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Modulation of Brain Stimulation Reward by GABA in the Ventromedial Nucleus of the Thalamus

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Abstract

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The reward system has generated a lot of interest in the field of neurobiology, specifically in regards to the mesolimbic dopamine system. While several studies hold the dopaminergic effect at the nucleus accumbens accountable for inducing the rewarding effect, the present study attempts to see where this “reward signal” goes after the accumbens. The objective of the study was to use the autotitration paradigm and to manipulate GABAergic effects in the ventromedial nucleus of the thalamus (VMT) in order to determine the role of the VMT in intracranial self-stimulation (ICSS) reward. In the following study, the GABAergic input to the VMT, which comes particularly from the substantia nigra pars reticulata (SNPR), was manipulated in a sample of eight rats through the intra-VMT injection of GABA antagonist picrotoxin and GABA agonist muscimol. The behavioral effects of the drugs were shown through their activity in autotitration ICSS. After injection of picrotoxin in the VMT, the rats showed vigorous motoric ability, but decreased ICSS responding and showed “earlier” resetting of the mean intensity, a behavioral pattern described as “decreased reward” in the theoretical autotitration ICSS scheme of Neill et al. (1982). Following muscimol injection, the locomotor activity of the rats decreased, and decreased ICSS responding was accompanied by “later” resetting, a behavioral pattern described as “decreased effort” by Neill et al. (1982). These results suggest an important involvement of GABA in the brain reward system.

Keywords: autotitration, intracranial self-stimulation, reward, nigrothalamic pathway, ventromedial nucleus of the thalamus

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ICSS and Anatomy

The field of neurobiology is constantly developing and changing as researchers delve deeper into the inner workings of the brain. One area in particular that has aroused significant interest is the reward system. Why do drugs of abuse such as cocaine, amphetamine, or nicotine cause an individual to experience feelings of euphoria and pleasure? Where exactly does the neurochemical basis of this "reward" originate? To answer these questions, researchers have studied rats and manipulated their brains for years in order to study the mechanism of reward. In particular, an operant paradigm known as intracranial self-stimulation (ICSS), introduced by James Olds and Peter Milner (Olds & Milner, 1954), has been used to analyze reward. ICSS allows researchers to arouse certain areas of the rat's brain with electrical stimulation, which the rodent perceives as pleasurable, as rats will engage in operant behaviors (e.g., pressing a lever or poking their nose through a hole) in order to receive electrical stimulation delivered by means of an electrode implanted in the brain. The introduction of ICSS offered a powerful approach to targeting and regulating certain brain regions related to the reward pathway(s).

As the search to investigate brain regions related to reward began, several studies emerged that identified certain sites in the brain particularly responsive to electrical stimulation. Of the many brain regions studied in ICSS, the ones yielding the most significant responding were the lateral hypothalamus (LH), ventral tegmental area (VTA), and medial forebrain bundle (MFB), which connects the LH to the VTA (Phillips & Fibiger, 1989). ICSS in these areas

produced intense responding. Much of the research on "reward systems" in the 1960s and 1970s was thus devoted to understanding the anatomy of ICSS.

As previously mentioned, studies on the hypothalamus found that axons within or near the MFB seemed crucially important for ICSS (Carlezon & Chartoff, 2007). After the anatomical studies, it became crucial to understand the neurochemistry of ICSS. These neurochemical studies showed the importance of the release of the neurotransmitter dopamine. Dopamine is a naturally produced molecule in the body that functions through neurotransmission and is responsible for functions related to motivation, reward, learning, interest, addiction, and drive (Wenzel & Cheer, 2017) The release of the dopamine neurotransmitter upon the nucleus accumbens, therefore, became the basis of the popular "mesolimbic dopamine pathway" theory of reward.

Dopamine and Reward

The mesolimbic dopamine pathway originates in the VTA of the midbrain and terminates in the nucleus accumbens septi (NAS) of the forebrain, where it releases dopamine. Phillips and Fibiger (1989) suggested that the mesolimbic system employed dopamine-releasing neurons that originated in the VTA or substantia nigra and projected to neuronal sites such as the nucleus accumbens, olfactory tubercle, amygdala, and dorsal striatum. Extensive research had been conducted regarding the role of dopamine in the neuronal basis of reward utilizing techniques such as drug injections, lesions, and chronoamperometry.

In order to study the reward-related pathway excited by dopamine, Ranaldi and Beninger (1994) conducted an ICSS study in which they injected dopamine agonists, quinpirole and A-77636, along with amphetamine into the caudal nucleus accumbens of rats self-stimulating at an

electrode in the VTA. They found that when injected in the accumbens, all three drugs reduced the threshold frequency needed to maintain ICSS, indicating greater brain stimulation reward (BSR). Their findings suggested that increased dopamine receptor activity enhanced the "rewarding effect" of ICSS, leading the rats to experience pleasure at lower stimulation frequencies than baseline (Ranaldi and Beninger, 1994). Similarly, Mogensen et al. (1979) studied the effect of the dopamine antagonist spiroperidol when injected into the nucleus accumbens while the electrode was implanted in the VTA. They found a reduction of ICSS in the ipsilateral VTA, but not the contralateral VTA, supporting the prior study's findings about the role of dopaminergic neurons in ICSS. These results were also mirrored in other studies as ICSS response decreased from the injection of dopamine antagonists SCH 23390 into the accumbens as the rat received stimulation in the VMT (Kurumiya & Nakajima, 1988).

The role of dopamine in ICSS behaviors and reward were also supported by other techniques. Fibiger et al. (1987) experimented with 6-hydroxydopamine lesions of the dopaminergic projections that originated in the VTA and terminated at the accumbens. As expected, preventing the release of dopamine resulted in a significant decrease in rat ICSS behaviors, indicating an inhibition of reward. Chronoamperometry, an electrochemical technique used by Phillips et al. (1989), supported prior findings regarding dopamine and reward. During ICSS, chronoamperometry can be used to quantify extracellular dopamine levels. Phillips et al. (1989) found that during self-stimulation and experimenter-administered stimulation in the VTA, there was a rise in dopamine levels in the nucleus accumbens.

There have been many papers published supporting the role of dopamine transmission in the nucleus accumbens as a vital component for brain reward in general, not just ICSS. However, there are few studies examining the question: "What circuitry is involved beyond the neurons

which respond to dopamine?" Few papers exist devoted to determining where the "reward signal" goes after the nucleus accumbens. Most of the forebrain neurons, which respond to dopamine, including those in the nucleus accumbens, send axons back down to the ventral midbrain (see Figure 1), where they synapse on cells in the VTA or SNPR. The SNPR, in turn, sends axons to synapse on cells in the VMT, the superior colliculus, and further down in the brainstem (Deniau, Menetrey & Thierry, 1994). The transmitter for all those projections is gamma-amino-butyric acid (GABA).

Ventromedial Nucleus of the Thalamus

The VMT was the region of interest for this particular study. In order to manipulate the SNPR-VMT projection, drugs that alter GABA transmission were injected into the VMT of rats performing ICSS of the VTA. GABA generally has an inhibitory effect on the neuronal firing of a postsynaptic neuron. Picrotoxin, functioning as a GABA antagonist, is commonly known to decrease the drug's effect by blocking the GABA receptor site. Muscimol, a GABA agonist, has the opposite outcome as it mimics GABA's effect at the synapses.

Most published studies concerning the VMT are focused on motor function. For instance, in a study that assessed the role of the VMT in relation to motor activity and stereotypic behaviors, the authors (Starr & Summerhayes, 1983) injected various GABAergic drugs unilaterally and bilaterally into the VMT, and measured locomotor activity by observing circling behavior as locomotor activity and stereotypic behaviors through observing repeated behaviors such as grooming, gnawing, teeth chattering, licking, etc. The results were quite significant in indicating that the VMT had minimal or almost no effect in regards to stereotypic behaviors no matter whether GABA agonists (muscimol), GABA antagonists (bicuculline and picrotoxin),

GABA-inhibiting drugs (4-amino-hex-5-enoic acid and *cis*-1,3-aminocyclohexane carboxylic acid), or GABA-potentiating drugs (flurazepam and procaine) were injected. However, locomotor effects were significant, indicating that GABA agonists altered motoric ability by producing activity ranging from slowing to cataleptic effects and GABA antagonists caused hyperactivity when the drug was injected bilaterally in the VMT (Starr and Summerhayes, 1983). Di Chara and colleagues (1979) supported the previous findings as they injected muscimol in the VMT and saw no effect on stereotyped behaviors, suggesting that naturally produced stereotypic behaviors had no role in the GABAergic systems downstream from the from the rat's striatum. Two additional studies, Young et al. (1995) and Klockgether et al. (1986) supported the previous information, showing the same cataleptic effect with muscimol injected into the VMT while the former also indicated an increase in locomotor activity when the GABA antagonist bicuculline was injected into the VMT.

Another study that utilized lesions in the VMT lends support to the prior claims. For example, in yet another study conducted by Starr and Summerhayes (1983), unilateral VMT electrolesions resulted in weak locomotor behaviors while bilateral lesions suppressed these behaviors. Chemically induced lesions, through kainic acid, in the VMT either unilaterally or bilaterally resulted in intense hypoactivity in the initial phase, but with time, the effect wore off and the rat's motor function returned. Stereotypic behavior was unaffected by lesions in the VMT (Starr and Summerhayes, 1983). To further delve into the relationship of the nigrothalamic GABAergic system, Timmerman and Westerink (1996) utilized a microdialysis analysis in which they monitored extracellular GABA levels in the rat's VMT before, during, and after they had received electrical stimulation in the SNPR. As expected, GABA levels significantly increased after receiving SNPR stimulation; however, in this 10-minute session, the effects

decreased and returned to baseline three minutes after initial stimulation (although they were still receiving electrical stimulation throughout the session). This finding suggests a "compensatory response" of the neuronal GABA release after SNPR stimulation (Timmerman and Westerink, 1996). In addition, local infusion of the GABA reuptake inhibitors nipecotic acid and SKF 89976-A into the VMT demonstrated a similar increase in extracellular GABA levels although this effect was absent when combined with electrical stimulation. Surprisingly, local infusion of the GABA antagonist bicuculline in the VMT did not affect levels of extracellular GABA; however, when combined with electrical stimulation, these regions also showed an increase in GABA levels in the VMT (Timmerman and Westerink, 1996). This suggested that GABA receptors might have had a role in the release of GABA from the nigrothalamic neurons.

This project examined the role of the VMT in brain reward by using ICSS as the behavioral measure of reward. If the VMT was truly a part of a "reward system," then manipulations of the region should affect ICSS. Only one paper in existence (Williams and Herberg, 1987) appeared to have reported the effect of these VMT injections on lateral hypothalamus ICSS. These authors found that injections of the GABA agonist muscimol in the VMT decreased ICSS responding, whereas injections of the GABA antagonist picrotoxin in the VMT increased responding. While these effects were clearly strong, one major concern was the possibility of multiple behavioral interpretations. For instance, the study could be criticized on behavioral grounds, based on their use of the simplest measure of ICSS reward: rate of single lever bar-pressing at a constant electrical intensity. Specifically, the decrease in responding with muscimol could be due to a "nonspecific" behavioral effect (e.g., decreased arousal or a motoric problem). Similarly, the increase in responding with picrotoxin could be due to a nonspecific increase in general behavioral activity. Since the VMT is part of the brain circuitry involved in

movement, the changes in ICSS responding reported by Williams and Herberg (1987) could simply reflect effects on the rat's ability to move.

The Autotitration Procedure

The challenge of the behavioral interpretation of VMT injection effects on ICSS was similar to that found in prior studies indicating that dopamine in the ventral anterior striatum (VAS) was involved in ICSS (Neill, Peay & Gold, 1978). In these studies, injection of dopamine agonists into the VAS increased ICSS responding, and although this effect might have reflected a reward change, further study (Neill, Gaar, Clark & Britt, 1982) using the "autotitration" ICSS method of Schaefer and Holtzman (1979) found that the effect reflected a change in "effort" instead of "reward." Therefore, the logic supporting ICSS studies became challenging when a different paradigm, namely autotitration, was utilized.

Autotitration is a relatively "rate-free" method of assessing ICSS reward. In this paradigm, rats are placed in an operant box in which there are two levers: the stimulation lever and the reset lever. Responses on the stimulation lever deliver brain stimulation, but the intensity of the stimulation progressively decreases. To bring the electrical stimulation intensity back to maximum, the rat must walk around a partition, press the reset lever, and walk back around the partition to the stimulation lever. In this study, every five lever presses produced a three-microampere decrease in intensity. Accordingly, if the rat started at an intensity of 100 microamperes, then the intensity would decrease to 70 microamperes after 50 lever presses on the stimulation lever. In order to bring the intensity back to 100 microamperes, the rat would have to press the reset lever. When the autotitration paradigm was first introduced by Stein and Ray (1959), they determined that each rat had a specific threshold frequency that they would

always reset at. For example, if a rat started at 110-microampere intensity, and reset at 80-microampere intensity, then altering the starting intensity would have no effect on the threshold frequency of 80 microamperes. Essentially, whether the rat's starting intensity was 120 amperes or 100 amperes, it would always reset at 80 microamperes. Easterling and Holtzman (1997) later disproved this theory and proposed that the rat's motivation to press the reset lever was based on a given amount of change in intensity. For instance, a rat may reset every time their starting intensity dropped by 30 microamperes.

Therefore, the autotitration paradigm is an acceptable way to distinguish whether the effects of ICSS are due to reward or some other motivational behavior. It is used to assess not only the rat's response, but also the average intensity at which the rat runs over to push the reset lever, the "reward threshold." The objective of the following experiment was to examine the effect of GABAergic manipulations of the VMT on ICSS, using the autotitration method.

Interpretation of Results

Following the theoretical scheme described by Neill et al. (1982) if a drug injection results in earlier resetting (a higher than normal reset intensity) intensity, along with an overall decrease in responding, then this effect is interpreted as reflecting reduced reward. From the paper of Williams and Herberg (1986), we already know that injection of muscimol into the VMT decreased ICSS responding. In autotitration, if the decreased responding is accompanied by "later" resetting, we can attribute the behavioral change to a decrease in motivation or motor or arousal alteration. In this case, the results would not support the hypothesis that the VMT is part of the reward circuitry. On the other hand, if the injection decreases responding but resetting

is “earlier” than normal, the behavioral change will be interpreted as decreased reward, and support the hypothesis that the VMT is involved in reward circuitry.

Purpose and Hypothesis:

As described above, the purpose of the present study was to use the autotitration method to determine the effect of GABAergic manipulations in the VMT of the brain on ICSS reward. Although Williams and Herberg (1986) examined this using ICSS, it is unclear whether their findings resulted from a change in reward value of the stimulation, the “effort” component described by Neill et al. (1982), or other aspects of behaviors. In order to appropriately support or refute the theory proposed by Williams and Herberg (1986), the lateral VMT was manipulated through GABAergic injections and the autotitration paradigm was used as a means to distinguish “reward” from other motivational components that may explain the rat’s behavior, using the theoretical scheme described by Neill et al. (1982). Since the VMT is associated with motor function in the rat (Wenger, Musch, & Mink, 1999), we postulated that the role of the VMT might be associated with locomotor activity rather than reward. We hypothesized that when injected with the GABA antagonist picrotoxin in the VMT, the rat will increase ICSS responding and press the reset the lever “earlier,” and when injected with muscimol, the rat will decrease ICSS responding and reset “later.”

Material and Methods

Animals

Eight adult male Sprague-Dawley rats, weighing 330 to 400 grams at the time of surgery were acquired from Envigo (Indianapolis, Indiana). They were placed in an environment where temperature and humidity were both controlled. Each box housing the rat also contained access

to one or two small toys that the rat could use. Food and water was constantly provided along with a 12-hour lighting cycle. When they arrived at the facility at Rollins Biomedical Research Building, they were allotted a week to adapt and familiarize themselves with the environment and cope with any stress that may have been induced from traveling. After the one-week adjustment period, the rats were then handled and weighed in order to accustom them to the researcher. They were then screened for locomotor activity in a novel environment to determine whether they would be classified as either a high-responding rat or low-responding rat to novelty (not considered as a variable in the present experiment). Rats then underwent electrode and cannula implantations, and were housed individually. All surgical and behavioral procedures in this experiment have been approved by the Emory University Institutional Animal Care and Use Committee.

Surgery

All instruments used in the surgical procedure were sterilized with an autoclave or a heat sterilizer prior to the surgery. Using isoflurane gas anesthesia, Dr. Neill performed all surgical procedures on the rats. Before surgery, 1 mg/kg of the analgesic metacam was given orally. After determining the rat was completely anesthetized (observing whether a toe pinch elicited a reaction in the rat), the rat's scalp was then shaved and he was positioned in a Kopf stereotaxic frame with blunt ear-bars. Iodine and alcohol were used to clean and disinfect the rat's scalp. After a midline incision was made on the top of the head, four hemostats were used to stretch the rat's skin and fascia. A scalpel was then used to scrape the exposed skull and the bone dried.

The implant coordinates were corrected for the size and weight of the rat by measuring the anterior-posterior location of bregma, which is the intersection of the frontal and parietal

bones. The variance from the atlas was then corrected by adding 0.6 times the error to the A-P coordinate. To protect the cleaned skull from subsequent bleeding from drilling the implant holes, a thin layer of *Grip* dental acrylic was spread over the exposed skull surface. Stainless steel 0-80 self-tapping screws were then screwed into holes in the skull, followed by a bipolar twisted-wire steel electrode (Plastics One) implanted unilaterally in the left VTA while bilateral guide cannulae were inserted into the lateral ventromedial nucleus of the thalamus. Everything was secured in place by a 50-50 mix of *Grip* and *Cranioplast*. After a few minutes, the cement had dried and a broad-spectrum antibiotic ointment was applied around the implant. The wound was closed with polyethylene sutures.

Device Implantation and Coordinates

One MS 303/1 bipolar twisted-wire electrode (0.2 millimeter diameter) and two C232G bilateral stainless steel cannulae (22-gauge) from Plastics One Co. were implanted in all rats. The ICSS electrodes were in the ventral tegmental area at AP 3.5mm, L 0.5mm, H 2.2mm. VMT cannulae coordinates were AP 6.6mm, L 2.0mm. H 4.0mm.

Post-Operative Care

After surgery, rats were closely monitored and placed in a recovery cage. Three milliliters of sterile isotonic saline was provided to each rat intraperitoneally. Once the anesthesia from the surgery started to wear off and the rats became conscious, they were returned to their original cage where they were given food pellets or wet food. Animal condition was documented at 15 minutes, 30 minutes, 24 hours, 48 hours, and 72 hours postoperatively. Testing for the rats began approximately a week after surgery.

ICSS Testing

After allowing the rats to recover from the post-operative effects of surgery for approximately a week, rats were trained for intracranial self-stimulation. They were initially placed in a chamber that was 30 cm wide x 26 cm deep x 24 cm high with a stainless steel lever that they had to press in order receive electrical stimulation in the VTA region of their brain. Steel levers were used to prevent the rat from damaging the lever through behaviors such as the gnawing that is not atypical for rats that receive stimulation in the VTA. The testing chamber used ventilated shells in order to shield the rat from extraneous noise that may disrupt the results. The two response levers were located 3 centimeters above the floor and 18 centimeters apart on the wall. Every depression of the stimulation lever required an approximate force of fifteen grams. For each response on the stimulation lever, the rat received a 0.15 second train of constant current biphasic square wave stimulation (0.5 millisecond pulses, 100 pulses per second) through the chronically implanted bipolar electrode aimed at the VTA. Only one of the two levers served as the stimulation lever and could provide electrical stimulation to the rat.

Autotitration

Both response levers were active in autotitration ICSS,, one being the stimulation lever that produced the brain stimulation, and the other being a reset lever, which brought the intensity back to its maximum value. For every 5 responses on the stimulation lever, the intensity of the brain stimulation dropped 3 μA. In order to reset the stimulation lever to maximum intensity, the rat had to walk over and depress the reset lever.

Once the rat successfully learned this procedure, a 10 cm Plexiglas© partition was added between the two levers. At any point during the autotitration session, the rat was able to run

around the partition to the reset lever. A response on the reset lever did not deliver any stimulation to the rat's VTA, but reset the stimulation on the stimulation lever to the maximum value for that rat.

A starting intensity was individually determined for each rat (intensity was set between 85 to 120 microamperes). The goal was to get the rats to press the lever approximately 50 times before stepping down to reset (8-12 steps down; 24-36 microamperes). This was set in order to have comparable numbers of resets across all the rats. Stimulation was delivered through an armored lead that delivered electrical stimulation through the means of an electrode. Rats were put though two 15-minute testing sessions (once during the morning, and the other in the afternoon) throughout the week. The computer program recorded the average intensity at which the rat performed the reset response (mean reset intensity) and the number of stimulation responses for the entire duration of the 15-minute autotitration session. There was minimal intervention from the experimenter during the experiment.

Microinjections

To inject a solution, the rat was held in an experimenter's lap, the inner cannulae removed, and a 30 ga stainless steel injector which protruded 1 mm beyond the tip of the guide tube as inserted. While the rat freely moved about in its cage, 0.5 µl of drug solution or the saline vehicle was infused into the brain over a 40 sec period. The injector was left in place for a further 30 sec to minimize backpressure, the inner cannulae were inserted, and the rat was immediately taken to the ICSS testing chamber.

Analysis of Results

After completion of the experiment, the rats were taken into the Division of Animal Resources (DAR) facility in the Rollins Biomedical Research Building and euthanized through exposure to carbon dioxide gas. After ensuring the rats were not breathing (by observing their reaction following a toe-pinch), the rats were intracardially perfused with isotonic saline and 10% formol-saline. The brains were then detached, placed in container, and left to fixate. After allowing a day for the rats' brains to fixate, several 50 micron thick sections of the frozen brain were taken from the area surrounding the guide cannulae. The sections were analyzed on glass sides and stained with thionine in order to confirm the position of the cannulae.

Dose-response curves for the effect of picrotoxin were examined for change in ICSS responding from baseline and change in mean reset intensity from baseline, using a repeated measures analysis of variance (ANOVA). Baseline was determined by taking the number of ICSS responses and resets prior to drug injection. A bar graph was generated for rats that demonstrated the total ICSS responses and mean reset intensity after muscimol injection, using baseline measures as a means of comparison.

Results

From a total population of eight rats that started training for the experiment, four rats successfully learned ICSS and autotitration behaviors. The four rats that did not self-stimulate were excluded from the study.

ICSS Responding Following Picrotoxin

A repeated measures analysis of variance (ANOVA) was conducted to examine the effects of intra-VMT injections across the single injection of saline vehicle and the three doses of picrotoxin (25ng, 50ng, 100ng) on change in ICSS total responses (see Figure 2). There was a statistically significant decrease in ICSS responding across the doses, ($F(3, 9) = 5.68$, $p < .05$).

Mean Reset Intensity in Picrotoxin

A repeated measures analysis of variance (ANOVA) was conducted to examine the effects of intra-VMT injections across the single injection of saline vehicle and the three doses of picrotoxin (25ng, 50ng, 100ng) on changes in mean reset intensity (see Figure 3). There was a statistically significant increase in mean reset intensity across the doses, ($F(3, 9) = 5.68$, $p < .05$).

ICSS Responding in Muscimol

A bar graph was generated to examine the effects of intra-VMT injections across the single injection of saline vehicle and the two doses of muscimol (5ng and 10 ng) on total ICSS responses for two of rats (rat 12 and rat 15) compared to baseline (see Figure 4). There was a decline in ICSS responding at the two doses. The other two rats (rat 2 and rat 16) showed complete cessation of responding with muscimol.

Mean Reset Intensity in Muscimol

A bar graph was generated to examine the effects of intra-VMT injections across the single injection of saline vehicle and the two doses of muscimol (5ng and 10 ng) on mean reset intensity for two of rats (rat 12 and rat 15) compared to baseline (see Figure 5). There was a

decline in mean reset intensity at the two doses. The other two rats (rat 2 and rat 16) did not press the reset lever.

Observation of Rat Behavior after Picrotoxin

Once the rats received intra-VMT injections of the three doses of picrotoxin, they immediately became hyperactive, running around their cage and attempting to jump outside before being placed in the operant chamber. In the operant chamber, they showed vigorous motoric behavior as they ran around the chamber, investigating the partition and corners of the chamber. Although there was a clear increase in locomotor activity, this was not accompanied with increased ICSS responding. Two of the rats (numbers 15 and 16) did perform in the 15 min post-injection, but at a lower response rate, whereas the other two rats (numbers 2 and 12) initially would not perform ICSS and the stimulation did not appear rewarding, although they showed some immediate reset responses. In the latter two rats, performance reappeared after 15 or 30 min, presumably because of drug diffusion decreasing the local concentration. The data from the 15 min period of performing was included in the analysis for these two rats.

Observation of Rat Behavior after Muscimol

Once the rats received intra-VMT injections of muscimol, they generally showed a decrease in locomotor activity, particularly at the highest doses. In the operant chamber, two rats would not perform. At the end of the session, at least two of the rats (numbers 2 and 16) were found sleeping on the floor of the chamber having made no resets and very few stimulation responses. The two rats that did perform showed large decreases in ICSS responding and later resetting (see Figure 4).

Histology:

As shown in Figure 6, the placements of the cannulae for the rats were on target, positioned bilaterally in the center of the VMT. The electrode was also accurate within the target region of the VTA. Only one rats (rat 15) demonstrated excessive gliosis due to damage from the drug injections.

Discussion

The purpose of the current study was to use the autotitration method to examine the effect of GABAergic manipulations of the VMT on ICSS. After considering the ICSS effects found by Williams and Herberg (1986) and relating it to prior studies indicating the locomotor effects when the VMT was manipulated, a hypothesis was made that injection of the GABA antagonist picrotoxin would result in an increase in "effort," indicated by increased ICSS responding and earlier resetting in autotitration (Neill et al., 1982). Correspondingly, when injected with the GABA agonist muscimol, the rat would show a decrease in effort, exhibited by decreased ICSS responding and later resetting. The results, however, were very surprising.

Behavioral Interpretation of Picrotoxin Results

With the injection of various doses of picrotoxin, the rats demonstrated decreased ICSS responding accompanied by earlier resetting. These findings were unexpected, but can be explained through behavioral and neural interpretations. Picrotoxin and other systemic GABA antagonists, which have been shown to induce hyperactivity in rats when injected into the VMT (Klockgether et al.; Starr & Summerhayes, 1983), led to reduced ICSS responding. When the rats were initially injected with picrotoxin, they showed increased activity, moving vigorously around their box (to the extent where two of them tried to escape and jump out of their cage) and

then actively explored the operant chamber when placed into it. This finding was consistent with that of prior studies that reported an increase in locomotor activity with the local injection of GABA antagonists (picrotoxin or bicuculline) into the VMT (Klockgether et al., 1985; McGee, 2014; Starr & Summerhayes, 1983; Williams & Herberg, 1986). In these studies, various methods were utilized to assess locomotor activity such as simply visually assessing the rat's movements in a circular test box or using an activity meter with electromagnetic fields. All the methods showed increased motor activity following injection of a GABA receptor antagonist in the VMT.

Despite this surge in locomotor activity, the rats did not increase the rate they pressed the stimulation lever; rather, the opposite effect was observed. What was even more surprising was that this decrease in ICSS responding was accompanied with earlier resetting. Following the autotitration ICSS outcome scheme interpreted by Neill et al. (1982), this effect would indicate a decrease in reward. As determined by visual observation, the rats did not appear to experience any form of hypoactivity (indicated through a decrease in movement, fatigue, sleepiness). Although rate of stimulation decreased, they had no problem running back and forth to the reset lever. From a behavioral standpoint, this is a significant indication that the stimulation did not appear to be as rewarding after the drug injection in the VMT. If ICSS stopped following picrotoxin, the rats were not interested in experimenter-delivered stimulation either. The behaviors of the rats were similar to when they were being trained and the stimulation wire broke, resulting in the rats pressing the lever, but acquiring no stimulation or reward from their action, leading them to stop lever-pressing entirely because of the loss of reward.

Neural Interpretation of Picrotoxin Results

In order to understand why picrotoxin acted in the way it did, it is important to understand the relation between the SNPR and VTA along with the work of MacLeod et al. (1980) on the SNPR. While the electrode in the present study was placed in the VTA, it is plausible for axons projecting from the SNPR to the VMT to be stimulated by the electrode activating the VTA. In this manner, the SNPR would essentially be activated. In studying the GABAergic nigrothalamic pathway in the rat, MacLeod et al. (1980) reported that when the SNPR was electrically stimulated, GABAergic axons projecting to the VMT induced an inhibitory effect on the VMT neuronal activity; however that inhibitory effect was often followed by a short period of increased firing. Thus, once the SNPR was stimulated, the GABA neurotransmitter was released into the VMT synaptic cleft, bound to the GABA receptor of the postsynaptic neuron, had its inhibitory effect, and was cleared through the reuptake mechanism, followed by a short burst of "rebound" excitability. Therefore, every time the SNPR was stimulated, that short burst of excitability may constitute the "reward" effect for the rat, leading it to press the lever again. However, when picrotoxin was injected into the VMT, it blocked the GABA receptors of the postsynaptic neuron, and by inhibiting the GABA action also inhibited the "rebound" of excitability, and accordingly, the reward.

While it was true that picrotoxin inhibited the GABAergic axons from having an effect, it was also true that by blocking GABA receptors, picrotoxin increased neuronal activity in the VMT. Although the neuronal activity was fundamentally higher than that of baseline, resulting in motoric activation, the reward effect was absent because picrotoxin eliminates the small burst of excitability following GABA inhibition. If the rat received little reward after pressing the lever for electrical stimulation, then why would the rat continue pressing the lever? This caused the rat

to become apathetic towards the lever and attempt to reset earlier to no avail, which explains the findings of the present study.

There was yet another interpretation of the neuronal basis of reward. Schultz (2007) described how unexpected rewarding stimuli specifically fire dopamine cells in the brain. Therefore, by implanting the electrode in the VTA, the VTA became activated and fired dopamine cells and a theoretical scheme followed as such: a rewarding stimulus would lead to the firing of dopamine cells in the VTA, which projected towards the nucleus accumbens, activating the accumbens, which initiated the release of GABA cells in SNPR thereby inhibiting the SNPR, which in turn caused the excitation of the VMT, and that was how reward was experienced (see Figure 1). VMT excitation was what can be causing reward; therefore, it was not the dopaminergic effect, but the GABAergic effect that lead to reward.

Picrotoxin blocked the GABA receptor and prevented GABA from binding. As a result, VMT inhibition initially caused by the GABA neurotransmitter binding to its receptor was turned off. This lead to VMT activation and firing as long as picrotoxin was having an effect. Therefore, if the VMT was already firing due to picrotoxin, further excitation that resulted from the loss of GABA because of SNPR inhibition essentially had no effect on the reward. Because the VMT was already activated from the picrotoxin, stimulation in the VTA that resulted in the excitation of VMT had no effect on the rats, leading them to decrease their rate of ICSS responding.

Behavioral Interpretation of Muscimol Results

The results of muscimol injection into the VMT were more variable than those of picrotoxin. With two of the rats, muscimol completely disrupted their behavior, leading to a huge

drop in ICSS responding accompanied with no resets. These rats proceeded to lie on the floor of the chamber and rest. The other two rats showed striking results as ICSS responding decreased but did not stop, and resetting came later than usual. Again, looking at the autotitration interpretation of Neill et al. (1982), this would be indicative of decreased effort. Although at first glance, the findings from muscimol injection in the VMT would lead some to interpret it as a clear indication that the VMT was responsible for effort or other motoric functions (Klockgether et al., 1985; Starr & Summerhayes, 1983; Williams & Herberg, 1986), this was not the case as the rats showing complete cessation of responding were not interested in experimenter-delivered stimulation either.

Neural Interpretation of Muscimol Results

Following a similar theoretical scheme to picrotoxin, when muscimol was injected into the lateral VMT, it activated the GABA receptors at a constant rate and prevented the GABA neurotransmitter from binding. This resulted in a constant inhibitory effect and prevented the brief excitatory response following GABA release. With little reward produced by electrical stimulation, the rat had less motivation to run back and forth from the partition and stimulate the lever if the rewarding aspect is suppressed by muscimol. The behavior of the rats (decreased ICSS and later resetting in two rats and cessation of responding in two) may have been attributable to a mixture of decreased reward and decreased effort.

Muscimol also played a role in the second interpretation of the neuronal basis of reward. By binding to the GABA receptor and keeping it constantly activated, muscimol prevented the excitation of the VMT that was caused by stimulation in the VTA. Muscimol inhibited the VMT and prevented its excitation, which lead to the suppression of reward.

Comparison to Williams and Herberg (1986)

Williams and Herberg (1986) also reported decreased ICSS in a single-lever task following muscimol in the VMT. However, they reported increased responding in response to picrotoxin, an effect opposite the present results. What might account for this disparity? Three major factors might explain this discrepancy in findings.

First, Williams and Herberg placed the electrode in the lateral hypothalamus while the electrode in this study was in the VTA. The lateral hypothalamus and the VTA are two very different pathways; therefore, electrical stimulation received in the LH takes a different route than electrical stimulation received in the VTA.

Second, the placements of the cannulae were also different. While Williams and Herberg (1986) put the cannulae in the medial VMT (L = 1.4mm), the current study placed the cannulae in the lateral VMT (L = 2.0mm). In her study, McGee (2014) analyzed the implications of placing the cannulae in the medial VMT versus the lateral VMT, hypothesizing that the lateral VMT was more directed towards motivational behaviors such as goal-related movements while the former was responsible for cognitive functions such as sustained attention.

Last, the manner in which electrical stimulation was given to rats was different. While for the rats in the experiment of Williams and Herberg (1986) electrical stimulation was available at randomly varied intervals with a mean of 10 seconds (variable interval 10 seconds schedule of reinforcement), the rats in the current study received electrical stimulation every time they pressed the lever. Different methodologies result in different results, which may explain the different effects of picrotoxin in both Williams and Herberg's (1986) study and the present study.

Limitations

Although our study demonstrated significant results that suggest a role of GABA in the VMT in the reward system, there were a few limitations that must be considered. First and foremost, the sample size ($N = 4$) was very small, due to the outcome in which only 50% of the implanted rats would self-stimulate. Although the results obtained tell a very important story in regards to the VMT, a larger sample size would have been appropriate for the experiment. Another limitation was that the finding that the muscimol doses which allowed at least some responding to occur are far lower than the doses found effective in other behavioral tests in the Neill laboratory (e.g., the 5-CSRTT attentional task) was not discovered until late in the experimentation, after numerous other injections of muscimol as well as picrotoxin. These low (5 ng and below) doses should be tested in rats that are receiving their first injections.

Implications and Future Directions

GABAergic manipulations in the VMT using the autotitration paradigm suggested that the VMT might be important for the mechanism of reward, at least for VTA electrodes. Countless studies have examined the mesolimbic pathway, speculating that the reward system functioned by stimulating dopamine cells to fire in the VTA and release dopamine in to the nucleus accumbens where they bind to the receptors of the postsynaptic neuron and have an excitatory effect (Schultz, 2007). However, while this “mesolimbic dopamine system” had been a hot topic in the field of neuroscience since the mid-1900s, it may be that this was not the entire story. Where did this “reward signal” travel after it reached the accumbens? That is where the findings of the current study were applicable. After the burst of dopamine release into the accumbens, the accumbens output axons traveled down to the SNPR and inhibited the SNPR,

which was responsible for inhibiting the VMT through GABA release; however, since the SNPR was inhibited, it had an excitatory effect on the VMT, which was what the current study implied as the sensation of reward. When the rats responded on the stimulation lever, the electrical stimulation was delivered to the VTA, excited the accumbens, inhibited the SNPR, and eventually excited the VMT, providing reward. Picrotoxin and muscimol disrupted the GABAergic pathway from the SNPR to the VMT and, essentially, inhibited reward. Because the picrotoxin drug resulted in increased motor activity, the diminished reward effect was obvious. Because muscimol decreased motor activity, the lost reward effect was much more to verify.

In yet another interpretation described earlier, the VMT did not have to be completely inhibited to have a rewarding effect. If the SNPR was activated by the electrode placed in the VTA, it could send GABAergic axons to the VMT, where they would bind to the receptor, inhibit the neuronal activity, be cleared out by the reuptake process, experience a brief burst of excitation that constitutes reward (depicted through a surge in neuronal activity), and then return to baseline (MacLeod et al., 1980). Every time the stimulation lever was pressed, this process repeated itself, explaining how GABA may be the true transmitter accountable for the reward sensation. When this endogenous SNPR-VMT pathway was disrupted either by blocking the GABA receptors (picrotoxin) or binding to the GABA receptors (muscimol), the brief burst of excitability was gone, and consequently, the reward. Both interpretations implied the role of the GABA neurotransmitter in reward and could explain the findings of the current study.

Also, as described previously, Williams and Herberg (1986) placed their ICSS electrodes in the lateral hypothalamus (LH) of the brain, and obtained an increase in ICSS responding with intra-VMT picrotoxin, the opposite of what was found in the present study. It may be interesting to follow William and Herberg's experimental design and position the electrode in the LH rather

than the VTA and see if the results change. Similarly, to assess the same pathway, researchers could even place the electrode in the accumbens, which may yield findings similar to those of the present study if the theoretical pathway of in Figure 1 was correct. Stimulating the accumbens would have an inhibitory effect on the SNPR, which would result in the excitation of the VMT, resulting in reward through the GABAergic nigrothalamic pathway. This reward should also be decreased by picrotoxin in the VMT.

In conclusion, through using autotitration ICSS, the effects of GABAergic manipulations in the VMT of the brain on ICSS reward yielded surprising results. These results were consistent with a "GABA Theory of Reward," expanding the knowledge of brain reward systems in general.

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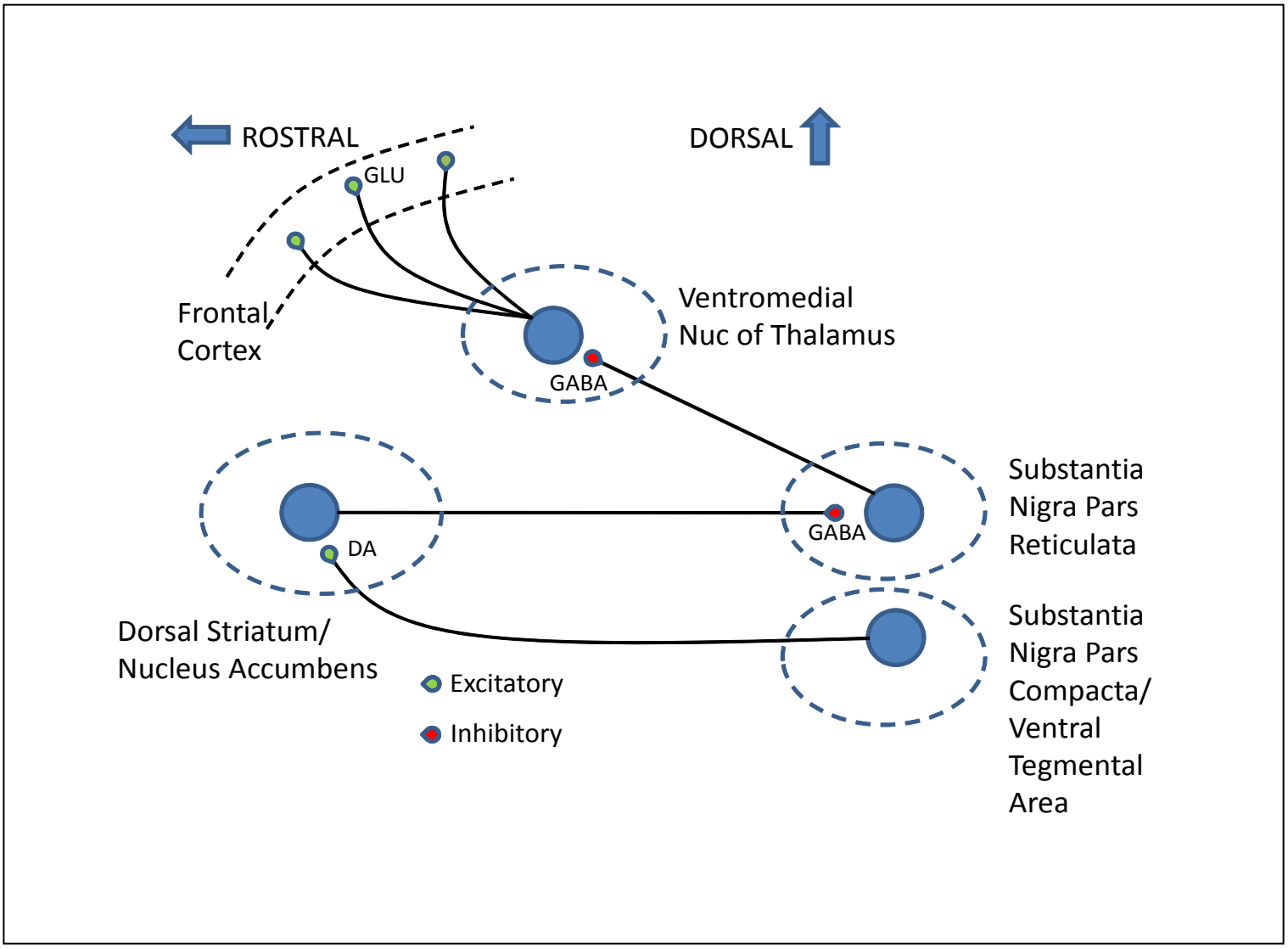

Figure 1. Lateral view of accumbens route to cortex.

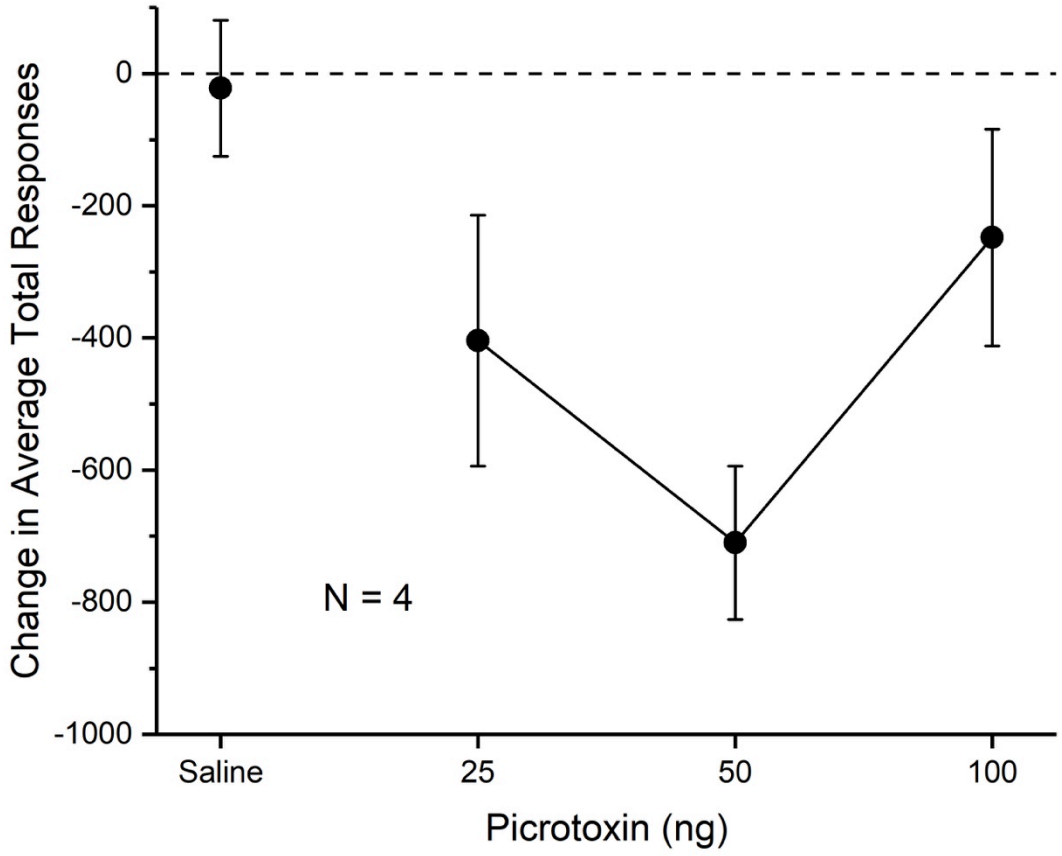

Figure 2. Change in average ICSS responding as a function of picrotoxin.

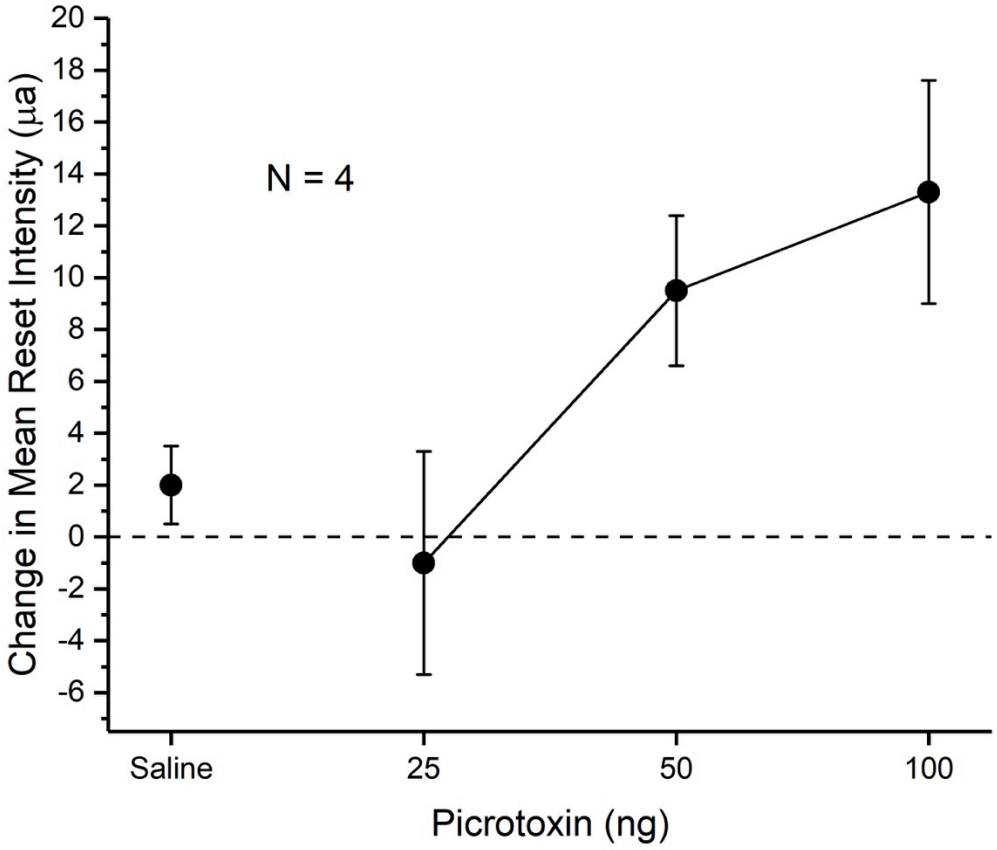

Figure 3. Change in mean reset intensity as a function of picrotoxin.

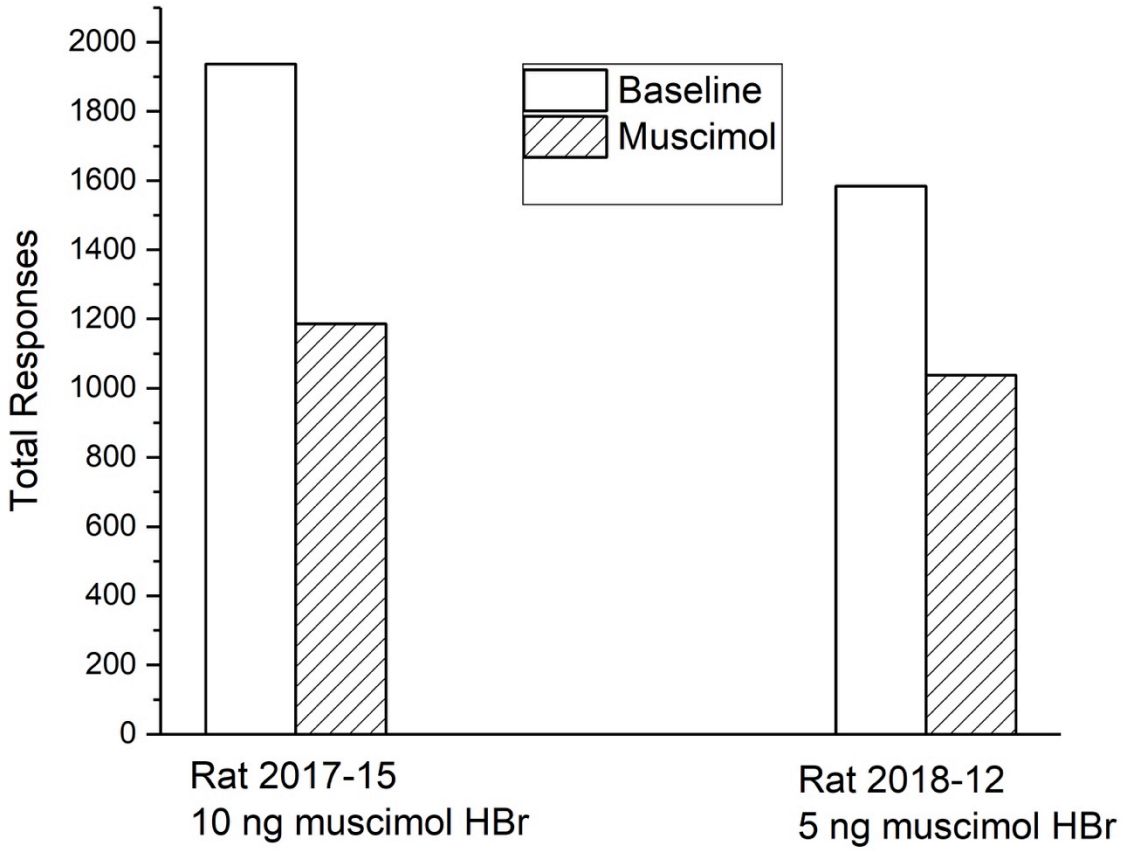

Figure 4. Change in total ICSS responding as a function of muscimol.

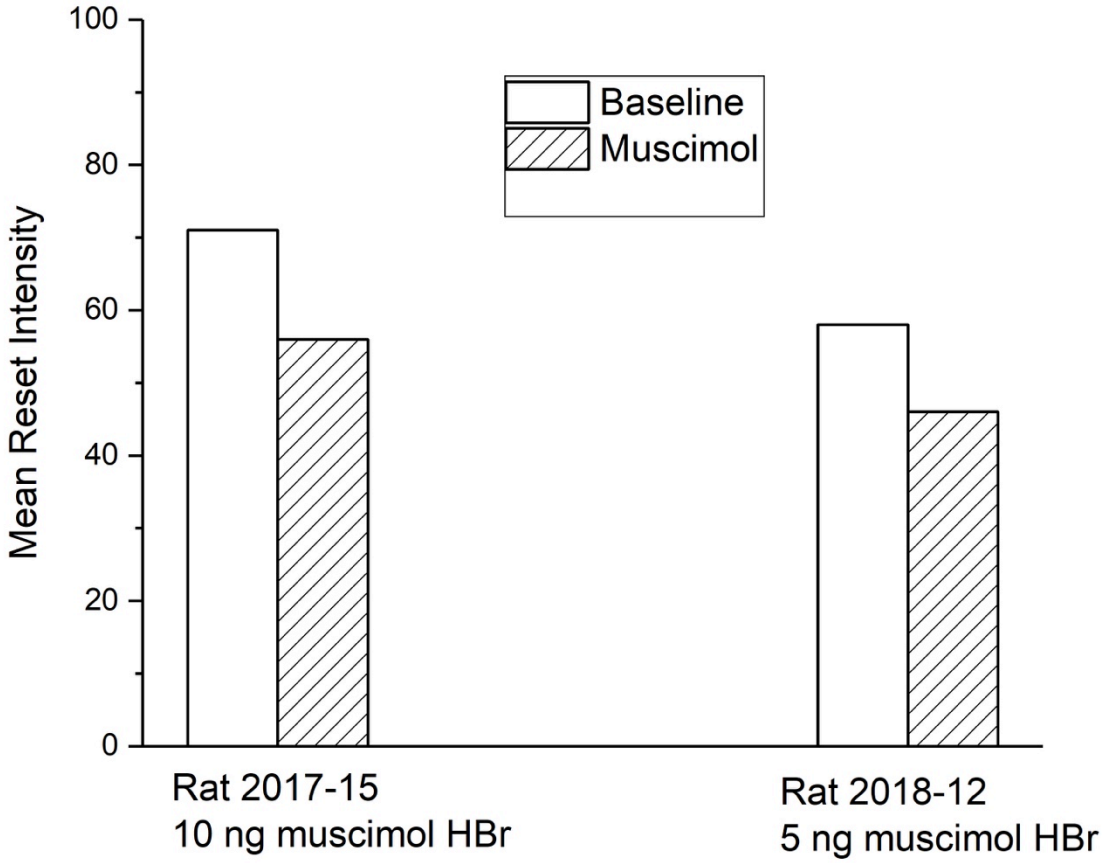

Figure 5. Change in mean reset intensity as a function of muscimol.

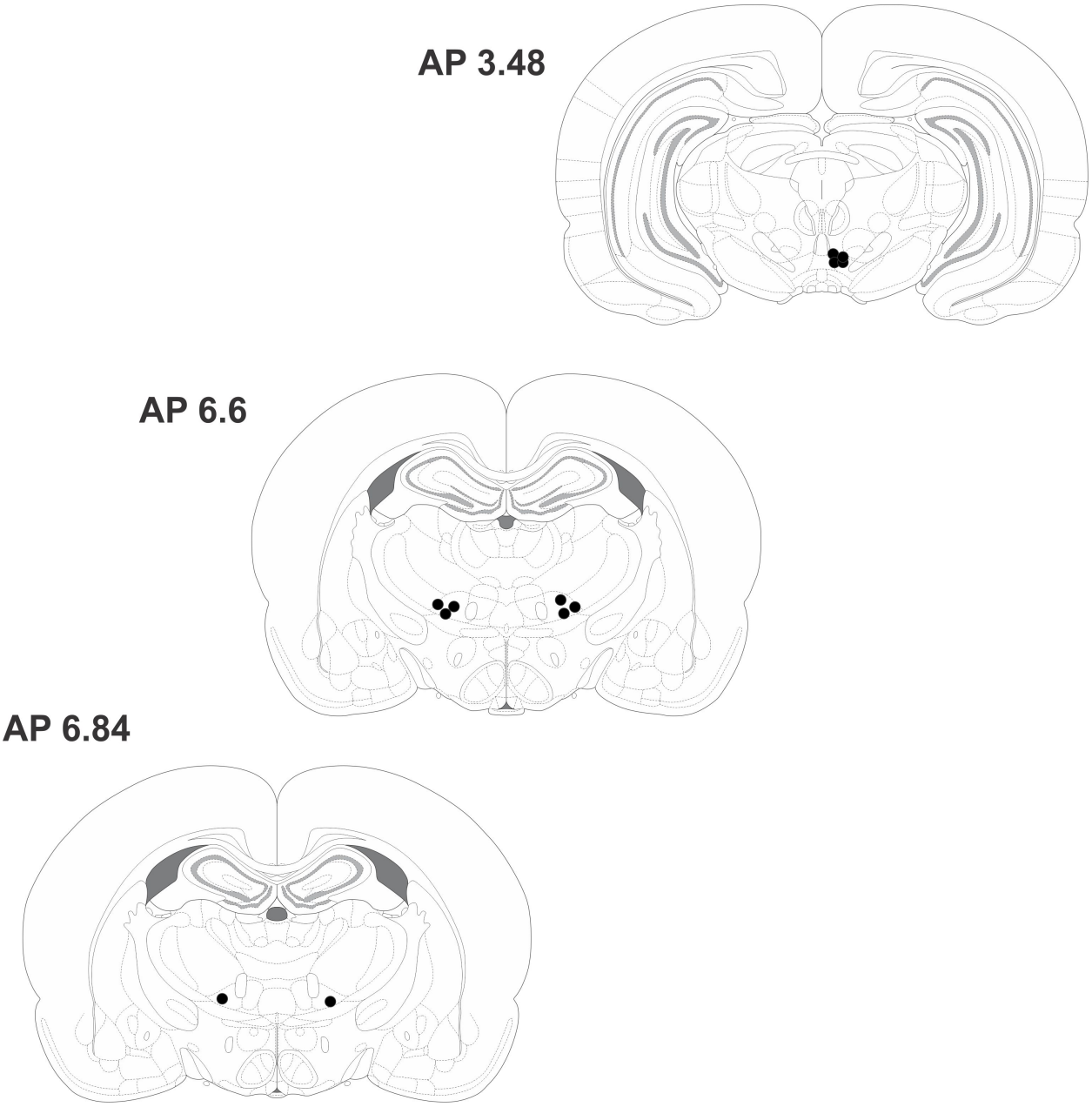

Figure 6. Placement of cannulae and electrode.