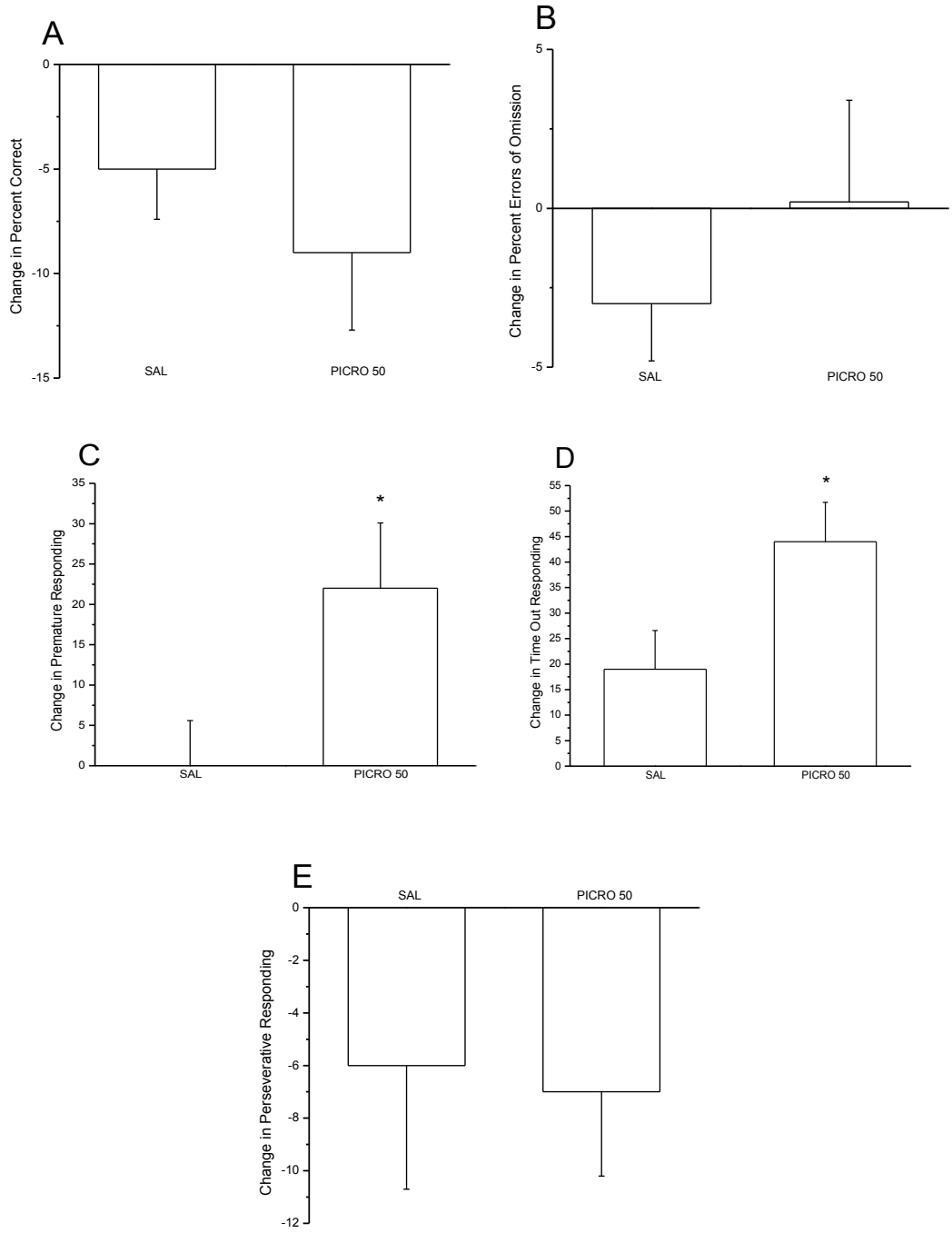

*Figure 13: Performance of rats with injections of picrotoxin into the lateral VMT. A: Choice accuracy, B: Errors of omission, C: Premature responding, D: Time out responding, E: Perseverative responding. Error bars indicate SEM. * represents significant differences from saline, p< 0.05.*

Response Latencies

As shown in Fig. 14A, the latency to correct response was unaffected by picrotoxin (t= 0.89, p=0.42). However, latency to incorrect response was significantly shorter than after vehicle (t= 3.74, p=0.02) (Fig. 14B); average latency to collect reward was not significantly affected by treatment (t=1.34, p=0.25) (see Fig. 14C).

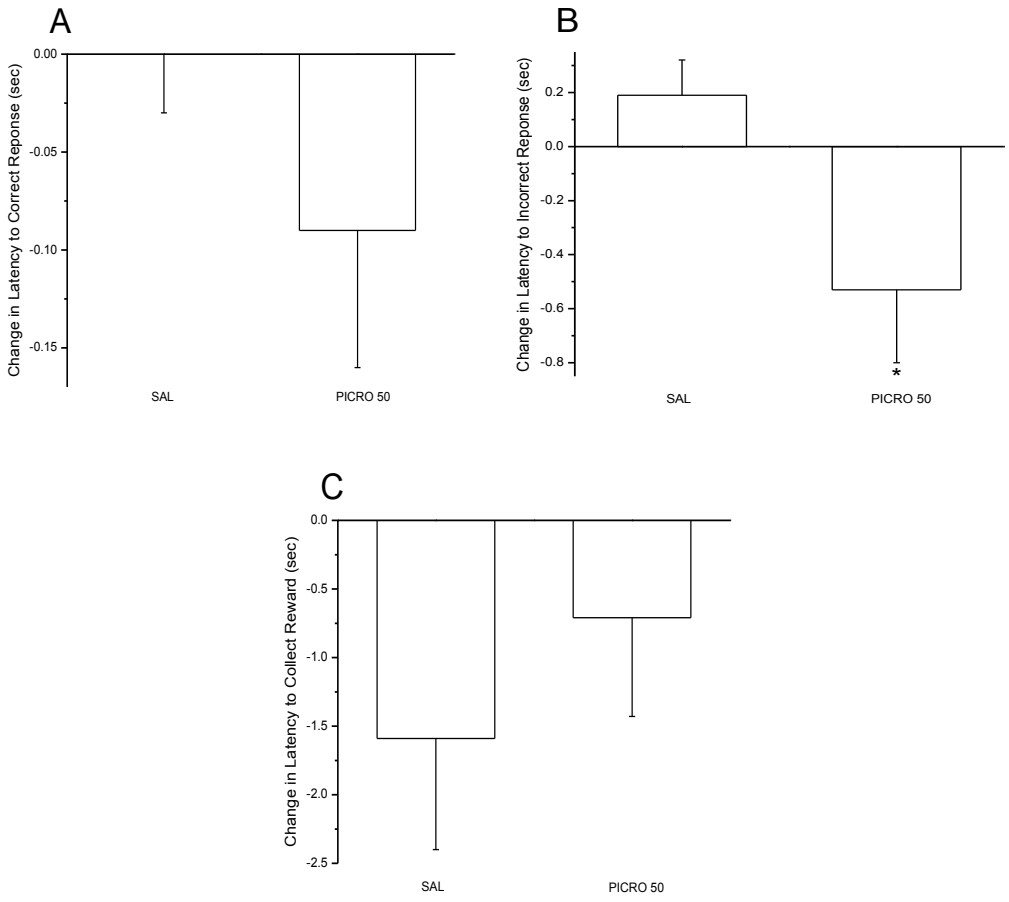

*Figure 14: Latency to respond after injection of picrotoxin in the lateral VMT. A: Latency to correct response, B: Latency to incorrect response, C: Latency to collect reward. Error bars indicate SEM. * p< 0.05.*

DISCUSSION

From McGee and Neill (unpublished) manipulations of the VMT causes hypo-hyperactivity in 5-CSRTT. More specifically, muscimol injected into the center-to-medial VMT increased premature responding, while inadvertently placed asymmetric injections, the medial VMT of one hemisphere and the lateral VMT of the other, showed large increases in errors of omission. Congruently, Aldes (1988) showed that the more medial VMT projects to the medial prefrontal cortex, while the lateral VMT projects to the somatic motor cortex in rats (Fig. 15). As such, the medial VMT is hypothesized to modulate executive control, while the more lateral VMT modulates sensorimotor functions. In other words, the medial VMT controls cognitive processes such as attention, inhibition, mental flexibility and planning, while the lateral VMT physically executes the required action.

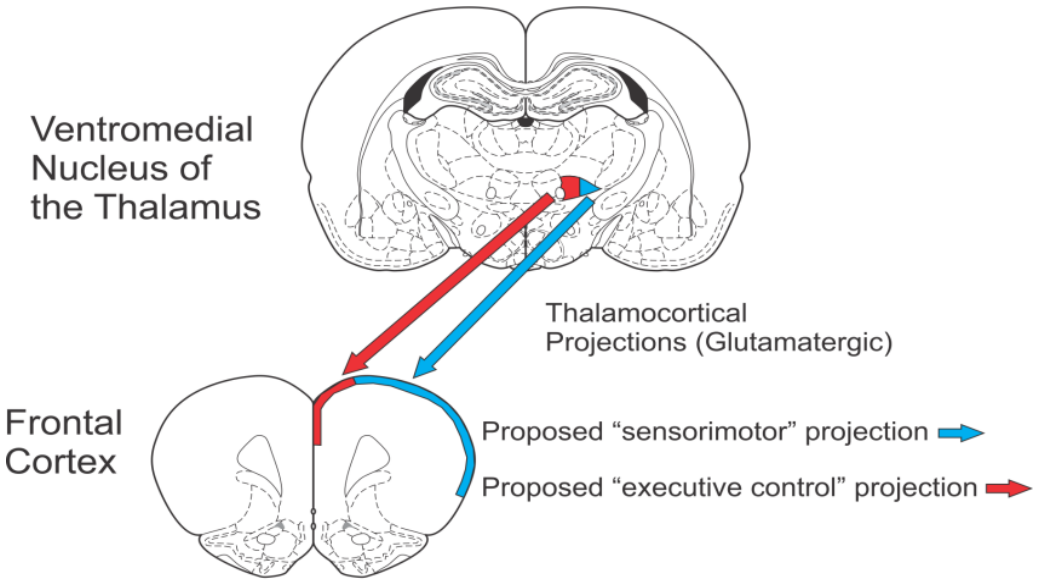

Figure 15: Cortical projections of sub-regions of the VMT

To investigate the proposed circuitry as shown in Fig 15, two experiments were conducted. Experiment 1 sought to characterize the function of the medial VMT after injections of muscimol, while experiment 2 sought to investigate the effect of muscimol and picrotoxin in the lateral VMT.

Experiment 1 tested the hypothesis that deactivation of the medial VMT by injection of 20 ng muscimol HBr in a volume of 0.5 µl would increase premature responding (Fig. 2C) whereas injection of the same dose in the same rat in a volume of 1.0 µl would increase errors of omission (Fig. 2B). The results of experiment 1 successfully replicated the findings of McGee and Neill (unpublished) in that injection of 20 ng muscimol into the medial VMT increased premature responding. As hypothesized, injection of the same dose in double the volume produced a very different pattern of effects characterized by an increase in errors of omission rather than premature responding. This pattern of results is consistent with the idea that the larger volume invades a nearby region, resulting in errors of omission.

Experiment 2 tested the hypothesis that the above-mentioned nearby region is the lateral VMT. First, direct injections of muscimol in the smaller volume (0.5 µl) into the lateral VMT were performed. The results strongly supported this hypothesis, showing a highly significant dose-related increase in errors of omission following lateral VMT injections.

Analysis of the pattern of behavioral change following muscimol injections in the medial VMT gives insight into the possible role of the medial VMT in behavior. The significant increases in premature and time out responding with deactivation of the medial VMT indicate that this region of the VMT normally functions to inhibit

inappropriate responding. In other words, the medial VMT function is one of "behavioral inhibition." Given that the medial VMT sends axons to the medial prefrontal cortex (Beckstead, 1979), and that medial prefrontal lesions have been reported to produce premature responding in the 5-CSRTT (Chudasama et al., 2001, 2003; Passetti et al., 2002), this result of medial VMT deactivation is not surprising.

An alternative explanation is that deactivation of the medial VMT enhances the reward of the food or the incentive salience of rewarding stimuli. Further experimentation might be able to test this explanation.

From a functional point of view, the results of the lateral VMT injections of muscimol are consistent with the known projection of this VMT region to the dorsolateral frontal cortex of rats (Desbois and Villanueva, 2001). Dorsolateral frontal cortex in rats is functionally sensorimotor cortex. Thus, deactivation of lateral VMT should deactivate sensorimotor cortex to some degree and reduce gross motor activity. This appears to be what happened following lateral VMT muscimol injections, in which errors of omission increased, premature responding tended to decrease, and time out responding significantly decreased (Fig. 6-8). In addition, video recordings made of some of the rats receiving lateral injections of muscimol showed slowed movements, but not problems with coordinated movements. Thus, the observed reduction in responding is less consistent with a motor deficit with regards to coordination, and more related to motoric slowing or loss of motivational attention. Interestingly, the latency data showed a significant increase in response time for incorrect responses (Fig. 11). Even under baseline conditions, times for incorrect responses were notably longer than for correct

responses. This result may reflect decision making by the rats, which may be more sensitive to disruption by lateral VMT deactivation.

Given that deactivation of the lateral VMT with muscimol slowed many aspects of responding, one would expect that activation of the lateral VMT with picrotoxin would have the opposite effect, i.e., increase aspects of responding. This expectation was supported by a significant increase in premature and time out responding (Fig. 13C-D), and a significant decrease in latencies for incorrect responses (Fig. 14B). That is, activation of the lateral VMT induced impulsivity.

As shown in Fig. 16, the medial VMT is notable for receiving major inputs from the medial substantia nigra pars reticulata (SNPR) of the ventral midbrain.

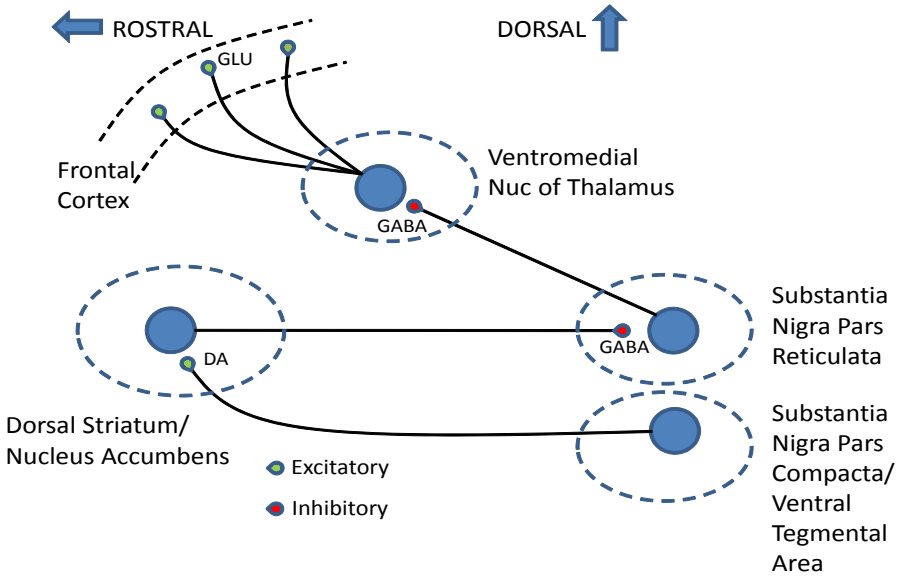

Figure 16: Afferents and efferent of the ventromedial thalamus (modified from Deniau et al., 1994).

The medial SNPR receives input from the medial nucleus accumbens. The nucleus accumbens is known to be involved in reward processes. Thus, the brain reward system is only two synapses away from the VMT, which is in turn one synapse away from the frontal cortex. One can speculate that this anatomy enables rewarding stimuli to reduce activity of the SNPR by stimulating the nucleus accumbens. This activity releases the inhibition on the medial VMT, thereby leading to the discharge of glutamate in the medial prefrontal cortex, stimulating brain systems for attention.

The results of the present study, in congruence with the hypothesized afferent/efferent connections (Fig. 16) of the VMT may help explain the well-known ability of psychostimulant drugs such as Adderall and Ritalin to enhance attention. These drugs facilitate dopaminergic transmission, including transmission in the accumbens. Fig. 16 shows that increasing accumbal output inhibits SNPR, removing inhibition over VMT, and should increase excitation of medial prefrontal cortex. Most notably, Aron et al., 2007 has shown that a decrease in the thalamocortical outputs in humans, and frontal and basal ganglia lesions in rodents cause impairment of stopping (impulsivity). In other words, the drugs should have an effect opposite to deactivation of the medial VMT, namely a reduction in impulsive behavior. This is one of the reported effects of psychostimulants in patients being treated for Attention Deficit Disorder Hyperactivity (ADHD).

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