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Akshay Goswami

Evaluation of Possible "Impulsivity" Following Ventromedial Thalamic Nucleus Deactivation in the Rat Using a Fixed Consecutive Number Task

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Abstract

Evaluation of Possible "Impulsivity" Following Ventromedial Thalamic Nucleus Deactivation in the Rat Using a Fixed Consecutive Number Task
By Akshay Goswami

This project was designed to assess the role of ventromedial thalamic nucleus (VMT), a thalamic structure which projects to prefrontal cortex, in impulse control as assessed by a fixed consecutive number (FCN) procedure. McGee (unpublished dissertation, 2014) demonstrated that transient deactivation via injection of the $GABA_A$ agonist muscimol into the VMT resulted in decreased efficiency of performance on a 5-choice serial reaction time test (5-CSRTT). Specifically, McGee observed an increase in premature responding, which was interpreted as an increase in impulsivity. This interpretation would be strengthened if premature responding could be demonstrated in a behavioral task other than the 5-CSRTT. The experiment reported here utilized the fixed consecutive number (FCN-8) task, in which the rat must complete 8 responses on one lever before a single response on a second lever in order to receive food reward; responding on the second lever before completion of 8 responses on the first lever does not deliver the reward, and the rat must start over. Stereotaxic procedures were used to implant bilateral guide cannulae above the medial VMT of six adult male Sprague-Dawley rats. These rats were subsequently food deprived and trained to perform the FCN-8 procedure. Doses of 5, 10, and 20 ng muscimol HBr, in a saline vehicle, or the vehicle alone, were injected

into the VMT prior to some test sessions. The muscimol resulted in a dose-related, statistically significant, increase in premature responding. However, the muscimol also resulted in a marked, dose-related decrease in overall responding. These results support the hypothesis that medial VMT modulates impulse control in the rat, although the decrease in overall responding indicates that the nature of the behavioral change is more complex than simply a change in impulse control.

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INTRODUCTION

Impulsive behaviors are composed of "actions which are poorly conceived, prematurely expressed, unduly risky or inappropriate to the situation and that often result in undesirable consequences" (Daruna & Barnes, 1993). Such behaviors are also defined as ones that are contrary to "responses to receive maximum reward" (Monterosso & Ainslie, 1999). Due to the complexity of impulsivity, many behavioral models currently exist that measure impulsive behavior in both humans and non-human subjects.

It is essential to consider both types of subjects to understand the neural mechanisms that play a role in such behavior. For example, self-report questionnaires and surveys are used to study behavior impulse control in humans. Other forms of measurements of behavioral impulse control in humans include the go/no-go task, the stop-signal reaction time (SSRT) task and the Circle Tracing task (CTT). The go/no-go task studies inhibitory processes. The subject begins knowing that when the "go" stimulus is presented they are to make a specific response (e.g., pressing a key in response to a stimulus on a screen). As the test continues, the "no-go" stimulus is presented either along with the "go" stimulus or before it. The subject must avoid responding prematurely to the "go" stimulus. The SSRT task also requires subjects to avoid responding prematurely to the "go stimulus". However the SSRT task differs from the go/no-go task as the "no go" stimulus is presented after the "go" stimulus. The findings of previous studies indicate that it is harder to avoid responding prematurely the sooner the "stop" signal is presented after the "go" signal. Therefore, impulsive subjects find it more difficult to avoid responding prematurely when presented with the "stop" stimulus (Band & Van Boxtel, 1999). The CTT task evaluates how slowly and precisely

the subject can trace the circle. The time it takes to complete this task the first and second time is generally smaller for impulsive subjects.

The behavioral tasks used to test impulsivity in laboratory animals, largely rats, are modified versions of tests used for human subjects. For example, the 5-choice serial reaction time task (5-CSRTT) is a test that examines visuo-spatial attention and impulsivity in an operant chamber (Robbins, 2002). It was adapted from the continuous performance test (CPT), which is widely used to evaluate performances based on attention in humans in clinical settings (Riccio, Waldrop, Reynolds, & Lowe, 2001). A food-deprived rat in the 5-CSRTT is presented with 5 holes in a curved wall. During a trial, a light comes on in one of the holes for 1.5 sec; a nose-poke into the hole within 5 sec of light onset results in delivery of a 45 mg food pellet into a lighted receptacle behind the rat. This is followed by a 5 sec delay during which the house light remains lit, after which another hole is illuminated for the next trial. This process continues for either 30 min or 100 trials.

There are three kinds of errors in this task. In the first (error of omission), the rat doesn't respond to illumination of a hole; this is followed by 5 sec of darkness in the chamber (time out), after which the chamber is illuminated and 5 sec later a hole is illuminated. In the second (error of commission), the rat pokes the wrong hole; this is followed by 5 sec of darkness and 5 sec of chamber illumination after which a hole is illuminated. In the third (premature response), the rat pokes a hole during the 5 sec chamber-illuminated period before a trial, when no hole is illuminated.

It has been reported that both lesions (Pasetti et al, 2002; Pezze et al., 2009) and transient (approx. 30 min) deactivation of neuron cell bodies in the medial prefrontal

cortex by local injection of the $GABA_A$ agonist muscimol results in increased premature responding, i.e., "impulsivity" in the 5-CSRTT (Paine et al., 2011). Unpublished studies in the Neill laboratory at Emory University have found that injection of muscimol into the medial VMT—the part projecting to medial prefrontal cortex (Arbuthnott et al., 1990)—also results in a large increase in premature responding.

The VMT is a longitudinally elongated nucleus of mainly medium to large rounded and multipolar closely packed cells (Herkenham, 1979). It is known that the VMT receives GABAergic afferents from the substantia nigra pars reticulata (SNr) (Bentivoglio, Van der Kooy, & Kuypers, 1979), and projects to layer 1 of frontal cortex (Herkenham, 1979). In the primate, the ventroanterior (VA) nucleus receives considerable input from the SNr; therefore the VA, specifically the ventral portion of it, can be considered a homologue of the rodent VMT.

It has been reported that excitotoxic lesions of the core of the accumbens, as with prefrontal damage, increase premature responding, although the test was not the 5-CSRTT (Cardinal et al., 2001); this effect has been specifically linked to the core subregion of the accumbens (Pothuizen et al., 2005). This makes sense because the projection from medial prefrontal cortex to accumbens utilizes the excitatory transmitter glutamate, so the accumbens function is probably similar to that of the prefrontal cortex; damage at either the cortical or accumbal level of this projection has similar effects. The accumbens sends GABAergic projections to the SNr; thus the prefrontal-accumbens-SNr-VMT-prefrontal circuit forms a "loop." One of the functions of this "cognitive" loop may be to regulate impulsivity. It is important to mention that there also exists a "motor" loop, which involves the motor cortex and deals with movement. For example, a loss of

dopaminergic signaling at the dorsal striatal level of the above loop is thought to be responsible for the appearance of the major motor components of Parkinson's Disease. Neurophysiological work on cortico-striato-thalamic loops, primarily from monkeys, has emphasized the parallel nature of these loops (e.g., Alexander et al., 1986; Alexander and Crutcher, 1990). This study is concerned with only the "cognitive" loop.

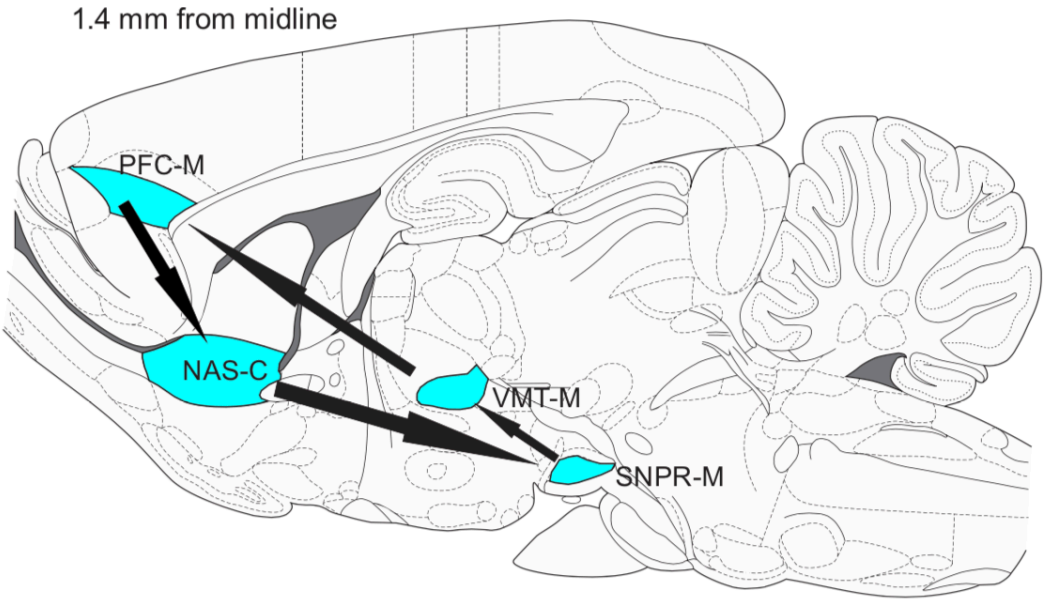

Figure 1: Schematic of the anatomy of a "cognitive" cortico-striatal-thalamic loop in rats

While the 5-CSRTT is a procedure widely used to assess "impulsivity" in rats by measuring premature responding, behavioral scientists are often reluctant to make a conclusion on the basis of one procedure. Therefore, the following experiment utilized a second procedure with which we have considerable experience. In the Fixed Consecutive Number 8 (FCN-8) procedure, the food-deprived rat is confronted with two response

levers on one wall of a test chamber. In the FCN-8, after the lever on the left (FCN lever) is pressed 8 times, a single press of the lever on the right (reward lever) delivers a food pellet. If the reward lever is pressed before 8 presses of the FCN lever (e.g., 6 presses), the press of the reward lever doesn't deliver a food pellet, and the rat has to begin the sequence anew. "Impulsive" responding in the FCN-8 consists of pressing the reward lever before the requisite number of responses on the response lever.

The goal of this study was to examine the effect of temporary deactivation of the medial VMT on FCN-8 performance in rats using transient muscimol (GABA agonist) injections via implanted medial VMT cannulae.

The hypothesis tested was that transient bilateral deactivation of VMT will increase the probability of the rat pressing the Reward Lever before pressing the FCN 1ever the requisite 8 times. i.e., "impulsive" responding.

MATERIALS AND METHODS

Subjects

Six adult male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) weighing between 320-350 grams prior to surgery served as subjects. All animals were housed individually. They were maintained on a 12 hour normal phase light-dark cycle (lights on 0700 hr) and received water *ad libitum.* All subjects were food deprived and chronically held at 90% of their free-feeding bodyweights. Behavioral procedures were conducted between 1200 and 2000 hrs. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Emory University and carried out in accordance with Emory's Division of Animal Resources (DAR) procedures. They were

also in compliance with National Institutes of Health guidelines for the care and use of laboratory animals.

Drugs

Bilateral infusions of vehicle (0.9% sterile saline) or the $GABA_A$ agonist muscimol HBr (Sigma-Aldrich Co., St. Louis, MO) dissolved in 0.9% sterile saline, in doses of 0, 5, 10, and 20 ng, based on the experience of the 5-CSRTT experiment (McGee, unpublished dissertation), were made with a pump-mounted microsyringe immediately prior to behavioral testing. All infusions were in a volume of 0.5 µl.

Apparatus

FCN procedures were conducted in clear, rectangular Plexiglas operant conditioning chambers (32 cm x 24.5 cm x 25 cm) placed into larger sound-attenuating wooden boxes (64 cm x 37 cm x 60 cm). Chamber floors were composed of 5 mm stainless steel rods positioned 1 cm apart. Two stainless steel levers (Med Associates Inc, St. Albans, VT) measuring 5 cm wide and spaced 13 cm apart center-to-center, protruded into the chamber 2.5 cm at a height of 6.5 cm above the chamber floor. The levers were located on either side of a centrally positioned small metal food bin through which food pellets could be delivered by an automatic feeder. Upon meeting the conditions of the FCN schedule (8 presses on the "FCN lever" on the left, followed by a single press of the "Reward lever" on the right, a single nutritionally balanced 45 mg dustless precision pellet (Bio-Serv, Frenchtown, NJ) was delivered to the food bin. A computer controlled all behavioral contingencies and monitored responses.

Surgical and Infusion Procedure

Stereotaxic surgeries were conducted under general anesthesia consisting of inhalation of 3-5% isoflurane gas through a nosecone. Anesthesia induction was preceded by orally administered meloxicam (1mg/kg) for analgesia All rats were implanted with a 22-gauge bilateral guide cannulae assembly with flush stylets and a center-to-center distance of 3 mm (Plastics One, Inc., Roanoke, VA); these devices had been sterilized by hydrogen peroxide gas. Guide cannulae tips were aimed to terminate 1 mm above the medial VMT (AP 10.4, L 1.5, H 2.6 mm above the interaural line) using the atlas of Paxinos and Watson (2007). The implants were secured to the skull with jeweler's screws and dental cement. The wounds were cleaned with an OTC antibiotic containing polymyxin B, bacitracin, and neomycin (CVS, Woonsocket, RI) and sutured in front and behind the cement skullcap with sterile polyethylene sutures. Flush 28 ga stylets were inserted in the cannulae. Food and water were available *ad libitum* during the seven-day recovery process.

A stainless steel infusion cannula (30 gauge; Plastics One, Inc., Roanoke, VA) was cut to protrude 1 mm beyond the tip of the guide cannulae into the medial VMT. The infusion cannula was securely attached to PE-10 standard wall cannula tubing (Clay-Adams, Parsipanny, NJ). 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 was administered to all subjects following a few sessions of training to induce the initial trauma from injector insertion. Once behavioral performance had stabilized, microinjection sessions were begun. Before these sessions, the flush stylets were

removed from the guide cannulae and the injector was lowered into each guide cannula. During the infusion procedure, the rats were allowed a small range of mobility in a non-bedded home cage replica. A 0.5 µl volume of drug was delivered into each medial VMT over an infusion period of approximately 60 seconds per hemisphere and the injector was left in place for 30 seconds after infusion to decrease backpressure and allow for diffusion into the brain tissue. Upon completion of drug injection, the flush stylets were reinserted into the guide cannulae to prevent drugs from entering the cannulae by back-flow, and the animal was immediately placed in a test chamber.

Behavioral Procedure: FCN-8 Training

The rats were progressively food-deprived to 90 percent of their free-feeding body weight. Since these were adult rats, the target weight was not adjusted to allow for growth. Operant sessions occurred once daily and were 20 minutes in duration. After initial training to press levers to obtain pellets, the rats had to press the left lever (FCN lever) once and then the right lever (Reward lever) once to obtain food (FCN-1 schedule). The rats performed this task until they earned 100 pellets within a session. Then, the FCN requirement task was increased to 2, and so on, until the rat performed with the required 8 FCN lever presses and 1 Reward lever press to receive the pellet. Premature responding, in which the rat pressed the Reward lever before completing the 8 presses on the FCN lever, did not yield a reward (food pellet) and the rat had to restart from the beginning. For each session, the number of lever presses (both FCN lever and Reward lever), food pellet rewards, and average chain length were recorded and computed by modification of a computer program obtained from Med-Associates, St. Albans, VT.

Performance Measures

The following parameters were recorded:

1. Responses on the FCN lever: This was defined as the total number of responses (bar presses) on the FCN lever (left lever) throughout the 20-minute session.
2. Responses on the Reward lever: This was defined as the total number of responses (bar presses) on the reward lever (right lever) throughout the 20-minute session.
3. Pellets Received: This was defined as the total number of pellets received throughout the 20-minute session.
4. Average Chain Length: This was defined as the average number of responses (bar presses) made on the FCN lever before switching to the reward lever. A decrease of this value is indicative of impulsivity (Evenden, 1998b).

Evaluation of Results and Histology

Dose-response curves for the effect of muscimol were analyzed for each behavioral measure by Analysis of Variance for Repeated Measures. Dose comparisons were subsequently made by the Newman-Keuls test. All statistics were performed by the Number Cruncher Statistical Systems (NCSS) software package.

After completion of all behavioral testing, the rats were euthanized by CO2 exposure in the Emory DAR facility in the Rollins Research Building. They were then intracardially perfused with isotonic saline followed by 10% formol-saline. The brains were removed, and after fixation, 50 micron-thick frozen sections were taken through the area of the guide cannulae. The sections were mounted on glass slides and subsequently stained with thionine and examined to confirm placement of cannulae. See Figure 2.

RESULTS

Histology

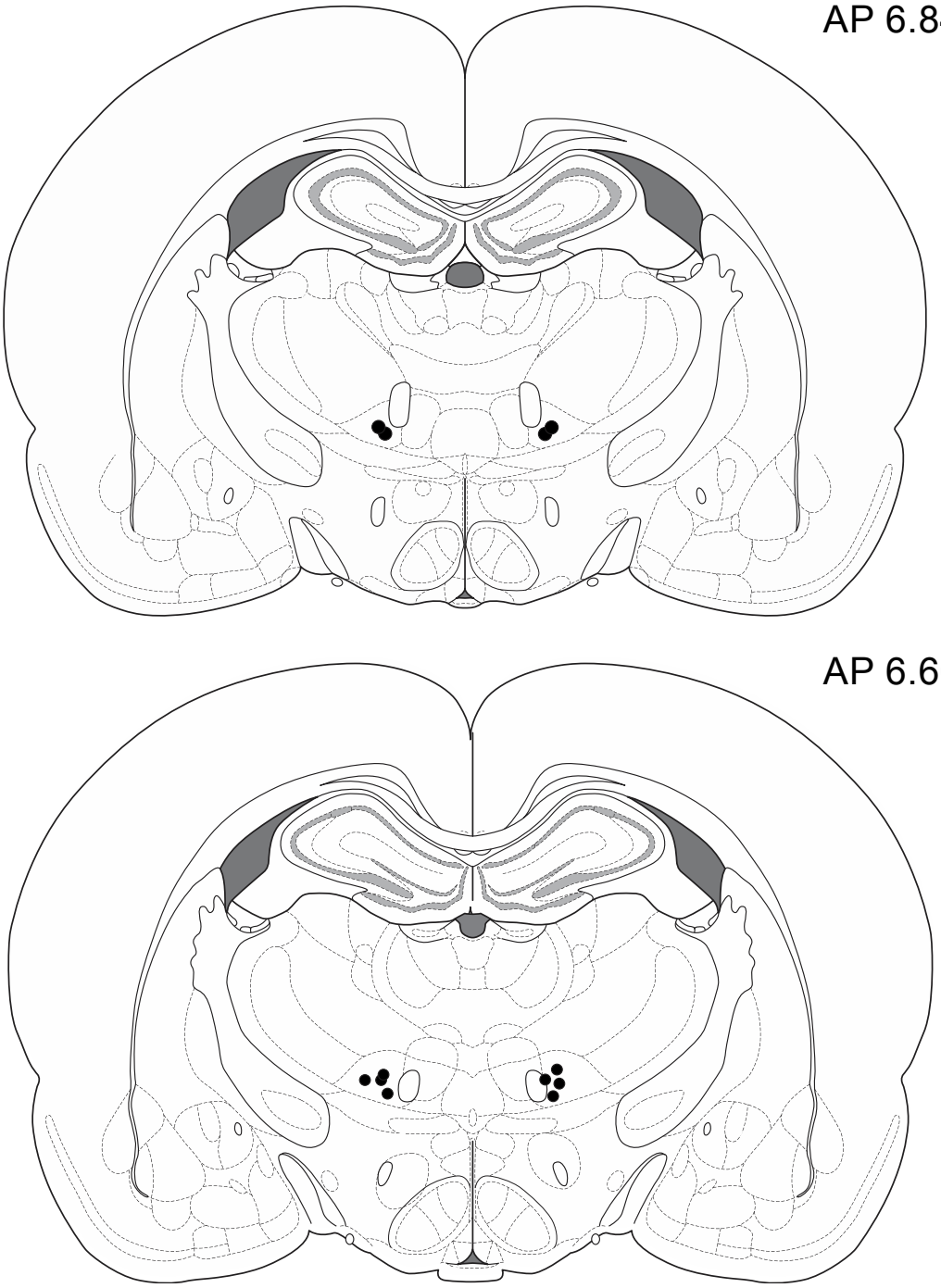

Figure 2: Histologic Injection Sites in the Rat VMT

FCN-8

Average Chain Length

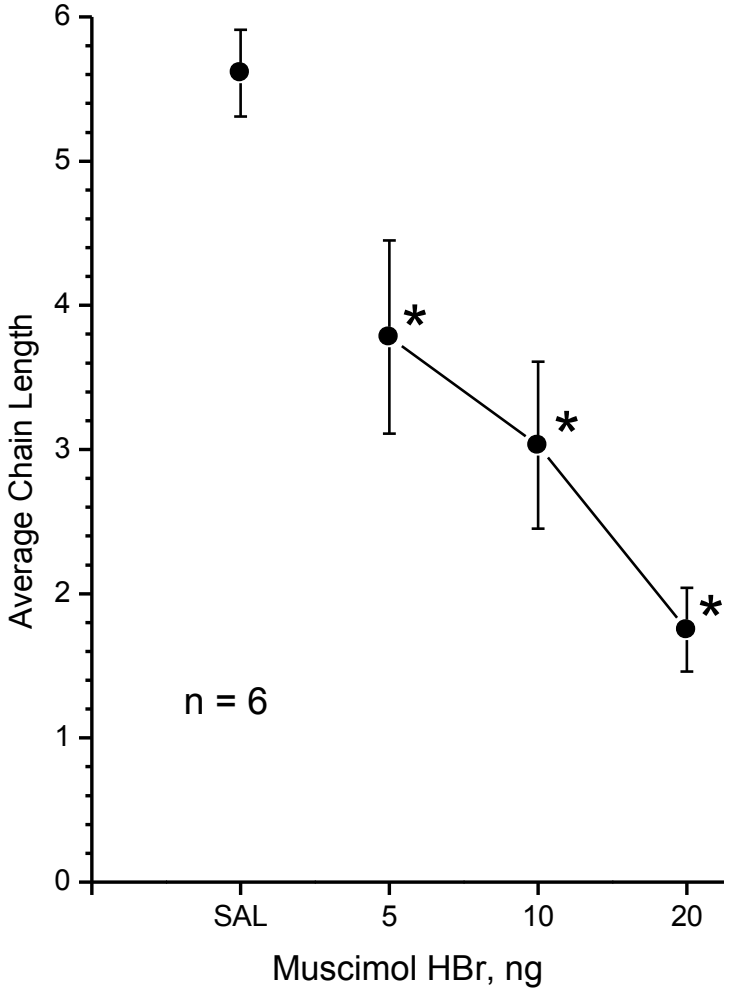

Figure 3: Average Chain Length

As illustrated in Fig. 3, repeated measures ANOVA on average chain length was significant, $F(3,15) = 12.34$, $p < 0.001$. The Newman-Keuls multiple comparison test showed that the Average Chain Length for all doses of muscimol was significantly lower than that following saline injection.

FCN lever Responses

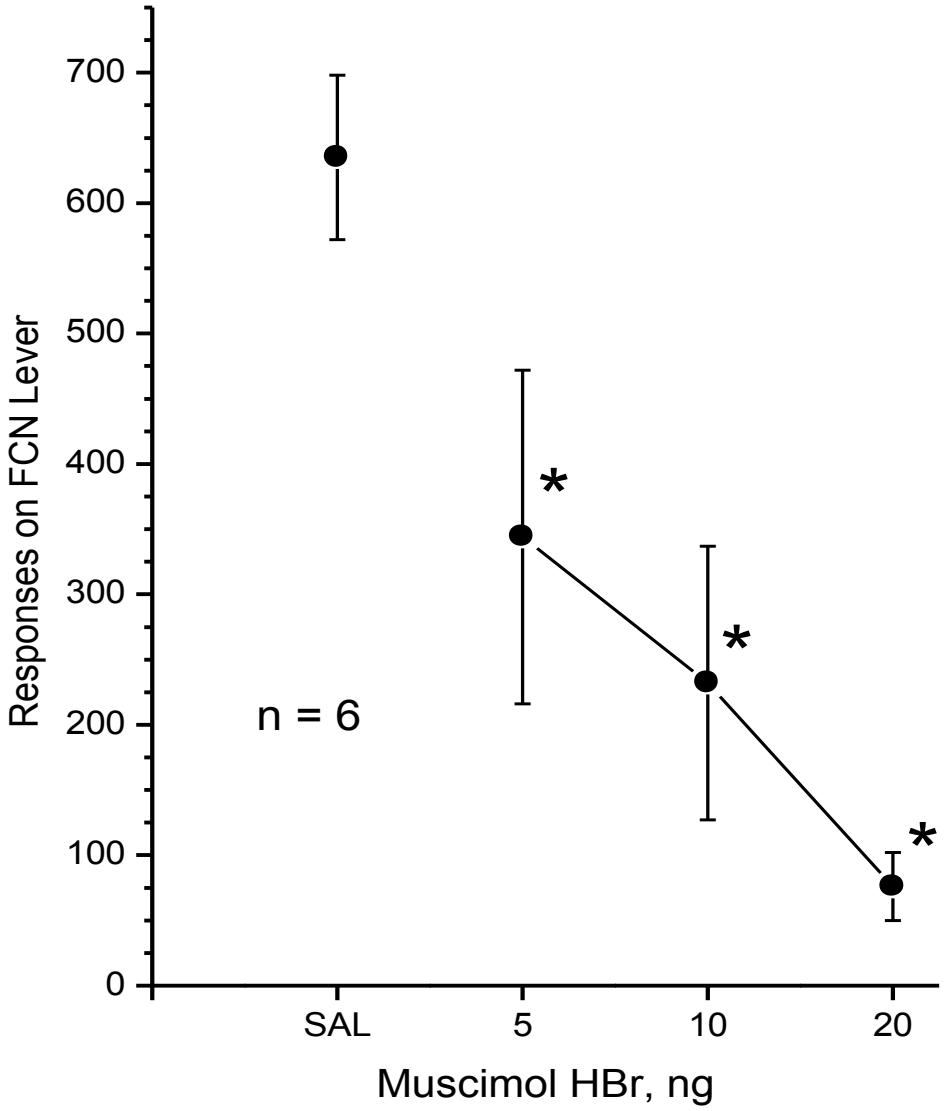

Figure 4: Responses on the FCN lever

As shown in Fig. 4, an analysis of variance for repeated measures on responses on the FCN lever was significant, $F(3,15) = 15.14$, $p < 0.001$. The Newman-Keuls multiple comparison test showed that responding on the FCN lever for all doses of muscimol was significantly lower than that following saline injection.

Reward lever Responses

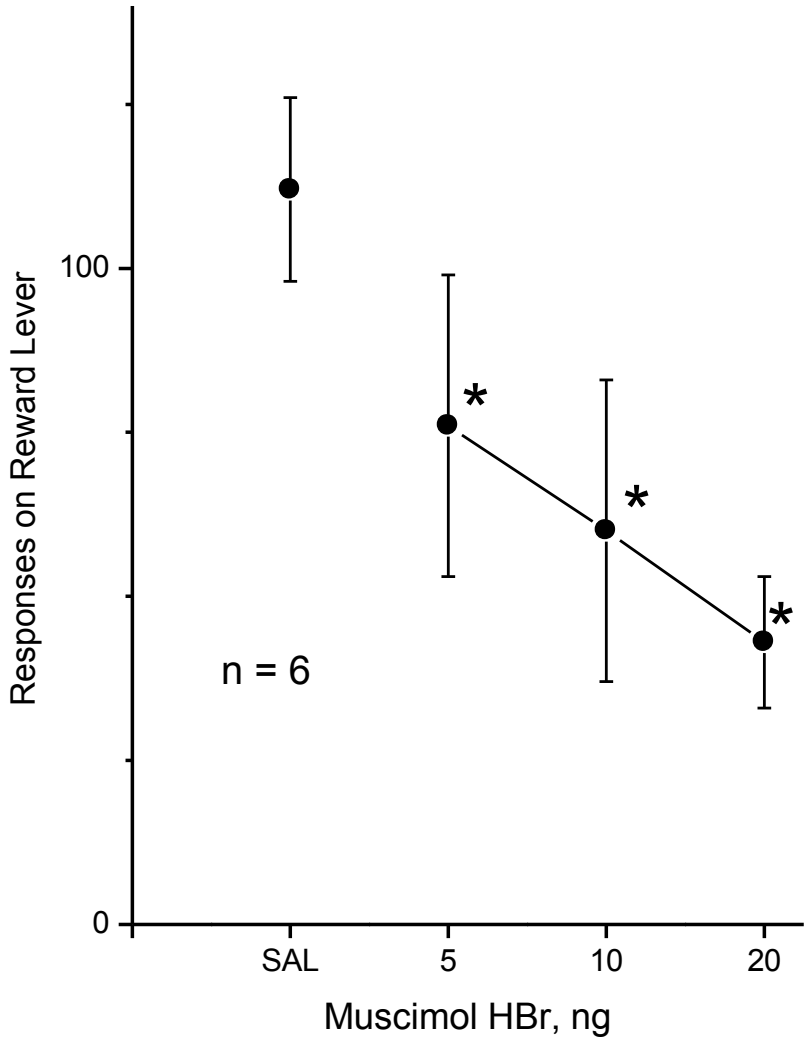

Figure 5: Responses on the Reward lever

As shown in Fig. 5, repeated measures ANOVA on the Reward lever was significant, $F(3,15) = 9.02$, $p < 0.01$. The Newman-Keuls multiple comparison test showed that responding on the Reward lever for all doses of muscimol was significantly lower than that following saline injection.

Pellets Received

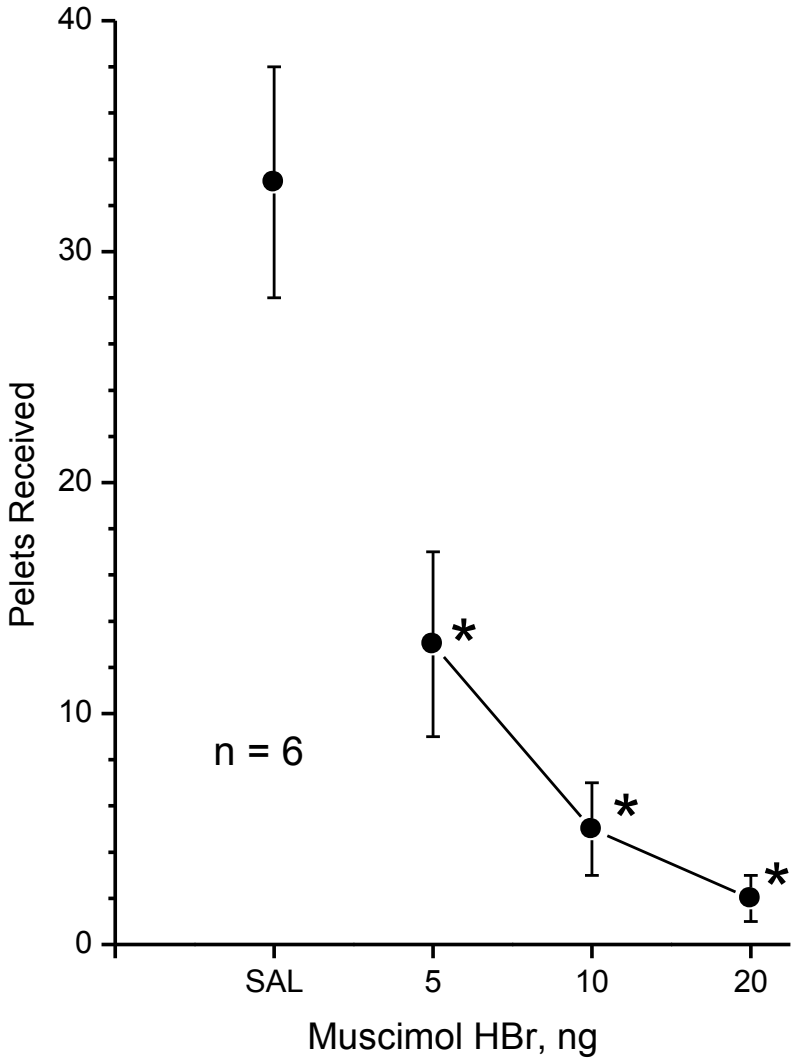

Figure 6: Pellets Received

As illustrated in Fig. 6, repeated measures ANOVA on pellets received was significant, $F(3, 15) = 16.93$, $p < 0.001$. The Newman-Keuls multiple comparison test showed that the Pellets received for all doses of muscimol were significantly lower than that following saline injection.

DISCUSSION

Summary of Results

In the FCN-8 procedure, the rat is trained to bar press 8 times on the FCN lever before bar pressing once on the Reward lever to get a reward (one pellet). Injections of muscimol into the medial VMT significantly decreased the average chain length on a FCN-8 schedule of reward. The decrease in average chain length means that the rat made fewer bar presses on the FCN lever before switching to bar press on the Reward lever. This type of behavior indicates that the drug induced impulsive behavior. As seen in Figure 3, the decrease in average chain length was found to increase as the dosage of muscimol increased from 5 ng to 10 ng and finally to 20 ng. The rats bar pressed the fewest amount of times (1.75 bar presses) on the FCN lever before pressing the Reward lever when infused with 20 ng of muscimol.

As seen in Figure 4, responding on the FCN lever decreased as the dosage of muscimol increased from 5 ng to 10 ng and finally to 20 ng. The overall bar presses on the FCN lever were the fewest (76 total bar presses) when the rats were infused with 20 ng of muscimol. In comparison, the rats bar pressed a total of 635 times when they were infused with saline.

As seen in Figure 5, responding on the Reward lever also decreased as the dosage of muscimol increased. The overall bar presses on the FCN lever was the fewest (43 total bar presses) when the rats were infused with 20 ng of muscimol. In comparison, the rats bar pressed a total of 112 times when they were infused with saline.

Finally, as seen in Figure 6, the number of pellets the rats received decreased as the dosage of muscimol increased. The rats received the fewest pellets (2) when they

were infused with 20 ng of muscimol. In comparison, the rats received a total of 33 pellets when they were infused with saline.

Therefore, the infusion of muscimol into the medial VMT produced statistically significant decreases in Average Chain Length, FCN lever responses, Reward lever responses, and total reward (pellets) received by the rats on the FCN-8 procedure. These observations are indicative of impulsive behavior due to the administration of muscimol in the medial VMT.

Interpretation of Results

In our society, the concept of impulsivity has usually been viewed as a personality trait. These traits are generally regarded as "risky or inappropriate to the situation and that often result in undesirable consequences" (Daruna & Barnes, 1993). From a neurobehavioral perspective, impulsivity is viewed in generally two distinct manners. The first view considers impulsivity as a singular behavior (Evenden, 1999), which would imply that a there exists a single neuronal pathway that dictates impulsivity. The opposing view considers impulsivity as an assortment of independent and unique behaviors that are dictated by the interaction of specific neuronal pathways (Buss and Plomin, 1975). Buss and Plomin described impulsivity as consisting of "more than one dimension of control" and believed that while "inhibitory control lies at the core of impulsivity, […] decision time, persistence and boredom or sensation seeking" were also important aspects of impulsivity.

Due to the complexity of impulsivity, many behavioral models currently exist that measure certain aspects of impulsive behavior. The Fixed Consecutive Number (FCN) procedure is an appropriate test of impulsivity, as it requires the subject to maintain

motivation and impulse control to continue responding accurately and receive maximal reward (food pellets). The FCN procedure is unique, as it is not based on the rate of responding and does not require the subject to maintain attention for a specific stimulus in the apparatus. These characteristics of the FCN procedure help minimize the effect outliers, like each subject's internal timing, level of impulsivity, and attention span, have on the final results. For example if two subjects had a large deviation between their respective attention spans and were performing the 5-choice serial reaction time task (5-CSRTT), then a lack of or delayed response to the stimulus (light) may be due to a lower level of attention span rather a sign of no impulsivity.

Previous studies in the Neill laboratory at Emory University have looked at the effect of temporary deactivation of the VMT via muscimol HBr on the 5-choice serial reaction time task (5-CSRTT). These studies found that injection of muscimol into the medial VMT, the part projecting to medial prefrontal cortex, resulted in a large increase in premature responding (Jennifer McGee, unpublished dissertation; Nicholai Henry, Honors thesis). Another study conducted at the Neill laboratory (Emily Watts, Honors Thesis) found that temporary deactivation of the medial Nucleus Accumbens (NAcc) via muscimol HBr on a FCN-8 procedure led to premature responding.

The current study supports the findings of the previous studies indicated above. The subjects in the current study were trained to respond 8 times on the FCN lever before pressing the Reward lever to successfully receive a reward (food pellet). Transient deactivation of the medial VMT via muscimol HBr led to the subjects behaving more impulsively and performing less efficiently on the FCN-8 procedure. The subjects pressed the Reward lever before pressing the FCN lever the required 8 times. Since

similar behavior was observed in the previous study using the 5-CSRTT, it can be concluded that such impulsive behavior isn't specific to only one procedure. Therefore, temporary deactivation of medial VMT via muscimol specifically leads to impulsive responding regardless of the procedure or task.

This impulsive responding is perhaps due to a decreased ability to perform an intermediary behavior in order to receive a reward. The transient deactivation of the medial VMT probably also causes the subject to have a change in motivation and impairs the subject's ability to remain focused on a particular task (bar pressing FCN lever 8 times) to receive a reward. Thus there are most likely a variety of reasons as to why the rat behaves in an impulsive manner.

The above conjectures for impulsive behavior revolve around the idea that the subject doesn't want to be delayed from receiving the reward and thus bar presses the Reward lever prematurely. This would imply that the subject is impatient and wants immediate reward. The decrease in persistently working (bar pressing) to receive reward in the future is evidence for altered motivation. Such altered motivation is most likely due a change from the subject wanting to perform a task for a reward to the subject being averse to waiting for the reward. This implies that impulsivity may not be as extremely negative as currently considered in our society. As impulsivity assures *some* type of response, it could be evolutionarily advantageous in certain circumstances where immediate decision or action is crucial. However the likelihood of such a response being consistently advantageous is debatable, especially in a civilized society where individuals are expected to behave in a logical manner.

Another interesting finding in this study involved an overall decrease in bar pressing of both FCN and Reward lever. Observation of the subjects during drug-injection sessions showed the rats did not seem to show any signs of being sick, having a motoric impairment, or being under duress or stress. Rather, the subjects seemed, in colloquial terms, "lazy." It seemed that they lost the motivation to bar press the FCN lever 8 times. At the start of drug-injection session, the rats were active and bar pressed readily. However after some time, they seemed to lose interest in bar pressing and began wandering around the box, which was observed for the remainder of the duration of the procedure. They occasionally bar pressed and ate their subsequent reward (food pellets), but this wasn't very common. The observed loss in subjects' motivation is perhaps due to a loss of appetitive behavior. Appetitive behavior involves the subject working in the present to receive a reward in the future (Dent and Neill, 2012). An influential theory by Berridge (1996) describes this behavioral state as "wanting." This is a good description of the type of behavior needed to bar press 8 times on the FCN lever, followed by 1 bar press on the Reward lever, to get the reward (food pellet). On the other hand, consummatory behavior involves the subject working to get an instantaneous reward (Dent and Neill, 2012). This is a good description of the type of behavior when the rat presses the Reward lever prematurely (it wants instant reward). The subjects stuck their noses into the hole, where the pellets drop, on numerous occasions, but showed no sustained signs of working (pressing the FCN lever 8 times) to make the pellet drop. Therefore the rats still demonstrated consummatory behavior, but not appetitive behavior. In other words, the rats were willing to eat the food pellets if they were presented right in front of them but not willing to work to get those food pellets. Based on previous studies,

Nucleus Accumbens (NA) activation influences appetitive behavior and allows subjects to be motivated to perform a certain task for future reward. If the NA is inhibited, then appetitive behavior is seen to drastically decrease. The NA does *not* influence consummatory behavior. Thus even if the NA was inhibited, the subjects would eat the food pellets presented in front of them. However they wouldn't work to get those food pellets. It appeared that a loss of appetitive behavior was observed in this study.

This particular finding can be further explained by looking at the previously (Fig. 1) illustrated 'cognitive' loop in the brain. The VMT projects to the medial Pre-frontal Cortex (PFC), which is the area that controls impulsive behavior (Pothuizen, 2005). Previous studies and neurophysiological work have shown the presence of 'loops,' which are essentially a circuit consisting of an axonal projection from a cortical area to a striatal area, then to thalamus, and back to cortex. Based on previous unpublished work in the Neill Laboratory at Emory University, one such loop involves connections between the medial PFC, the Nucleus Accumbens (NAcc), the medial Substanitia Nigra Pars Reticulata (SNPR), and the medial VMT.

Due to the presence of the prefrontal area, manipulations within this loop would lead to changes in aspects of behavior associated with the medial prefrontal cortex, like response inhibition. In this study, the decrease in overall bar pressing due to inhibition of medial VMT most likely involved the structures that are part of this loop. Based on Figure 7, any changes in the activity of VMT would affect the activity of PFC, which would thereby affect NA. Therefore the inhibition of medial VMT probably affects the activity of NA, which leads to the loss of appetitive behavior (Neill, 2002). It is important to note that this study looked only at the medial VMT, not the lateral VMT. The medial

VMT has been shown to project to the medial frontal area of the rat brain, which is considered to be the pre-frontal area, while the lateral VMT has been shown to project to the lateral frontal area, which is more involved with motor and sensory responses (Arbuthnott et al., 1990). Therefore, the impulsive behavior observed in this study is probably not due to any motor problems because the lateral VMT was not involved. The exact mechanism, which provides an understanding as to why this loss of appetitive behavior occurred, is shown below.

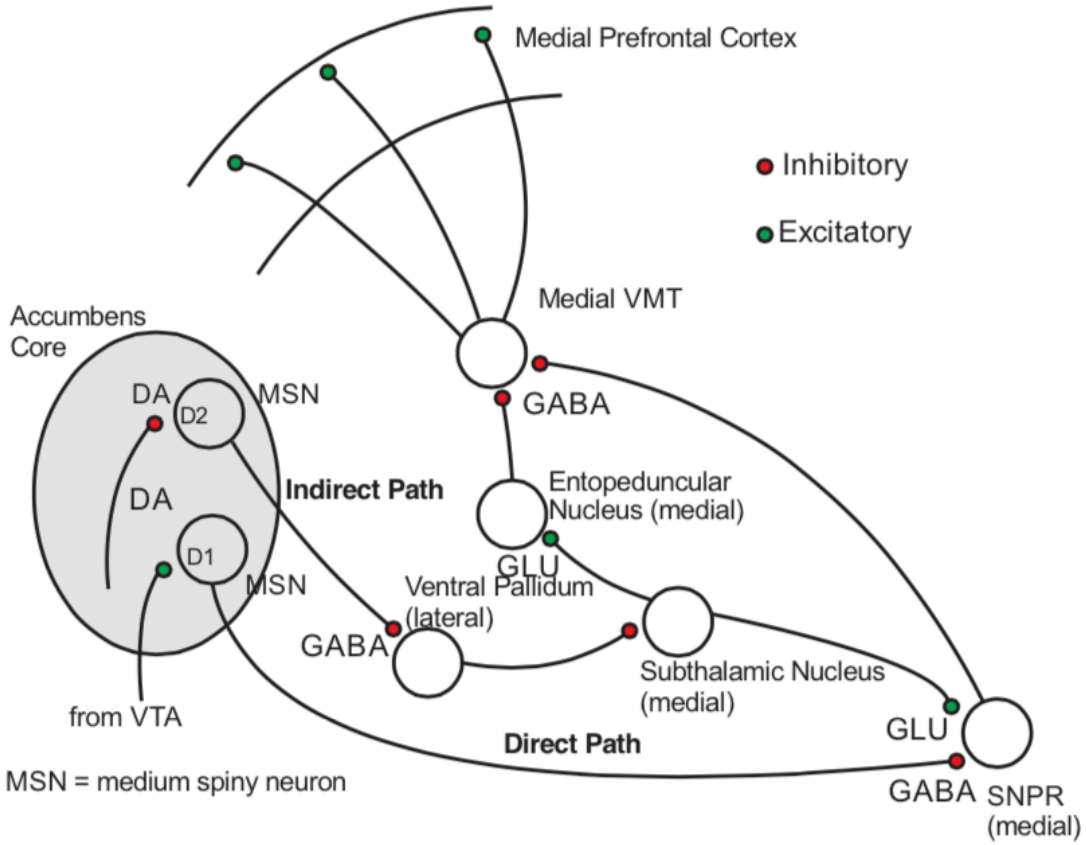

Figure 7: Detail of axonal connections and neurotransmitters in a "cognitive" loop, lateral view.

A considerable literature has focused on the existence of two distinct output pathways of the dorsal striatum, termed the "direct" and "indirect" pathways (Albin et al., 1989; DeLong, 1990). In this study, we are only concerned with the 'direct' pathway. The

"direct path" loop goes from nucleus accumbens (NAcc) to medial SNPR, from medial SNR to medial VMT, and finally from medial VMT to medial PFC (Gerfen and Surmier, 2011). As seen in Figure 7, medial VMT is GABAergic, but releases excitatory impulses that *increase* activity of the medial PFC. Therefore a decrease in activity of medial VMT would cause the medial PFC to not receive such excitatory impulses (Rubio-Garrido, 2009). A decrease in excitatory impulse would lead to the subject not receiving the same level of 'reward' from an activity as it did prior to any manipulations. A decrease in 'reward' would cause the subject to decrease its involvement in the said activity. In this study, muscimol HBr (GABA agonist) was injected into the medial VMT and the subsequent transient deactivation of medial VMT lead to overall decrease in responding by subjects. The subjects didn't get as much excitatory impulses to their medial PFC after the VMT deactivation as they did before the VMT deactivation, which lead to a decrease in responding. Thus this decrease in responding can be explained by the above mechanism and supports previous findings that VMT is in fact connected to the medial PFC.

Moreover the PFC is connected to NA, as shown in Figure 1, and thus the level of activity of PFC would influence the level of activity of NA. As there is decreased activity in the medial PFC, due to transient deactivation of the medial VMT via muscimol, there will also be decreased activity of the NA (Rubio-Garrido, 2009). As stated previously, NA activation affects appetitive behavior. A decrease of NA activation would lead to a loss of appetitive behavior, which is what occurs in this study. Therefore, the transient deactivation of the medial VMT leads to deactivation of NA, which leads to the observed

loss of appetitive behavior in the subjects. This reasoning explains why the rats lost interest in bar pressing 8 times (appetitive behavior) to receive the reward (food pellet).

In addition, the increase in premature responding can also be explained based on this mechanism. A decrease in excitatory impulses to the medial PFC would cause the subject to lose motivation to continually bar press for a reward. This reasoning is bolstered by the fact that NA is also most likely being inhibited due to a lack of excitatory impulses from PFC. A decrease in NA excitement results in a decrease in release of dopamine (DA) by NA, which would reduce the overall feeling of 'reward' in the subjects (Neill, 2002). In other words, the subjects would lose motivation to continually bar press (appetitive behavior) to receive the food pellet as they experience decreased dopamine levels. This alteration of motivation is probably what causes the subjects to become more impulsive and want the reward (press the Reward lever) before completely finishing the task (pressing the FCN lever 8 times) they were trained to perform. This conjecture assumes that the rats want to reach the same level of 'reward' they had *before* the medial VMT was inhibited, as the inhibition of medial VMT leads to the inhibition of NA, which causes a decrease in DA release by NA. As the pellets cause the rats to get excited and experience a feeling of 'reward', they perhaps respond prematurely to experience such a feeling more readily when the VMT is inhibited. Therefore, premature responding (pressing the Reward lever early) takes place most likely due to NA inhibition, which is caused by VMT inhibition in this study.

Furthermore, simply pressing the Reward lever once isn't indicative of appetitive behavior as a single bar press doesn't particularly require a lot of work. It is a simple task that is performed prior to receiving the pellet, given that the FCN lever is pressed 8 times

first. Thus perhaps the rats press the Reward lever in hopes to receive reward (pellet) early rather than wait to perform a task and then receive the reward, which is an example of consumatory behavior. The rats respond prematurely as they don't want to work for the reward due to a loss of appetitive behavior caused by NA inhibition. The idea mentioned earlier about the subject being aversive to waiting for the reward is another effect of the above conjecture.

This study provides strong evidence that transient deactivation of the medial VMT leads subjects to respond prematurely, which indicates impulsive behavior. This behavior is perhaps due to an alternation in the motivation of subjects, which is based on the effect medial VMT has on medial PFC in the 'loops' mechanism. Based on this mechanism, one reason for overall decreased and premature responding may be due to loss of appetitive behavior because of NA inhibition, which occurs due to VMT inhibition. However the specific nature of behavioral change is much more complex, and probably involves multiple neuronal mechanisms, than a simple change in impulse control.

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