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GABAergic Transmission in the Nucleus Accumbens-Ventral Pallidum Path

and DRL Task Efficiency in Rats

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Bachelor of Science

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Abstract

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By Marina Georgia Wheeler

The nucleus accumbens (NAc) has been implicated in behavioral response inhibition yet the roles of its efferent projections has yet to be explored. One of the major projections from the NAc is to the ventral pallidum (VP). In experiment 1, bilateral VP infusions of the GABAergic antagonist picrotoxin decreased task efficiency in rats on a Differential Reinforcement of Low rates (DRL) 10 second schedule in a dose-dependent fashion. In experiment 2, contralateral disruption of the NAc-VP path via unilateral NAc infusions of muscimol, a GABAergic agonist, and VP infusions of picrotoxin decreased DRL 10 second task efficiency; ipsilateral infusions did not. These findings suggest an intact NAc-VP GABAergic pathway may be involved in behavioral response inhibition as measured by DRL.

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GABAergic Transmission in the Nucleus Accumbens-Ventral Pallidum Path and DRL Task Efficiency in Rats.

Behavioral response inhibition is the ability to withhold a physical response to a stimulus in order to receive reinforcement; conversely, behavioral response disinhibition is the inability to withhold responding for reinforcement (Evenden, 1999; Pothuizen, Jongen-Relo, Feldon & Yee, 2005). In humans the prefrontal cortex (PFC) is correlated with decisions to delay reward (McCLure, Laibson, Loewenstein & Cohen, 2004; Torregrossa, Quinn & Taylor, 2008). The role of the orbital frontal cortex (OFC) in behavioral response inhibition may be related to the actions of the modulatory neurotransmitter dopamine (DA) within the OFC (Winstanleym Theobald, Dalley, Cardinal & Robbins, 2006). The basolateral amygdala (BLA) may also be involved in behavioral response inhibition and this may be related to the actions of serotonin (5HT) within the BLA (Winstanley, Theobald, Cardinal & Robbins, 2004; Meyers-Lindenberg, et. al. 2006). Both the OFC and the BLA project to the nucleus accumbens (NAc) (Kelley, Domesick & Nauta, 1982, Robinson & Beart, 1988; Sesack, Deutch, Roth, & Bunney, 1989; Brog, Salyapongse, Deutch & Zahm, 1993; Gorelova & Yang, 1997; Winstanley, Theobald, Cardinal & Robbins, 2004; Meyers-Lindenberg, et. al. 2006).

The NAc is a component of the ventral striatum which can be separated into core and shell subregions (Deutch, Bourdelais & Zahm, 1993). The NAc has been intensively studied for its involvement in motivation and reward processes. A small relatively, recent literature indicates that in addition to these roles, the NAc core may also be involved in behavioral response inhibition (Ikemoto & Panksepp, 1999; Winstanley, Theobald, Cardinal & Robbins, 2004; Berridge, 2007; Mingote et al. 2008). Lesions of

the NAc core significantly increase the likelihood that a rat will select a small immediately available reinforcer instead of a larger but delayed reinforcer (Cardinal, Pennicote, Sugathapala, Robbins, & Everitt, 2001). Previous studies have also shown that lesions of the core but not shell region of the NAc lower response efficiency (a ratio comparison of the number of reinforced responses made to the total number of reinforced and unreinforced responses) on a DRL18 second task (Pothuizen, Jongen-Relo, Feldo & Yee, 2005).

Involvement of the NAc in behavioral response inhibition may seem surprising given the prominent role of the structure in behavioral activation (Mogenson, Jones & Yim, 1980). Yet a role in behavioral response inhibition may not be surprising after all given that both the OFC and BLA send glutamatergic projections to the NAc (Kelley, Domesick & Nauta, 1982, Robinson & Beart, 1988; Sesack, Deutch, Roth, & Bunney, 1989; Brog, Salyapongse, Deutch & Zahm, 1993; Gorelova & Yang, 1997). Therefore, it seems likely that the NAc may be perfectly situated as a part of a neural pathway involved in behavioral response inhibition.

The afferent inputs into the NAc are distinct from those which innervate the remaining portion of the ventral striatum (Ikemoto & Panksepp, 1996; Holt, Graybiel & Saper, 1997), and the NAc may therefore be considered a part of the ventral striatal complex with specialized function (Deutch, Bourdelais, & Zahm, 1993). There are two subregions of the NAc, termed "core" and "shell" (Deutch, Bourdelais & Zahm, 1993). Both of these subregions are innervated by dopaminergic neurons from the ventral tegmental area (VTA) and both regions send discrete projections to the ventral pallidum (VP) (Ikemoto & Panksepp, 1999). The projection neurons from the NAc core are called

medium spiny neurons (MSNs) and release γ-amino butyric acid (GABA), a primary inhibitory neurotransmitter (Koob and Swerdlow, 1988; Zahm & Brog, 1992).

The activity of the MSNs within the NAc may be important in behavioral response inhibition (Robbins & Everitt, 2007). The axons of the NAc MSNs form a GABAergic projection which innervates the VP (Zahm & Brog, 1992, Mingote et al. 2008). This inhibitory projection is one of the major NAc efferent pathways (Koob & Swerdlow, 1988). Activity of the MSNs in the NAc could therefore affect behavior by influencing the VP via this NAc-VP GABAergic pathway (Berridge, 2007; Mingote, 2008).

The VP is found below the anterior commissure between the NAc and the lateral hypothalamus (Paxinos, 1997). It can be separated into lateral and medial components (Zahm & Brog, 1992; Deutch, Bourdelais, & Zahm, 1993; Maurice, Deniau, Menetrey, Glowinski & Thierry, 1997). The core and shell of the NAc send discrete projections to the VP (Robledo & Koob, 1993). The core of the NAc sends an efferent projection to the lateral portion of the VP and the shell of the NAc projects to the medial portion of the VP (Zahm & Brog, 1992). The VP then sends discrete projections from the lateral and medial portions elsewhere. The lateral VP projects to the subthalamic nucleus and the medial portion of the VP projects to the VTA and mediodorsal thalamus (Deutch, Bourdelais, & Zahm, 1993). VP neurons are primarily GABAergic and are tonically active (Yang & Mogenson, 1989). Given that the VP is a major recipient of the NAc GABAergic MSN projection and that the VP acts as the interface between higher mesocorticolimbic structures and the brainstem, the potential role of the VP in behavioral response inhibition is intriguing yet currently not well understood.

Differential Reinforcement of Low rates (DRL) is an operant schedule of reinforcement which has been used to test behavioral response inhibition (Kramer & Rilling, 1970; Evenden, 1999; Monterosso & Ainslie, 1999; Pothuizen, Jongen-Relo, Feldon & Yee, 2005). The DRL schedule requires the delay of a behavioral response (e.g. lever pressing) for a predetermined period of time (e.g. 10 seconds) in return for the delivery of a reinforcer (e.g. food pellet). Premature responses are punished with the non-delivery of a reinforcer and the timer is reset to zero, the subject must then delay responding for the set amount of time again (Kramer & Rilling, 1970).

Performance on a DRL schedule can be evaluated by measuring the total number of responses, reinforcements, and overall task efficiency. The task efficiency score identifies the ratio of reinforced responses to the total number of responses made, both reinforced and unreinforced. It is computed as:

$$Task\ Efficiency = \left(\frac{total\ number\ of\ reinforcements}{total\ number\ of\ responses}\right) * 100$$

The DRL schedule has been used extensively to study the effects of pharmacological agents such as d-amphetamine, benzodiazepines, dopamine, catecholaminergic stimulants and anticholinergic drugs (Sanger, Key & Blackman, 1974; Neill, 1976), and has been considered a measure of behavioral response inhibition (Kramer & Rilling, 1970).

The task efficiency score is an important measure of changes in behavioral performance on a DRL 10 second schedule. Yet task efficiency alone may not be sufficient for understanding the underlying nature of a behavioral change. As has already been mentioned, the total number of responses made is a measure of both reinforced and unreinforced responses whereas the total number of reinforcements is a measure of reinforced responses only. A decrease in task efficiency may therefore be due to an

increase in unreinforced responding which could be suggestive of an increase in motor activity. Alternatively, a decrease in reinforced responding if unaccompanied by an increase in total responding might imply an issue of timing. Changes in both responding and reinforcements may indicate reasons for behavior modifications which would be missed by looking only at the task efficiency score. It is therefore necessary to include all three measures in analyses.

The role of the NAc in motivation and effortful behavior has in recent years been an area of interest (Monterosso & Ainslie, 1999). Previous work in the Neill lab (M.A. thesis of D. Romine, 2007) has shown that transient inactivation of the NAc core via infusions of the GABAergic agonist muscimol, decreased task efficiency on a DRL10 second task in rats. While evidence strongly suggests that the NAc core is involved in behavioral response inhibition, little research has been conducted to understand how the NAc is contributing to behavioral response inhibition via its effects on downstream pathways such as the innervation of the VP. Inactivation of the NAc core via infusions of muscimol or lesions may decrease task efficiency on a DRL schedule by preventing the NAc GABAergic MSNs from releasing GABA into the VP (Figure 1). The following 2 experiments were designed to test the hypothesis that the VP is involved in behavioral response inhibition as observed on a DRL 10 second schedule, and that this involvement is modulated by the MSN GABAergic projection coming from the NAc core to the VP.

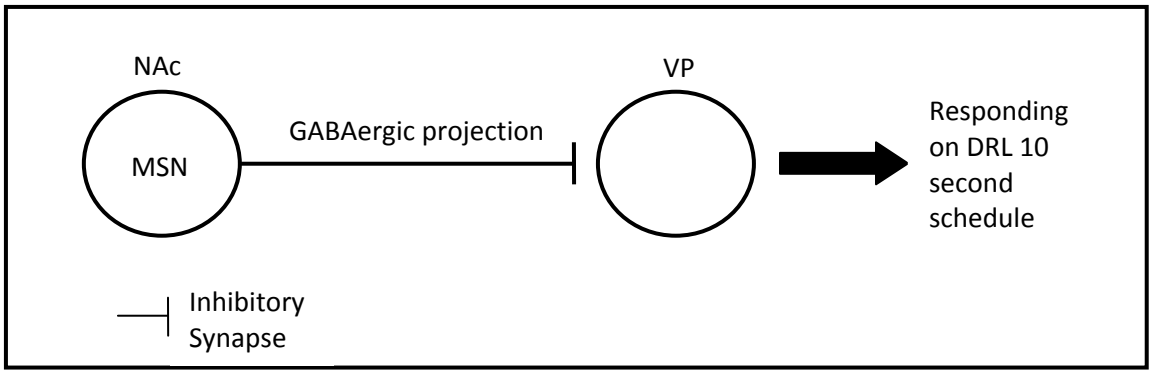


Figure1. Muscimol infusions in the NAc decrease efficiency on a DRL 10 second schedule. Infusions of picrotoxin in the VP should also decrease task efficiency on a DRL 10 second schedule.

Experiment 1

If muscimol in the NAc impairs the ability to withhold responding by inhibiting the action of GABAergic MSN projection neurons, it should be possible to impair behavioral response inhibition in rats by blocking the effects of GABA in the VP. In this within-subjects experiment a dose response curve was established by administering bilateral infusions of the $GABA_A$ non-competitive antagonist picrotoxin into the VP at 25 ng, 50 ng, and 100 ng doses. The effects of these doses were compared to DRL 10 sec task performance during a vehicle condition. The predictions were that task efficiency would decrease in a dose related way and that higher doses would produce greater deficits in efficiency when compared to vehicle.

Experiment 2

If the NAc-VP GABAergic path is important for the modulation of behavioral response inhibition, then disruption of this pathway at either the NAc core or VP level should reliably decrease behavioral response inhibition. Rats were placed in 4 conditions on a DRL 10 second schedule using a within subjects design. In the contralateral condition rats were given a unilateral infusion of muscimol into the NAc core of one

hemisphere and a unilateral infusion of picrotoxin into the VP of the opposite hemisphere. If the NAc-VP path comprised of the MSN GABAergic projection is involved in behavioral response inhibition, this contralateral condition should result in a decrease in task efficiency on a DRL 10 second schedule (Figure 2). In the ipsilateral condition unilateral infusions of muscimol into the NAc core and picrotoxin into the VP of the same hemisphere should not impair performance on a DRL 10 second schedule because the NAc-VP path of the opposite hemisphere remained functional. The results of experiment 1 were verified in experiment 2 by the inclusion of a bilateral condition in which picrotoxin was infused bilaterally into the VP (Figure 2). The effects of the contralateral, ipsilateral and bilateral conditions on responding, reinforcements, and overall task efficiency were compared to the results obtained during the vehicle condition. The predictions were that task efficiency would be decreased in the bilateral and contralateral conditions when compared to vehicle condition because of the bilateral disruption of the NAc-VP path. Task efficiency in the ipsilateral condition should not significantly differ from the task efficiency in the vehicle condition because the NAc-VP path remains operative in both of these conditions.

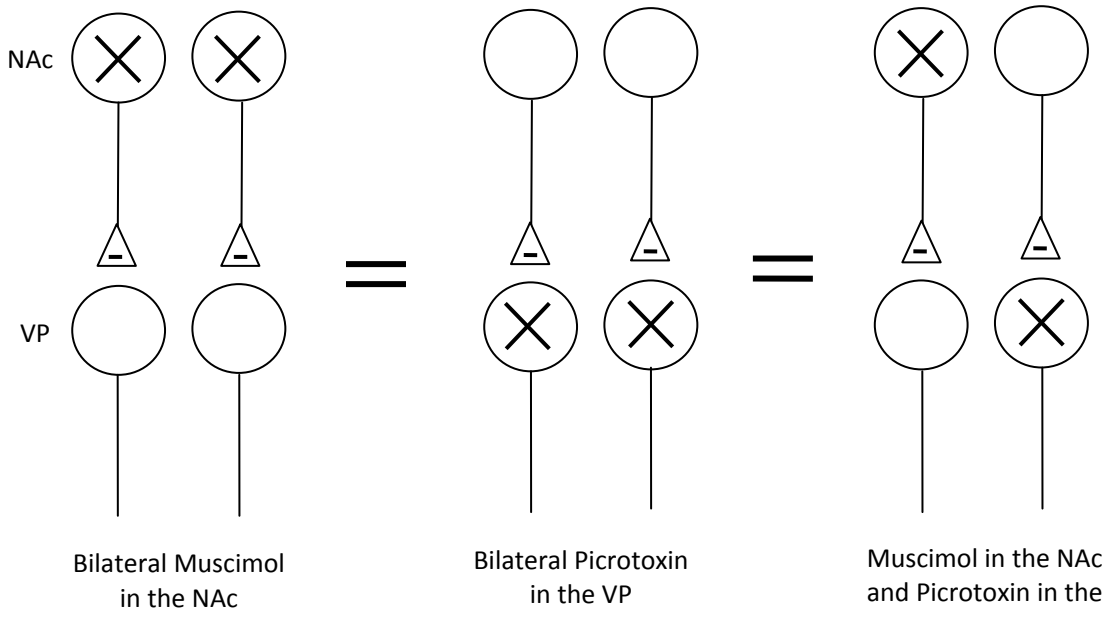


Figure 2. Depiction of the bilateral infusions of muscimol in the NAc and the prediction that picrotoxin infused into the VP as in experiment 1 and contralateral infusion of muscimol in the NAc and picrotoxin in the VP of experiment 2 will produce comparable results because of the functional connection between the two regions.

Methods

Experiment 1

The goal of experiment 1 was to determine if bilateral infusions of picrotoxin into the VP would produce a dose-related decline in task efficiency on a DRL 10 second schedule. Performance in the vehicle condition in which vehicle (0.9% sterile saline) was infused bilaterally into the VP was compared to performance at each drug concentration in order to detect changes in the number of responses, reinforcements, and task efficiency.

Subjects: Sixteen male Sprague-Dawley-derived rats (Harlan Sprague Dawley, Indianapolis, IN) weighing between 250 g and 300 g upon arrival were used. Rats were individually housed in clear Plexiglas cages with corn cob bedding and one enrichment device on 12 hour light-dark cycle (lights on at 7:00 am). Animals had access to water *ad*

libitum and were food restricted to 90% of their free-fed body weight by way of a monitored diet of Purina Lab Rat Chow (approximately 15 grams per day). Food was supplied each day after testing. Daily records of individual weights were maintained over the course of the study for each animal. All procedures were approved by the Emory University Institutional Animal Care and Use Committee (IACUC).

Differential Reinforcement of Low Rates 10 second (DRL 10). The DRL10 second schedule required each rat to delay responding for reinforcement by ten seconds. If a response was made before the ten seconds passed, no pellet was delivered and the timer was reset to zero. Each rat was placed in an operant response chamber and the number of responses (lever presses) and reinforcements (45 mg food pellet) delivered during a 20 minute testing period was recorded.

Four response chambers constructed of Plexiglas (30.5 cm wide, 25.0 cm tall, 31.0 cm deep) were used in experiment 1. Each response chamber was mounted with an external pellet dispenser and internal food hopper placed in the center of the right side wall. A response lever mounted to the right of the food hopper protruded into the response chamber 4 cm above the floor. The lever required approx 15 gm of pressure to close. Response chambers were individually housed inside an outer wooden chamber box (48.5 cm wide, 38.0 cm tall and 39.0 cm deep). The outer chamber box was closed during testing to block external visual and auditory cues. A light was affixed to the center of the ceiling of the outer chamber and a video camera was mounted on the inside center of the outer chamber back wall to record behavior.

Training. Rats were trained to respond on a DRL 10 schedule to receive reinforcement. Training sessions lasted 20 minutes and took place once a day between

1200 and 1700 hours 6 days a week. Each rat was assigned to a response chamber at the beginning of training and was henceforth trained only in that chamber. Training began with the introduction of the food reinforcer in the animal's home cage. On each subsequent day following the introduction of the reinforcer, each rat was placed in the testing apparatus and one food pellet was dispensed into the food hopper automatically every 60 seconds. Rats that did not eat the food pellets from the feeder remained on this schedule on each successive day until food pellets were readily consumed. Rats were then placed on a fixed-ratio 1 (FR1) schedule with one pellet delivered for every lever press. The FR1 schedule of delivery continued until the rats consistently lever pressed a minimum of 100 times per 20 minute session. Once the FR1 criterion was met, animals were introduced to DRL schedules of progressively longer interval delays. Rats were first exposed to the DRL 4 second schedule. On this schedule rats needed to withhold a response for 4 seconds before responding would yield reinforcement. Once a criterion of 15% task efficiency was achieved this delay was extended to a DRL 8 second schedule. The DRL 8 second schedule was maintained until 15% task efficiency scores were observed. Following the DRL 8 second schedule rats were placed on the DRL 10 second schedule. Once a 15% efficiency score on the DRL 10 second schedule was reached and maintained consistently across a number of days the rats were prepared for cannulae implantation.

Surgery. Prior to surgery the rats were taken off of food deprivation for a minimum of one week. On the day of surgery, rats were anesthetized with a Ketamine-Acepromazine-Xylazine (KAX) anesthetic [2ml/kg, intraperitoneal (i.p.)]. This anesthetic was used because of its greater margin of safety compared to other available

injectable anesthetics such as barbiturates. Once the rat to be implanted displayed no motoric reflex and respiration was slowed, the site for incision on the top of the rats head was shaved. The rat was then secured into a stereotax with sterilized ear bars. The stereotaxic apparatus was used to implant bilateral 22-gauge cannulae (Plastics One, Roanoke, VA.) aimed at the VP (8.7 mm AP, 3.0 mm H, 2.5 mm L). Implanted cannulae were held in position by anchoring 2 jewelers screws to the skull which were overlaid with cranioplastic cement. Once the implantation was completed each rat was given an injection of 0.9% sterile saline [3 cc, subcutaneous (s.c.)] and the analgesic *Metacam* (Henry Schein; 1 mg/kg, s.c.).

Post-operatively rats were kept in a clear Plexiglas cage on top of a heated mat for a minimum of two hours or until consciousness was regained. A post-operative inspection of the incision site was conducted 24 hours after surgery to look for signs of infection and to assess the overall well-being of the animal. If infections were observed the site was treated with topical antibiotics.

Testing and Intracranial Infusion. Not less than one week after surgery rats were placed back on a food restricted diet to maintain a body weight of 90% of the post-operative free-fed weight. Each rat was then reintroduced to the response chambers on a DRL 10 second schedule for one 20 minute session occurring each day between 1200 and 1700 hours. Testing continued daily until animals were performing consistently across days as measured by response and task efficiency scores. Once animals displayed consistent performance the first intracranial infusion was delivered. Bilateral VP drug infusions of either vehicle (0.5 µl; 0.9% sterile saline], or picrotoxin (0.5 µl; 25ng, 50 ng, or 100ng) were delivered in a randomized order.

Drug doses were infused into the VP though a 30-gauge microinjection needle that extended 1.0 mm past the end of the cannula. The microinjector was attached to a Hamilton syringe (Hamilton Company, Reno, NV) by PE-10 tubing (Clay-Adams, Parsipanny, NJ) and mounted on a microsyringe pump (Sage Instruments, Cambridge, MA). The microinjection needle was left in place for 30 seconds following the completion of each drug infusion to ensure the drug diffused completely out of the injector. Following drug infusion the rat was placed into the response chamber and the 20 minute testing session began. Drug infusions were separated by a minimum of two days of behavioral testing with no drug infusions in which the animal displayed consistent performance.

Histology. Once testing was completed, animals were euthanized with a lethal overdose of KAX and perfused intracardially with a solution of 0.9% saline followed by 10% formalin. Brains were stored in 10% formalin until sectioned using a microtome. Sections through the region of the cannulae tracks were mounted and stained using thionin. These sections were then examined to determine the placement of cannulae. Animals were excluded from the analysis if it was discovered that the placement of cannulae was outside of the specified region of interest.

Statistics and Sensitivity Analyses. A binary regression comparing the data of those animals missing one or more data points (n = 3) against the data of animals with no missing data points (n = 7) was carried out. This was done to identify any systematic differences between the two groups which would require the exclusion of animals that did not complete all four of the testing conditions. Alternatively, a non significant result

from the binary regression would support the inclusion of animals with missing data in the analyses.

Poisson loglinear analyses (PLA) of the total number of responses, reinforcements, and task efficiency were conducted. The PLA was selected because, as is often the case with frequency counts and percentages, these data were highly skewed and followed a Poisson distribution. These analyses of the dependent variables were each conducted three times to consider the impact of animals in experiment 1 which received only 2 of the four doses (n = 1), 3 of the four doses (n = 2), and all of the four doses (n = 7). A nested model that examined dose within rat was used. This generalized linear approach allowed all animals to be included in analyses despite missing data. A post hoc analysis of planned comparisons using a Bonferroni analysis of simple contrasts was performed on significant findings to determine which of the 3 picrotoxin doses significantly changed DRL task performance compared to vehicle as measured by total number of responses, reinforcements, and task efficiency.

Experiment 2

The goal of experiment 2 was to test the hypothesis that the nucleus accumbens controls DRL responding via a functional GABAergic NAc-VP path.

Subjects. Eleven rats were used in this experiment. These rats were obtained from the same supplier as in Experiment 1 and maintained as in Experiment 1. Only rats with appropriate cannulae placement in the NAc and VP were included in the data set for analyses.

DRL 10 second schedule training. The same procedures used to train animals in experiment 1 were used to train animals in experiment 2.

Surgery. In addition to the bilateral cannulae aimed at the VP (8.7 mm AP, 3.0 mm H, 2.5 mm L), a unilateral 22-gauge cannula was implanted over the NAc (10.5 mm AP, 3.7 mm H, 1.5 mm L). The cannula aimed at the NAc was counter-balanced across rats for placement in the left and right hemispheres.

Testing and Intracranial Infusions. Intracranial infusions of muscimol [40 ng in 1.0 µl] or vehicle (1.0 µl of 0.9% sterile saline) were delivered into the core of the NAc. All unilateral muscimol infusions into the NAc core were accompanied by a unilateral infusion of picrotoxin (0.5 µl: 100 ng) into the left or right hemisphere VP. In the vehicle condition 0.9% sterile saline (1.0 µl in the NAc and 0.5 µl in the VP) was infused into the NAc and bilaterally into the VP. The muscimol dose which was infused into the NAc core was selected based upon previous research. Specifically, work in the Neill lab (D. Romine, unpublished M.A thesis) showed that bilateral NAc infusions of 40 ng muscimol in 1 µl decreased behavioral response inhibition. This dose and volume were therefore selected. The selection of the 100 ng dose of picrotoxin in a 0.5 µl volume to be infused into the VP was based upon the results of experiment 1. The 0.5 µl volume was chosen to minimize the distribution radius of the drug once it was infused into the VP.

In experiment 2, vehicle was delivered to the NAc and the VP and the effects of the other 3 conditions were compared to this condition. In the bilateral condition, drug infusions of picrotoxin (100 ng) were delivered to the VP. These injections were included in experiment 2 to confirm that these animals behaved like those in experiment 1. A contralateral condition in which muscimol was unilaterally infused into the NAc core (40 ng) and picrotoxin (100 ng) was infused into the VP of the opposite hemisphere tested the necessity of an inhibitory NAc-VP path in behavioral response inhibition. The

fourth condition was an ipsilateral infusion of muscimol (40 ng) into the NAc and picrotoxin (100 ng) into the VP of the same hemisphere. No change in task efficiency was expected in the ipsilateral condition when compared to vehicle because the NAc-VP path remained intact in the opposite hemisphere. The order in which these 4 conditions were delivered was varied across rats.

Histology. Animals were euthanized with a lethal overdose of KAX and perfused intracardially with a solution of 0.9% saline followed by 10% formalin. Brains were stored in 10% formalin until sectioned using a microtome. Sections from the VP and NAc were mounted and stained using thionin. Mounted samples were then examined by an observer blind to the behavioral responses of each animal to determine the correct placement of cannulae. Animals for which it was discovered that the placement of cannulae was outside of the specified regions of interest were excluded from the data analyses.

Statistics and Sensitivity Analyses. A binary regression comparing the data of those animals missing one or more data points (n = 4) against the data of animals with no missing data points (n = 5) was carried out to identify the possible occurrence of systematic differences between the two groups which would require the exclusion of animals that did not complete all four of the testing conditions.

As in experiment 1, a PLA analysis using a nested model of condition within rat was used to analyze the total number of responses, reinforcements, and task efficiency. This was done because of the skewed nature of data so common to count and percentage data. These analyses were each carried out 3 times to assess the impact of animals which received only 1 of the four conditions (n = 1), 3 of the four conditions (n = 3), and all of

the four conditions (n = 5) on the results of the experiment. A post hoc analysis of planned comparisons using Bonferroni simple contrasts was performed on significant findings to determine if the contralateral, ipsilateral, and bilateral conditions significantly differed from vehicle. A direct comparison of the ipsilateral and contralateral conditions could not be performed within the PLA model because the independent contrasts for the PLA were designed to compare orthogonal data. Additionally the small number of subjects in this experiment made it unsuitable to assess the data using independent contrasts. Because the direct comparison between the ipsilateral and contralateral conditions was so important to understanding the results of experiment 2, an additional analysis of the 5 animals with complete data sets was carried out using a repeated measure Analysis of Variance (ANOVA) with a post hoc Newman-Keuls test of significant differences. This analysis directly compared the ipsilateral and contralateral conditions to detect significant differences.

Results

Analysis of Incomplete Data sets.

One animal from experiment 1 completed the vehicle and 25 ng picrotoxin conditions before being euthanized due to the development of an abscess on the lower right jaw. An additional 2 animals from experiment 1 completed 3 of the four conditions; both were missing the 25 ng picrotoxin condition. A binary logistic regression was done comparing the total number of responses, reinforcements, and task efficiency to animals with any missing data points and animals missing no data points. The results indicated no significant difference between the two groups in the total number of responses $\chi 2(10, N=40) = .126$, $p < .05$, reinforcements $\chi^2(10, N=40) = 3.363$, $p < .05$, and overall task

efficiency $\chi 2(10, N=40) = 3.037$, $p < .05$. Please refer to Appendix A for the binary table. While the binary regression suggested no significant difference between animals with complete data sets and those incomplete data sets, the results of experiment 1 were analyzed three times to include all animals N = 10), to exclude the 1 animal missing 2 data points (N = 9), and a third time to include only animals with completed data sets (N = 7) to be aware of the impact that the different groupings could potentially have on the results.

One animal from experiment 2 was euthanized after receiving a probably contaminated dose of picrotoxin. The only condition completed by this animal was the bilateral VP condition. Another 3 animals in this study were missing 1 of the 4 conditions. One was lacking the bilateral condition, one the ipsilateral condition, and the third was missing the contralateral condition. A binary regression was performed in experiment 2 to compare the data for animals with any missing data (n = 4) to animals missing no data points (n = 5). The results of the binary logistic regression suggest systematic differences in the total number of responses $\chi 2(9, N=36) = .6.303$, $p > .05$, reinforcements $\chi^2(9, N=36) = 7.681$, $p > .05$, and overall task efficiency $\chi 2(9, N=36) = 7.104$, $p > .05$. Please refer to appendix B for the Binary Table. For experiment 2 it may therefore be more appropriate to consider the statistical outcome of only those animals with complete data sets. However, analyses of the data for all 9 animal, 8 animals excluding the one missing 3 data points, and only the 5 animals with completed data sets are presented here.

Experiment 1

Experiment 1 tested the hypothesis that VP infusions of the GABAergic antagonist picrotoxin would decrease behavioral response inhibition in a dose dependent way as measured on a DRL 10 second schedule.

Histology

Tissue samples of the location of cannulae were analyzed by an observer blind to the behavioral responses of each animal. Analysis indicated that of the 16 animals tested in experiment 1, 10 had proper cannulae placement in the VP. Figure 3 demonstrates the locations of the VP infusions for this experiment in the 10 animals whose data was analyzed.

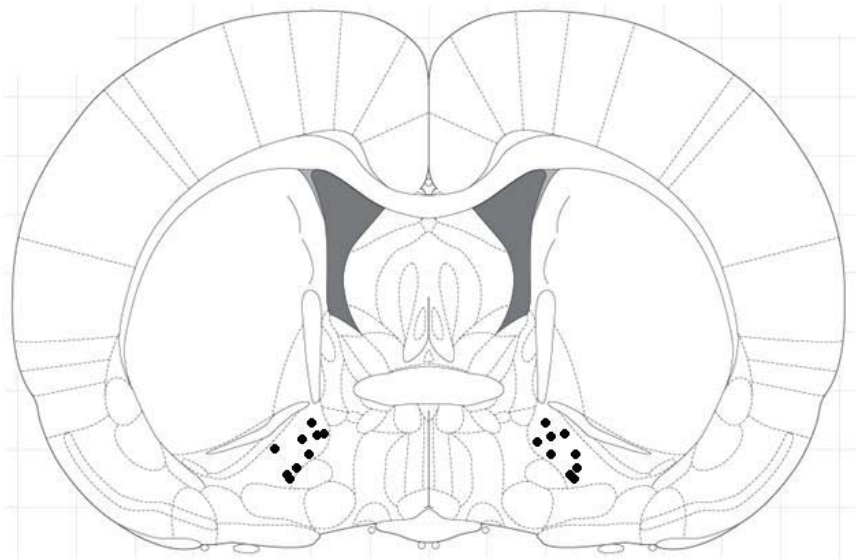


Figure 3. Bilateral guide cannulae were implanted above the VP. Circles in the VP denote the placement of the injector tip as determined during histology for those animals with placement within the VP for experiment 1.

Sensitivity analyses

The effects of the 3 doses of the GABAergic antagonist picrotoxin (25 ng, 50 ng, 100 ng) on the total number of responses, reinforcements, and task efficiency during 20

minute testing session on a DRL10 second schedule were analyzed. Three separate analyses were conducted for each of the three dependent measures (response, reinforcement, and efficiency) to test the impact of those animals which did not have complete data sets on the overall outcome of the study. In the first analysis 7 animals with complete data sets, 2 animals missing 1 data point, and 1 animal missing 2 data points were included (n = 10). In the second analysis the one animal which was perfused after receiving only a vehicle infusion and a 25 ng picrotoxin dose was excluded (n = 9). In the third analysis only those animals with complete data sets were included (n = 7). The three analyses for each of the three measures are reported below.

Responses

A PLA of all 10 animals data indicated a significant difference in the total number of responses made $\chi 2$ (10, N = 36) = 1296.046, $p < .05$. A Bonferroni simple contrast was used to compare the mean number of responses made at each of the three doses of picrotoxin to the vehicle condition. The mean number of responses for animals given the 25 ng dose [$\chi 2$ (10, N = 36) = 1729.173, $p < .05$], 50 ng dose [$\chi 2$ (10, N = 36) = 773.483, $p < .05$] and 100 ng dose [$\chi 2$ (10, N = 36) = 372.553, $p < .05$] were significantly increased compared to the mean number of responses made in the vehicle condition (figure 4a).

In the second analysis (n = 9) the one animal with only two data points was excluded. Results of the PLA revealed a significant difference in the number of responses made $\chi 2$ (9, N = 34) = 826.136, $p < .05$. A Bonferroni post hoc analysis indicated that the mean number of responses made when the animal was given a 25 ng bilateral infusion of picrotoxin did not significantly differ from vehicle [$\chi 2$ (9, N = 34) =

.489, $p = 1.000$]. The mean numbers of responses made for animals in the 50 ng dose [$\chi 2$ (9, N = 34) = 102.619, $p < .05$] and 100 ng dose [$\chi 2$ (9, N = 34) = 121.934, $p < .05$] conditions were significantly greater than the mean number of responses made during the vehicle condition (figure 4b).

In the third analysis which included only those animals with complete data sets (n = 7) a PLA identified a significant difference in the number of responses made across the four conditions $\chi 2$ (7, N = 28) = 713.524, $p < .05$. A Bonferroni simple contrasts comparison showed that the mean numbers of responses made in the 25 ng dose condition [$\chi 2$ (7, N = 28) = 4.045, p =.133] did not significantly differ from the mean number of responses made in the vehicle condition. The mean numbers of responses made in the 50 ng [$\chi 2$ (7, N = 28) = 86.355, $p < .05$] and 100 ng dose conditions [$\chi 2$ (7, N = 28) = 135.591, $p < .05$] were significantly greater than the numbers of responses in the vehicle condition (figure 4c).

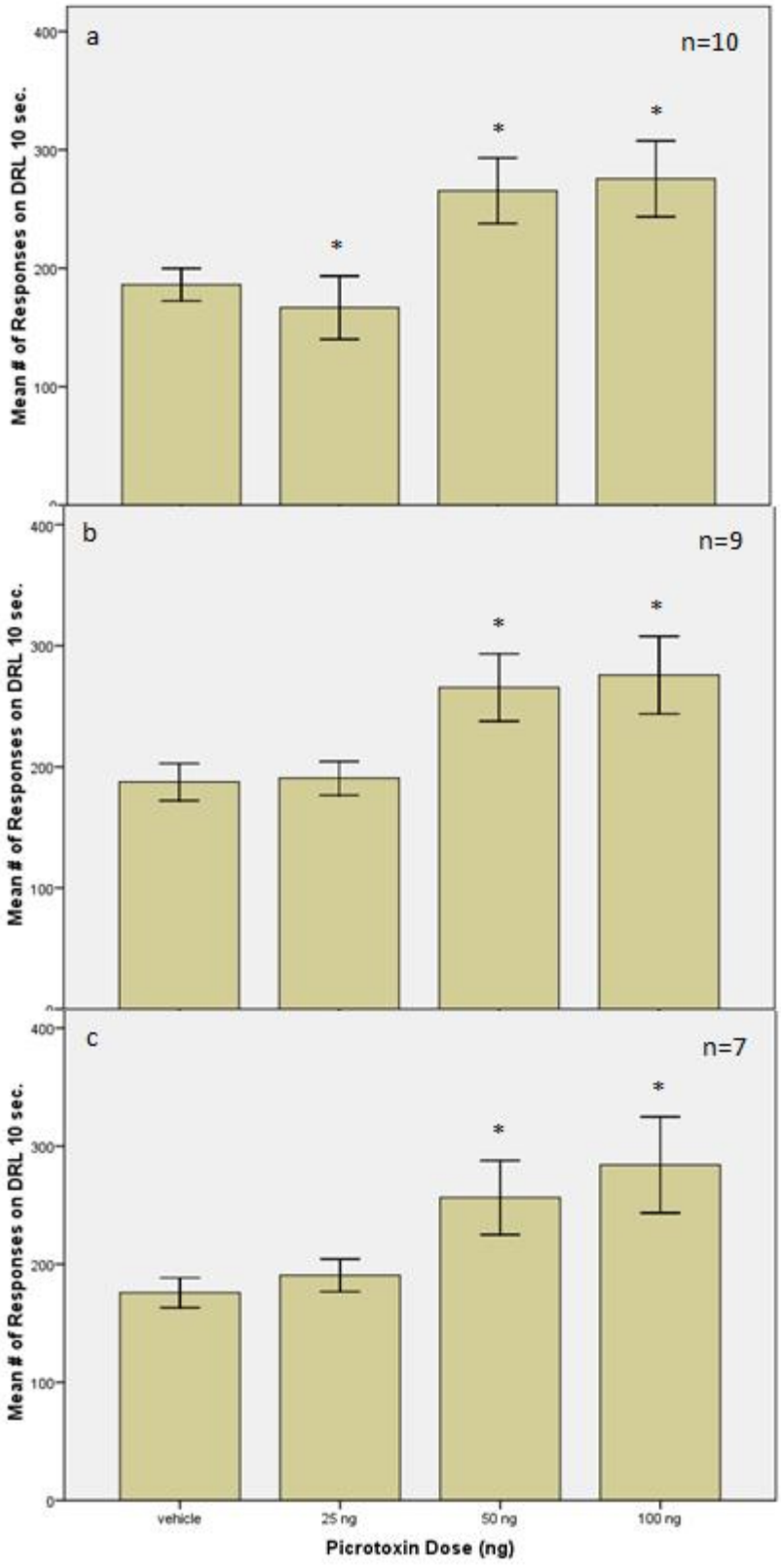


Figure 4. Mean response rate and SEM (+/- 1) on a DRL 10 second schedule for 3 doses of picrotoxin. Response rates significantly different from vehicle ($p. < .05$) are denoted by the * symbol.

Reinforcements

In the first analysis (n = 10), a PLA revealed a significant difference in the number of reinforcements obtained $\chi 2(10, N = 36) = 237.004$, $p < .05$ on the DRL 10 second schedule. A Bonferroni simple contrast tests indicated that the mean number of reinforcements obtained was significantly decreased in the 25 ng [$\chi 2(10, N = 36) = 505.125$, $p < .05$], 50 ng [$\chi 2(10, N = 36) = 33.339$, $p < .05$], and 100 ng [$\chi 2(10, N = 36) = 34.224$, $p < .05$] dose conditions when compared to vehicle (figure 5a).

When the animal that received only 2 of the 4 conditions was excluded from the data set a PLA found a significant difference amongst the four conditions $\chi 2(9, N = 34) = 147.704$, $p < .05$. Further post hoc Bonferroni analysis suggested that the mean number of reinforcements delivered to animals in the 25 ng dose condition [$\chi 2(9, N = 34) = 1.026$, $p = .933$] did not significantly differ from the mean number of reinforcements delivered in the vehicle condition. The mean number of reinforcements obtained in the 50 ng [$\chi 2(9, N = 34) = 36.600$, $p < .05$] and 100 ng dose conditions [$\chi 2(9, N = 34) = 37.497$, $p < .05$] was significantly reduced when compared to the mean number of reinforcements obtained in the vehicle condition (figure 5b).

When only the data of those animals which had complete data sets were analyzed, a significant difference in the number of reinforcements delivered was observed $\chi 2(7, N = 28) = 125.568$, $p < .05$. Post hoc analysis revealed that the mean number of responses made for animals in the 25 ng dose condition did not significantly differ from the mean number of responses made in the vehicle condition [$\chi 2(7, N = 28) = 2.598$, $p = .321$]. However a significant reduction in the mean number of reinforcements delivered in the 50 ng [$\chi 2(7, N = 28) = 29.100$, $p < .05$] and 100 ng dose [$\chi 2(7, N = 28) =$

39.165, $p < .05$] conditions was observed when each condition was compared to vehicle (figure 5c).

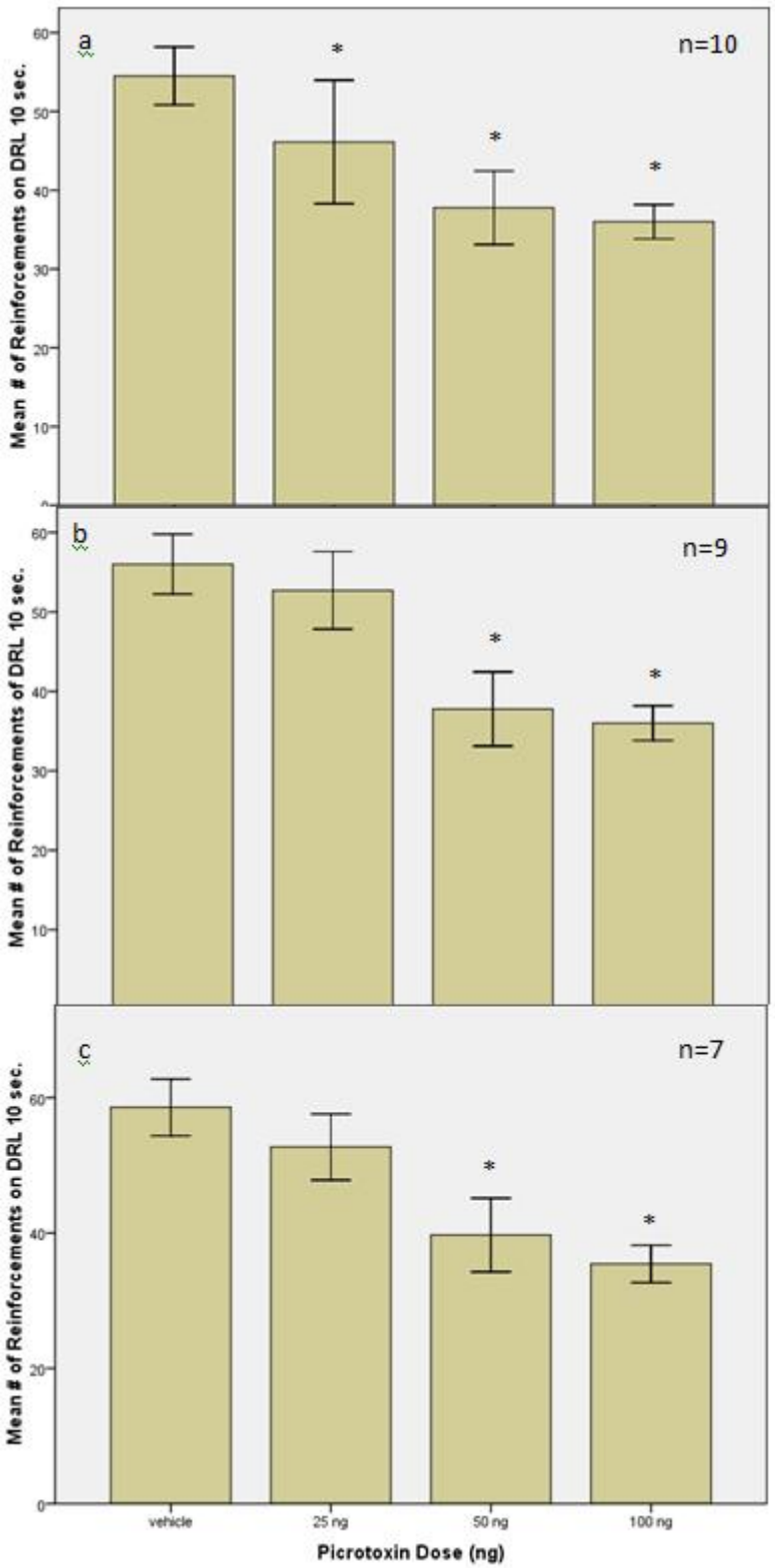


Figure 5. Mean number of reinforcements and SEM (+/- 1) on a DRL 10 second schedule for 3 doses of picrotoxin. Mean numbers of reinforcements which were significantly different from vehicle ($p. < .05$) are denoted by the * symbol.

Efficiency

Task efficiency was measured as a percentage and was defined by the division of the number of reinforcers delivered over the total number of responses, multiplied by 100. In the first PLA (n = 10) a significant difference was found in task efficiency $\chi 2(10, N = 35) = 205.057$, $p < .05$. Post hoc analysis of Bonferroni simple contrasts indicated that task efficiency was significantly reduced in the 25 ng [$\chi 2(10, N = 35) = 253.133$, $p < .05$], 50 ng [$\chi 2(10, N = 35) = 47.491$, $p < .05$], and 100 ng [$\chi 2(10, N = 35) = 55.406$, $p < .05$] dose conditions when compared to vehicle (figure 6a).

In the second analysis which excluded the data of the 1 animal (n = 9) the results of the Poisson log linear analysis were significant $\chi 2(9, N= 34) = 161.460$, $p < .05$. Post hoc analysis for simple contrasts indicated that the difference in task efficiency in the 25 ng dose condition was not significantly different from vehicle [$\chi 2(9, N= 34) = .938$, $p = .998$]. Task efficiency was significantly reduced in the 50 ng [$\chi 2(9, N= 34) = 47.491$, $p < .05$] and100 ng [$\chi 2(9, N= 34) = 55.406$, $p < .05$] dose conditions when compared to vehicle (figure 6b).

The Poisson analysis of only those animals with complete data sets (n=7) was significant $\chi 2(7, N = 28) = 137.280$, $p < .05$. Post hoc analysis for simple contrasts indicated that task efficiency in the 25 ng dose condition did not significantly differ from task efficiency in the vehicle condition [$\chi 2(7, N = 28) = 3.720$, $p = .161$]. Task efficiency was significantly decreased in the 50 ng [$\chi 2(7, N = 28) = 42.772$, $p < .05$] and 100 ng [$\chi 2(7, N = 28) = 60.760$, $p < .05$] dose conditions when compared to vehicle (figure 6c).

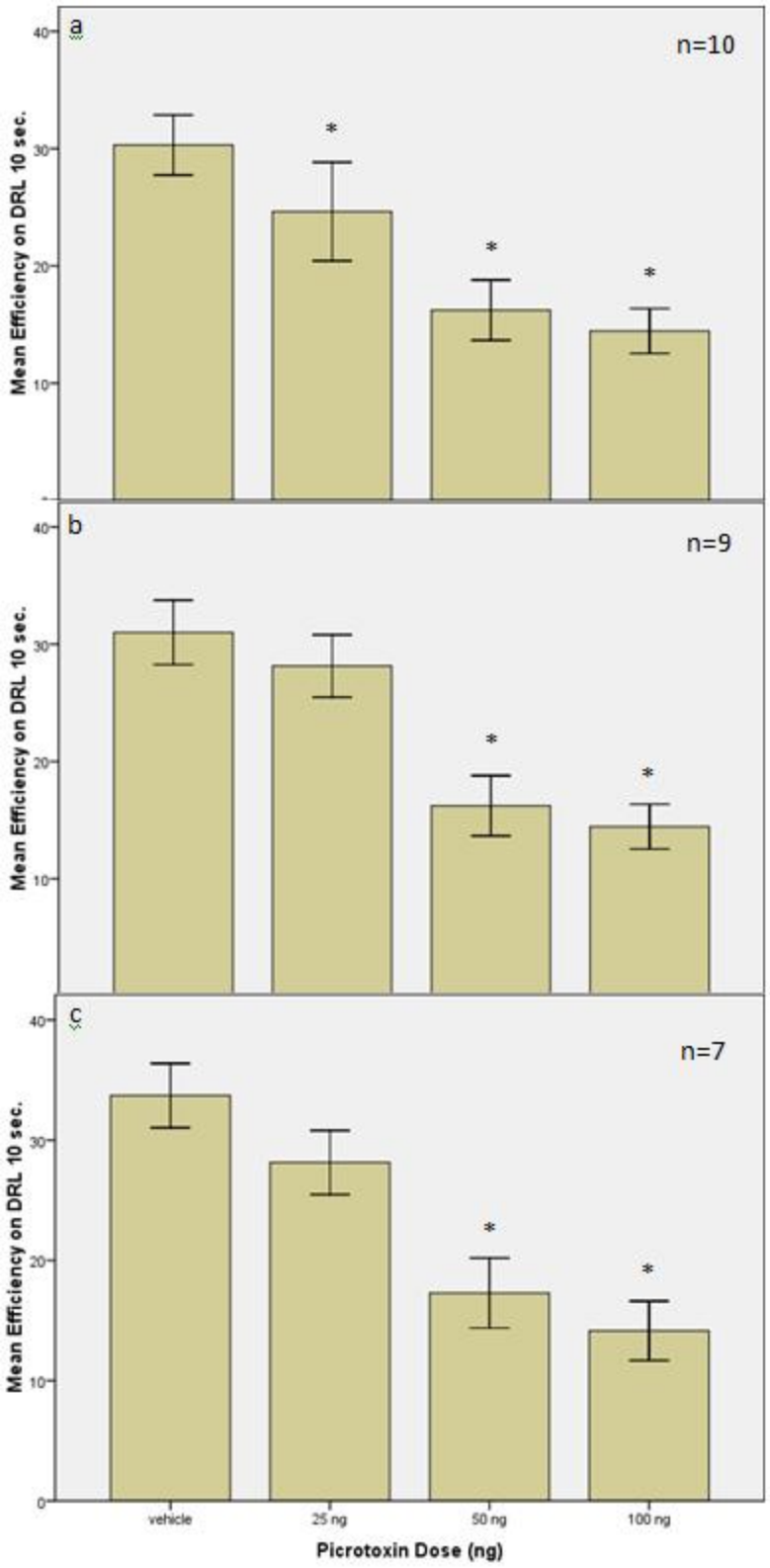


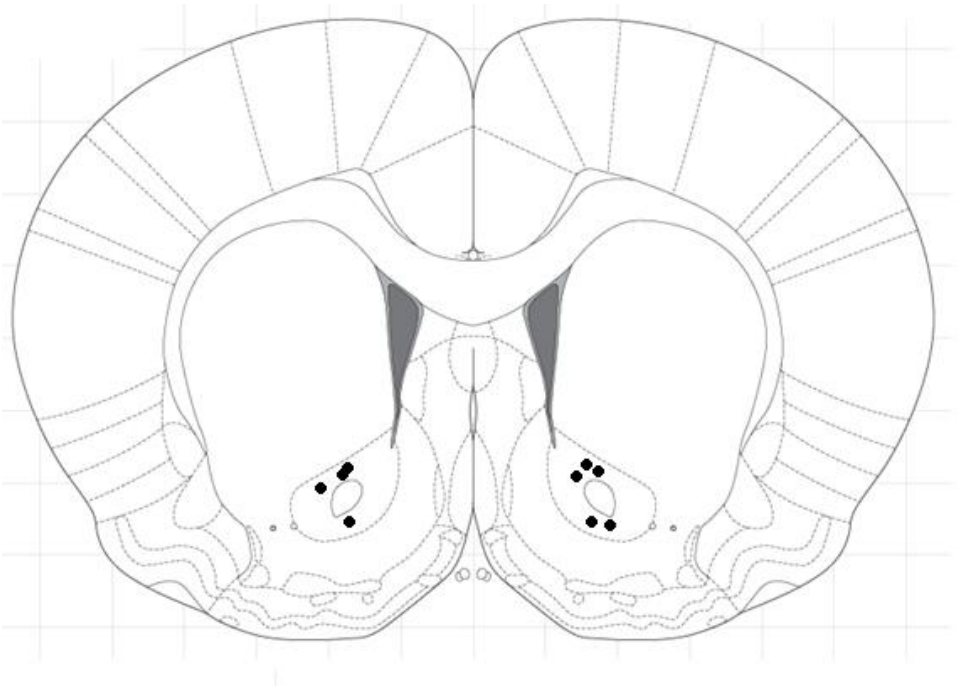
Figure 6. Mean task efficiency and SEM (+/- 1) on a DRL 10 second schedule for 3 doses of picrotoxin. Mean task efficiencies which were significantly different from vehicle (p. < .05) are denoted by the * symbol.

Experiment 2

In experiment 2, the hypothesis that the NAc-VP path may be involved in behavioral response inhibition as measured on a DRL 10 second schedule was tested. The GABAergic projection from the NAc to the VP was therefore inhibited in one of two ways. Pre-synaptic infusions of the GABAergic agonist muscimol (40 ng) in the NAc core which would hyperpolarize the MSNs preventing the post-synaptic release of GABA in the VP. Post-synaptically, the effects of GABA release in the VP were blocked via infusion of the GABAergic antagonist picrotoxin (100 ng). Using these two manipulations the necessity of the NAc-VP path was tested in two conditions. In the first condition called the Ipsilateral condition muscimol (NAc) and picrotoxin (VP) were infused unilaterally into the same hemisphere. In the second condition called the contralateral condition, contralateral infusions of muscimol into the NAc of one hemisphere and picrotoxin into the VP of the opposite hemisphere were delivered. These two conditions allowed for the direct analysis of the necessity of a functioning NAc-VP GABAergic path. Additionally, in the bilateral condition picrotoxin was infused into the VP of both hemispheres. In the vehicle condition saline was infused bilaterally into the VP and unilaterally into the NAc.

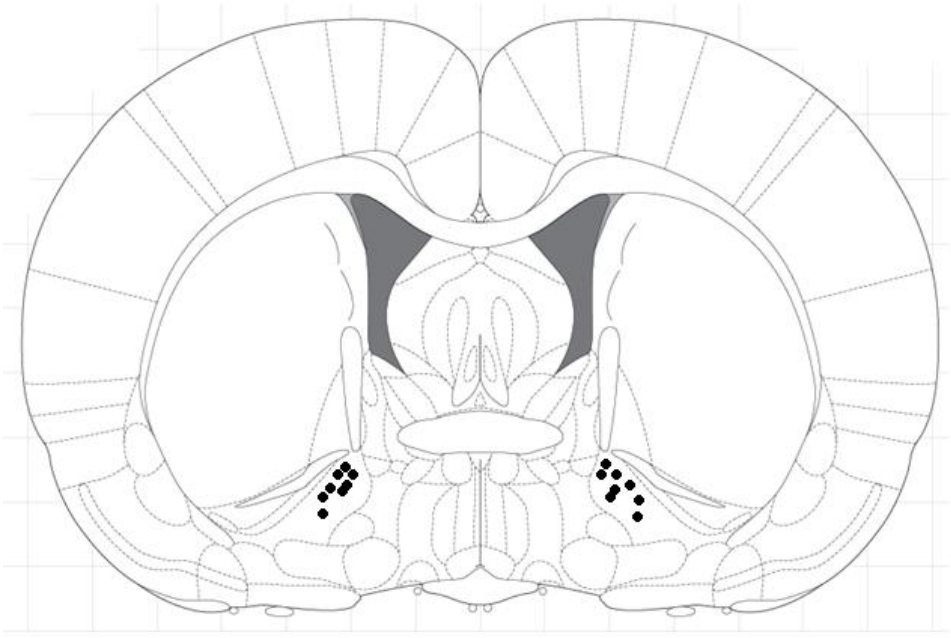
Histology

Tissue samples of the location of cannulae were analyzed by an observer blind to the behavioral responses of each animal. Analysis indicated that of the 13 animals in experiment 2, 9 animals had proper cannulae placement in the NAc and VP. Figure 7 demonstrates the relative location of the NAc and VP infusions for this experiment in the 9 animals included in the analyses.



AP

10.5 mm



AP 8.7 mm

Figure 7. Bilateral cannulae were implanted above the NAc and VP. The demarcations in the NAc denote placement of the injector tip. Cannulae in the NAc were unilateral and counterbalanced between the right and left hemispheres across rats (4 in the right hemisphere and 5 in the left hemisphere) NAc section is 10.5 mm AP. Marks in the VP denote the placement of the injector tip as determined during histology for those animals with placement determined to be within the VP (Section is 8.7 mm AP).

Sensitivity analyses

The effect of the four conditions: vehicle, contralateral, ipsilateral, and bilateral infusions on the total number of responses made, reinforcements delivered, and task efficiency during 20 minute testing sessions on a DRL10 second schedule were analyzed. As in experiment 1, 3 separate analyses were conducted for each of these measures (response, reinforcement, and efficiency) to assess the impact of those animals which did not have complete data sets on the overall outcome of the study. In the first analysis 5 animals with complete data sets, 3 animals missing 1 data point, and 1 animal missing 3 data points were included (n = 9). In the second analysis the one animal which was perfused due to illness after receiving only the bilateral VP 100 ng condition was excluded (n = 8). In the third analysis only those animals with complete data sets were included (n = 5) excluding the 3 animals missing each missing 1 condition (ipsilateral, contralateral, and bilateral respectively).

Responses

A PLA indicated a significant difference in the number of responses made amongst the four conditions of experiment 2 when the data from all animals (n = 9) were included $\chi 2(9, N = 30) = 125.851$, $p < .05$. Post hoc Bonferroni planned comparisons revealed no significant difference in the number of responses made in the contralateral [$\chi 2(9, N = 30) = 4.170$, $p = .123$] or ipsilateral conditions [$\chi 2(9, N = 30) = 0.391$, $p = 1.000$] when compared to the vehicle condition. The number of responses made in the bilateral condition [$\chi 2(9, N = 30) = 9.326$, $p = .007$] was significantly increased when compared to the vehicle condition (figure 8a).

When the one animal which received only the bilateral condition was excluded from the analysis (n = 8), the PLA was significant $\chi 2(8, N = 29) = 120.427$, $p < .05$. Post

hoc Bonferroni analyses suggested that responding was significantly increased in the bilateral condition when compared to vehicle [$\chi 2(8, N = 29) = 9.599$, $p < .05$]. Responding in the ipsilateral condition [$\chi 2(8, N = 29) = .391$, $p = 1.000$] and contralateral condition [$\chi 2(8, N = 29) = 4.170$, $p = .123$] did not significantly differ from the vehicle condition (figure 8b).

When only the data from animals that completed each of the four conditions (n = 5) were analyzed the results of the PLA were significant $\chi 2(5, N = 20) = 64.920$, $p < .05$. Post hoc Bonferroni tests revealed that responding in the contralateral [$\chi 2(5, N = 20) = 5.400$, $p = .060$] and ipsilateral [$\chi 2(5, N = 20) = 1.005$, $p = .949$] conditions did not significantly differ from vehicle. Responding in the bilateral condition however, was significantly increased [$\chi 2(5, N = 20) = 12.863$, $p < .05$] (figure 8c).

Additional Analysis

A repeated measures ANOVA showed no significant difference in the number of responses made in any of the 4 conditions, $F(3, 20)=2.64$, $p = 0.09$ when only those animals with complete data sets were analyzed.

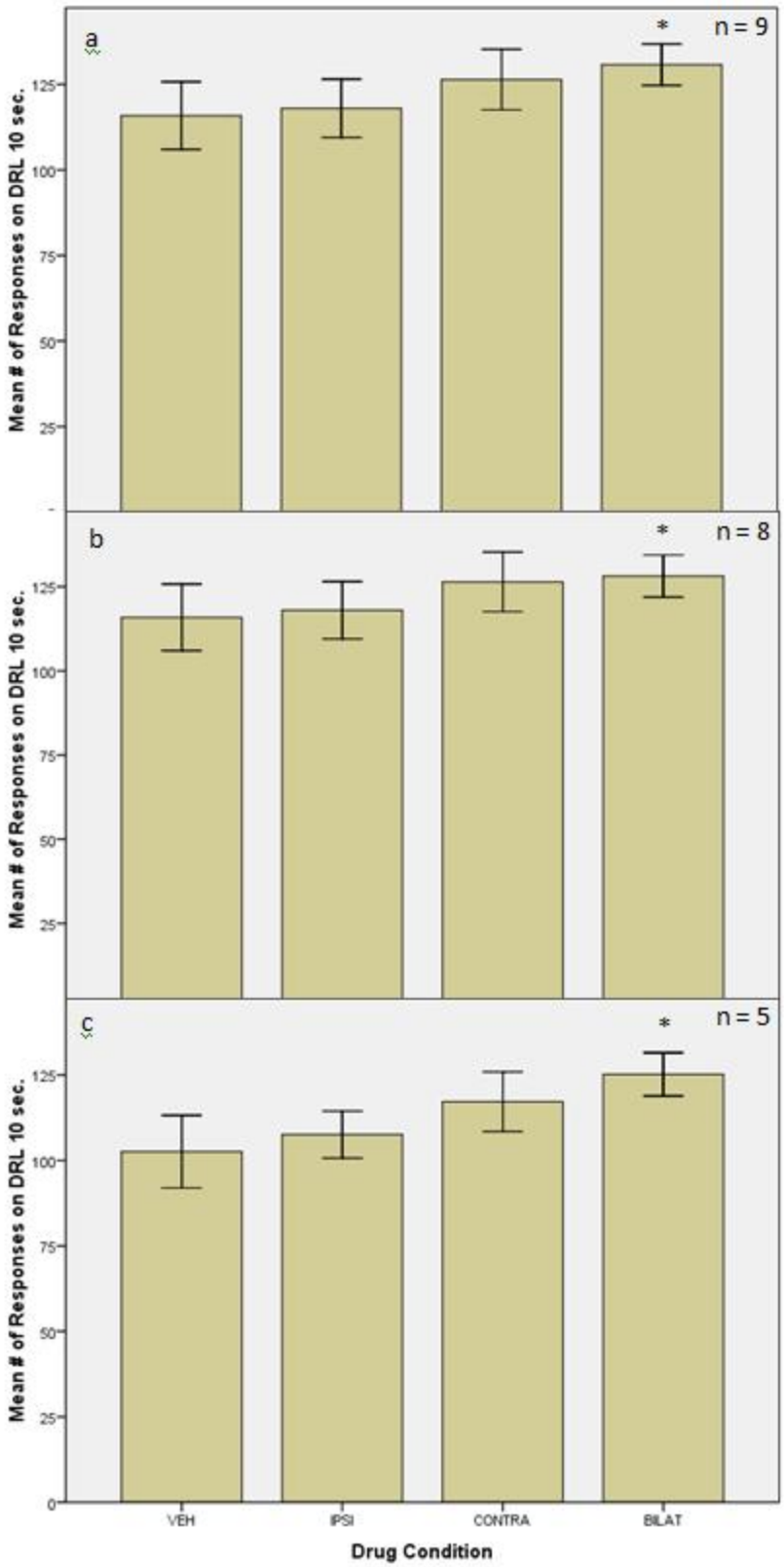


Figure 8. Mean response rate and SEM (+/- 1) on a DRL 10 second schedule for 4 test conditions. Response rate for conditions which were significantly different from vehicle ($p. < .05$) are denoted by the * symbol.

Reinforcements

A PLA looking at the total number of reinforcements delivered in the vehicle, ipsilateral, contralateral, and bilateral conditions for all 9 animals was significant $\chi 2(9, N = 30) = 217.297$, $p < .05$. Post hoc Bonferroni analyses of the results indicated a significant decrease in the number of reinforcements obtained in the contralateral [$\chi 2(9, N = 30) = 47.106$, $p < .05$] and bilateral conditions [$\chi 2(9, N = 30) = 9.599$, $p < 05$] when compared to vehicle. No significant difference in the number of reinforcements delivered in the ipsilateral condition was observed in comparison to the vehicle condition [$\chi 2(9, N = 30) = .002$, $p = 1.000$] (figure 9a).

When the one animal who only received the bilateral condition was removed from the data set, the results of a PLA were significant $\chi 2(8, N = 29) = 201.402$, $p < .05$. Post hoc analysis showed a significant decrease in the number of reinforcements obtained in the contralateral condition [$\chi 2(8, N = 29) = 47.106$, $p < .05$] when compared to vehicle. No significant difference in the number of reinforcements was found when the bilateral condition [$\chi 2(8, N = 29) = 3.858$, $p = .149$] or ipsilateral condition [$\chi 2(8, N = 29) = .002$, $p = 1.000$] were compared to vehicle (figure 9b).

When only the data from the 5 animals which completed all 4 of the conditions was analyzed with a PLA, the results were statistically significant $\chi 2(5, N = 20) = 43.821$, $p < .05$. Post hoc Bonferroni analysis of the planned comparisons revealed that the number of reinforcements delivered in the contralateral condition [$\chi 2(5, N = 20) = 5.474$, $p < .05$] were significantly reduced when compared to vehicle. No significant reduction in the number of reinforcements delivered was observed in the ipsilateral [$\chi 2(5, N = 20)$

= .791, $p = 1.000$] or bilateral [$\chi 2(5, N = 20) = 3.838$, $p = .150$] conditions when compared to vehicle (figure 9c).

Additional Analysis

A repeated measures ANOVA for animals with complete data sets showed a significant decrease $F(3, 20)=10.65$, $p < .01$ in reinforcements. Post hoc Newman-Keuls analysis indicated that the number of reinforcers obtained in the contralateral condition did not significantly differ from the number of reinforcers obtained in the bilateral condition. The number of reinforcers obtained in the bilateral and contralateral conditions was significantly less than the number of reinforcers obtained in the ipsilateral condition. Post-hoc analyses also revealed the number of reinforcers delivered in the ipsilateral condition did not significantly differ from the vehicle condition.

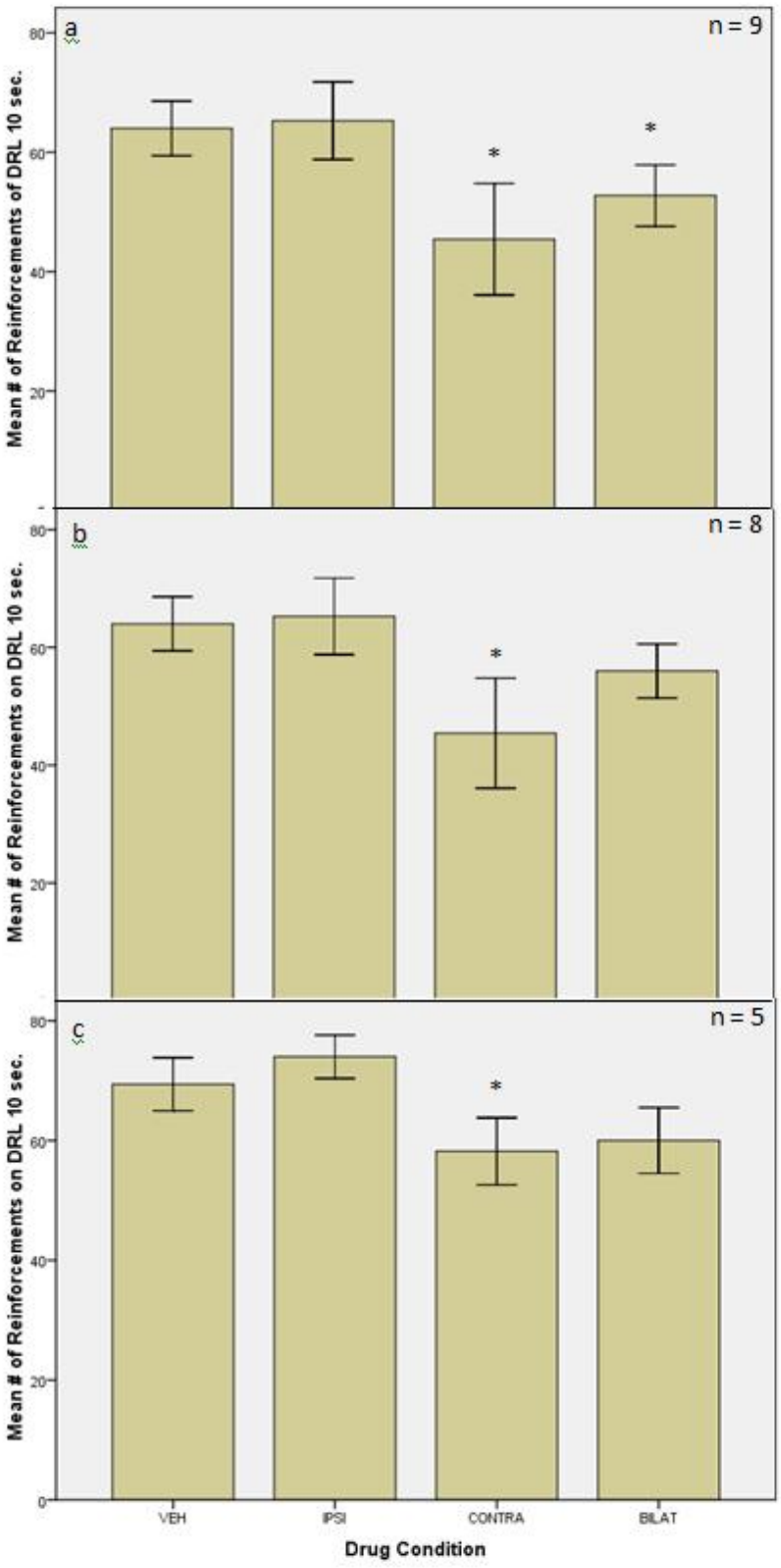


Figure 9. Mean # of reinforcements and SEM (+/- 1) on a DRL 10 second schedule for 4 conditions. Mean # of reinforcements which were significantly different from vehicle ($p. < .05$) are denoted by the * symbol.

Efficiency

A PLA of task efficiency for all 9 animals found a significant difference $\chi 2(9, N = 28) = 274.115$, $p < .05$. A post hoc Bonferroni analysis of planned comparisons suggested a significant decrease in task efficiency in the contralateral condition [$\chi 2(9, N = 28) = 40.012$, $p < .05$] and bilateral condition [$\chi 2(9, N = 28) = 20.748$, $p < .05$] when compared to vehicle. No significant difference was observed in task efficiency between the ipsilateral and vehicle conditions [$\chi 2(9, N = 28) = .083$, $p = 1.000$] (figure 10a).

When the data from the one animal which received only the bilateral condition were removed from the data set (n = 8) the results of a PLA remained significant $\chi 2(8, N = 27) = 249.266$, $p < .05$. Post hoc analysis revealed a significant decrease in task efficiency in the contralateral condition [$\chi 2(8, N = 27) = 40.012$, $p < .05$] and bilateral condition [$\chi 2(8, N = 27) = 10.882$, $p < .05$] when compared to vehicle. No significant difference between the ipsilateral and vehicle conditions was identified [$\chi 2(8, N = 27) = .083$, $p = 1.000$] (figure 10b).

When only those animals with complete data sets (n = 5) were included in the analysis the results of the PLA were significant $\chi 2(5, N = 20) = 86.216$, $p < .05$. Post hoc Bonferroni simple contrasts revealed that task efficiency was significantly reduced in the contralateral [$\chi 2(5, N = 20) = 17.239$, $p < .05$] and bilateral conditions [$\chi 2(5, N = 20) = 18.574$, $p < .05$] when compared to vehicle. No significant difference was detected in the level of task efficiency between the ipsilateral and vehicle conditions [$\chi 2(5, N = 20) = .020$, $p = 1.000$] (figure 10c).

Additional Analyses

Analysis with repeated measures ANOVA indicated a significant decrease in task efficiency for animals with complete data sets, $F(3, 20) = 9.24$, $p < .01$. Post hoc Newman-Keuls testing indicated that the contralateral condition and bilateral conditions did not significantly differ but that both of these conditions were significantly lower than the bilateral condition and vehicle conditions. Furthermore, the ipsilateral and vehicle conditions did not significantly differ from each other.

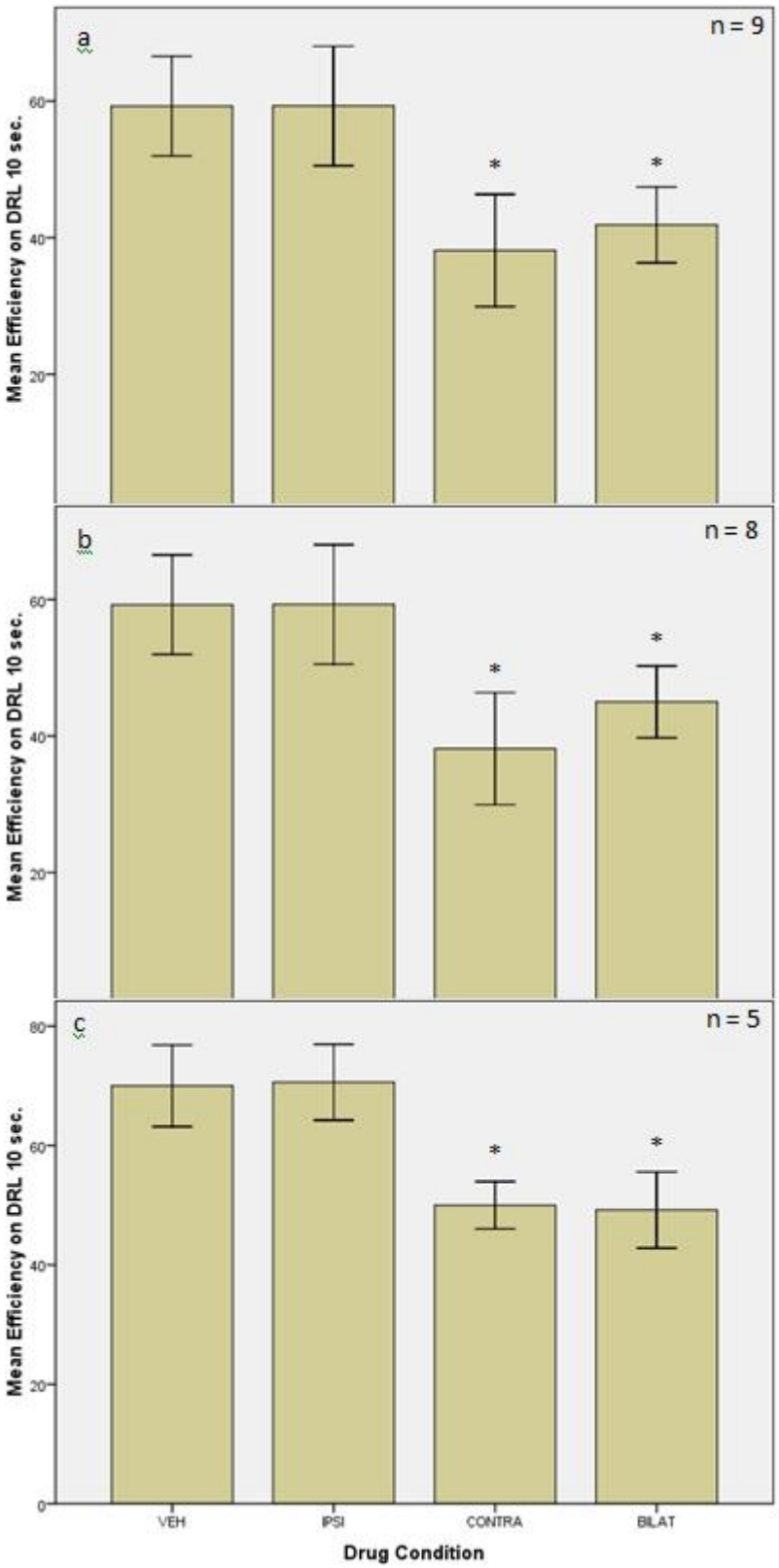


Figure 10. Mean task efficiency and SEM on a DRL 10 second schedule for 4 test conditions. Efficiency score for conditions which were significantly different from vehicle ($p. < .05$) are denoted by the * symbol.

Discussion

One animal in experiment 1 was sacrificed after completing only the vehicle and 25 ng dose of the dose response curve after an inspection of the animal revealed an abscess in the right side of the jaw. In accordance with IACUC regulations the animal was perfused to prevent unnecessary suffering. One animal in experiment 2 with only one condition completed became ill on the day immediately following a bilateral VP infusion of 100 ng of picrotoxin. It is our belief that the animal was unintentionally exposed to a bacterium which had contaminated the stock solution of picrotoxin. In agreement with IACUC procedures the animal was immediately perfused and the picrotoxin solution believed to be contaminated was destroyed. A new stock solution of picrotoxin was made to replace it and all PE-10 tubing used during drug infusion was replaced. Furthermore the syringes used with the Hamilton syringe pump to deliver the drug conditions were flushed with ethanol to ensure that any bacterium which may have been present in them was destroyed. The 3 analysis were conducted for each of the dependent variables in experiment 1 and experiment 2 in order to assess the impact of data from animals missing one or more data points upon the results.

Poisson Loglinear Analysis

While Analyses of Variance (ANOVA) are common in psychological research a disadvantage of these statistics is the increased risk of committing type II error and the significant impact of missing data on the reliability of the outcome of these statistics (Thompson, 1986). Linear analyses have the advantage of being less susceptible to type II error. This is because rather than analyzing all Pairwise comparisons only a specified number of planned comparisons determined by the degrees of freedom for that analysis

are carried out. For these reasons, the PLA was the main statistic used to analyze the data. The additional analysis in experiment 2 using the repeated measures ANOVA was only performed on the data from animals with complete data sets to avoid issues of missing data and because the result of the Binary regression suggest that there may have been systematic differences between the animals with complete data sets and incomplete data sets in experiment 2.

The data were not normally distributed. The skewed distribution of the data was expected given the fact that it was count and percent scores and skew is often found in this type of data. Because of the non-normal distribution a PLA with a nested model design was the most appropriate choice of linear analyses as it was designed to assess data with a non-normal distribution. It should be noted that the interpretation of a Poisson analysis when the sample size is small and there are missing data values can be difficult. Smaller effects may not be detected in these smaller and incomplete data sets; this is a limitation of the present analyses and perhaps explains the detection of significant differences by the repeated measure ANOVA in experiment 2 which were not detected by the PLA.

Experiment 1

A DRL 10 second schedule was used in experiment 1 to evaluate changes in behavioral response inhibition which were dependent upon bilateral VP infusions of picrotoxin. Changes in behavioral response inhibition were measured by changes in the total number of responses made, reinforcements delivered, and overall task efficiency. The results from experiment 1 support the hypothesis that bilateral infusions of picrotoxin would significantly reduce behavioral response inhibition in a dose dependent way. A

significant increase in the number of responses made was observed regardless of whether or not the animal missing two data points, or the animal missing one data point, were included in the analyses. The planned comparisons revealed that when the one animal missing the two data points was excluded from the analysis the 50 ng and 100 ng picrotoxin dose conditions resulted in an increase in the total number of responses emitted when compared to vehicle. The numbers of reinforcements obtained by animals in the 50 ng and 100 ng picrotoxin dose conditions were significantly reduced in all three analyses. Overall task efficiency in experiment 1 was reduced in a dose dependent manner. In all three of the sensitivity analyses task efficiency was significantly reduced in the 50 ng and 100 ng dose conditions. These results suggest that preventing GABA receptor coupling in the VP results in a decrease in the ability to withhold responding for reinforcement on a DRL 10 second schedule.

The highest concentration of picrotoxin given in experiment 1 was 100 ng. While the behavioral effects of both the 50 ng and 100 ng doses were significantly different from vehicle no post hoc planned comparison was performed to compare 50 ng and 100 ng doses due to the limitation on the total number of comparisons which could be made (3). It was decided that the 100 ng dose of picrotoxin would be used in experiment 2 and that a bilateral condition in experiment 2 would allow for the confirmation of the results obtained in experiment 1 for this dose.

Experiment 2

Previous work has implicated a role of the NAc in behavioral response inhibition. When coupled with these previous findings that the agonistic effects of muscimol in the NAc decrease behavioral response inhibition, it seems likely that the effect of muscimol,

consistent with its pharmacological action, is to inhibit the GABAergic MSN neurons which send a major inhibitory projection to the VP.

The hypothesis that the NAc's effect on behavioral response inhibition is via the VP was supported by the results of experiment 2. In experiment 2 the PLA of the effects of the bilateral, contralateral, ipsilateral, and vehicle infusions on responding, reinforcements, and task efficiency were significant regardless of whether or not the 1 animal with only the bilateral condition was included. The significant effects of all three measures remained when only the 5 animals with complete data sets were analyzed. A post hoc planned comparison compared the results of the three variables for the contralateral, ipsilateral, and bilateral conditions to the vehicle condition. For the total number of responses, neither the contralateral or ipsilateral conditions significantly differed from the vehicle condition nor did this result vary across the three sensitivity analyses. It is still important to note that the results of the five animals alone with complete data sets were the most conservative because the results of the binary regression suggest that systematic differences may have occurred between animals with complete data sets and animals without complete data sets.

A significant increase in the total number of responses made was observed when the bilateral condition was compared to the vehicle condition (please refer to figures 7, 8 & 9). This result was intriguing, particularly because the significant increase in responding was not detected when the data were analyzed using the repeated measures ANOVA. On the one hand, the increased responding detected by the PLA in the bilateral VP picrotoxin infusion supports the results of experiment 1. The non-significant difference in responding between the contralateral condition and vehicle condition is

therefore somewhat surprising. However the increase in the number of responses made when animals were in the contralateral conditions appears to be trending towards significance particularly in the analysis of only the 5 animals with complete data sets. It is therefore possible that with an increased sample size this result would be significantly different from vehicle.

The significant decreases in the number of reinforcements obtained and the overall task efficiency are the most intriguing results. In particular, the repeated measures ANOVA indicated that in the group of animals with complete data sets (n = 5) the decreases in reinforcements and task efficiency for the contralateral condition were significantly greater when comparing the results to ipsilateral condition. These results strongly support the hypothesis which motivated the study suggesting that a functioning NAc-VP GABAergic path is involved in behavioral response inhibition. Across all 3 of the sensitivity analyses using the PLA there was a significant decrease in task efficiency for the contralateral and bilateral conditions but not for the ipsilateral condition. These results strongly suggest the role of not only the NAc or VP but of the functional GABAergic NAc-VP pathway in behavioral response inhibition.

Interpretations

Torregrossa, Quinn and Taylor (2008) proposed that impulsivity is a multi-dimensional behavior characterized by three components. The three components of impulsivity are a) the inability to consider the consequences of action when deciding which action is to be taken, b) the inability to delay responding for a small immediately available reward to receive a greater reward later, and c) a decrease in the ability to withhold a behavioral response. The data collected in the two experiments presented in

this study suggest the involvement of the inhibitory NAc-VP GABAergic pathway in the last dimension of what is defined as impulsive behavior. It is possible that the NAc-VP GABAergic pathway is also involved in the other aspects of impulsivity. However, the two experiments described here were not designed to address these components and so the involvement of the NAc-VP path in these other aspects of impulsivity cannot be extrapolated from the present data set. Future studies should be designed to test the role of the NAc-VP GABAergic path in the ability to delay responding for a small reward in order to receive a greater one, and in the ability to evaluate action-outcome scenarios.

When the inhibitory action of GABA was blocked within the VP, an increase in responding was reliably observed in both experiments 1 and experiment 2. This increase in responding was accompanied by a significant decrease in the number of reinforcements which the animal was able to obtain and an overall decrease in task efficiency. Decreases in task efficiency were interpreted as a change in the animal's ability to withhold responding, specifically, the inability to withhold responding. When the actions of GABA were sufficiently blocked in the VP animals displayed behavioral response disinhibition. Behavioral disinhibition was observed even though the animal was punished by the absence of the reinforcer when responding was premature.

The decrease in task efficiency observed in experiment 2 in both the contralateral and bilateral, but not ipsilateral conditions when compared to the vehicle condition is compatible with the notion that a decrease in behavioral response inhibition occurs when the NAc-VP path is bilaterally disrupted. These results strongly support the idea that the NAc-VP inhibitory path is involved in response inhibition and that by preventing the

activity of the inhibitory pathway the ability to withhold responding is significantly reduced.

The MSNs of the medial core of the NAc send a significant projection to the lateral VP (Zahm & Brog, 1992). Other structures innervated by the medial core include the medial Substantia Nigra Pars Reticulata (SNPR), Substantia Nigra Pars Compacta (SNPC), and the lateral hypothalamus (LH) (Zahm & Brog, 1992; Usuda, Tanaka & Chiba, 1998). Given the location of the VP as a downstream structure in the PFC-striatal path, and the role of the VP in motor control, it is not surprising that the VP is also involved in behavioral response inhibition (Mogenson, Jones & Yim, 1980; Maurice, et al., 1997). The role of GABA in the VP and the NAc-VP pathway were therefore the focus of the two experiments presented here. It is possible that these other structures innervated by the NAc may also be involved in behavioral response inhibition yet this was not the focus of the current study and further experimentation involving these efferent's would have to be conducted to properly address this question.

While impulsivity is a multidimensional behavior the results of experiment 1 and 2 suggest that the NAc-VP path may be involved in at least one aspect of impulsivity. As has been previously mentioned the OFC is thought to be involved in the inhibition of behavioral responses. This disruption of normal functioning within the OFC can result in an increase in impulsive choices (Mobini et al, 2002). Since the NAc-VP path is a part of the downstream path from the OFC, the present results from experiments 1 and 2 fit well with the existing data on brain regions involved with impulsive-like behavior.

Impulsivity is also a core feature of drug addiction (Allen, Moeller, Rhoades & Cherek, 1998), personality disorders (Mulder, Joyce, P. R., Sullivan, P. F., Bulik, C. M.

& Carter, 1999), attention deficit hyperactivity disorder (ADHD) (Casey et al. 1997; Sagvolden, Johansenm Aase & Russel, 2005), and aggression (Myers-Lindenberg, et al., 2006). The impulsive features of drug addiction may be related to dysfunction of the frontostriatal pathway. This has been experimentally shown by disrupting the connection from the frontal cortex to the NAc in both rodents and primates resulting in a decrease in response inhibition (Jentsch & Taylor, 1999). In personality disorders and ADHD it is also thought that a the frontostriatal path is disrupted and that this disruption is the cause of the increase in impulsive-like behaviors (Mulder, Joyce, P. R., Sullivan, P. F., Bulik, C. M. & Carter, 1999; Sagvolden, Johansenm, Aase & Russel, 2005). An increase in the available serotonin in the BLA has also been linked to an increase in impulsive behaviors (Meyers-Lindenberg, et al. 2006).

Evidence that dysfunction of the OFC, frontostriatal path, and the BLA fit with the results of this study. The glutamatergic inputs from the OFC and BLA release glutamate into the NAc which binds to AMPA and NMDA receptors in the NAc core (McFarland, Lapish & Kalivas, 2003). The cells in the NAc are the GABAergic MSNs. In a properly functioning system therefore, the result of glutamatergic binding to AMPA and NMDA receptors would increase the likelihood of an excitatory post-synaptic potential (EPSP) of the MSN neurons. The excited MSNs would then release the inhibitory neurotransmitter GABA into the VP and in this way facilitate behavioral response inhibition. Conversely, preventing the inhibitory influence of the NAc-VP path either by inhibiting the MSNs in the NAc, blocking the effects of GABA in the VP, or some combination of both methods should result in a decrease in behavioral response inhibition. This is what was found in the current study.

An alternative hypothesis could be that inhibition of the GABAergic NAc-VP path might alter the value of the 45 mg food pellet reinforcer used in this study (Hanlon, Baldo, Sadeghian & Kelley, 2003; Inui, Shimura & Yamamoto, 2007). There is evidence to suggest that stimulation of GABA in the NAc in combination with other neurotransmitter systems may affect food intake by changing the motivational state to obtain it. However this increase in valuation of a food reinforcer was observed when muscimol was infused into the shell and not core region of the NAc (Hanlon, Baldo, Sadeghian & Kelley, 2003)

The NAc and VP are two regions involved in motor activity (Mogenson, Jones & Yim, 1980). It is therefore possible that the increase in responding observed in experiment 1 and experiment 2 was related to an increase in motor activity and was not related to the rats' desire to obtain a reinforcer. Fixed consecutive number (FCN) is a schedule of reinforcement in which a set number of responses must be performed on one lever before responding on a second lever will result in the delivery of a reinforcer (Evenden, 1998). The subject's motivation to respond in order to receive reinforcement is more readily apparent on an FCN schedule because responding for the reinforcer is distinct from the reset lever. Poor performance on a FCN schedule under the same drug conditions described in experiment 2 would provide additional support for the hypothesis that the poor performance observed on the DRL 10 second schedule when the NAc-VP GABAergic path is inhibited was the result of a failure to withhold responding and was not simply the result of hyperactivity. Future directions of the current study would therefore include and FCN schedule.

Conclusion

In conclusion impulsivity is a behavioral phenomenon comprised of several factors. One of these factors is the inability to withhold responding for reinforcement. Extensive evidence implicates a role of the OFC and BLA in impulsive-like behavior. These regions send glutamatergic projections to the NAc. Several disorders such as ADHD, drug abuse, personality disorders, and increased aggression are marked by the trait of impulsivity. All of these disorders are associated with dysfunctions in brain regions which send glutamatergic efferent projections to the NAc. It is therefore not surprising that a body of literature exists which suggests that the NAc is involved in behavioral response inhibition. Specifically, it may be the inhibition/disinhibition of the MSNs in the NAc core which modulate the ability to withhold a response. The two experiments presented here extend this research by presenting evidence supporting the hypothesis that the involvement of the NAc in behavioral response inhibition is at least in part through its effects on the VP. Based on these results, the NAc-VP path may play an important role in behavioral response inhibition and this function is deserving of future investigation.

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Appendix A

Binary Regression Table for Experiment 1

Measure	B	Standard Error	Wald Value	Significance Value
Responses	.002	.005	.126	.723
Reinforcements	.058	.032	3.363	.067
Efficiency	.085	.047	3.307	.069

The results of the Binary regression for the number of responses, reinforcements, and DRL task efficiency indicate no significant differences suggesting that no systematic differences between the group occurred therefore making the data comparable.

Appendix B

Binary Regression Table for Experiment 2

Measure	B	Standard Error	Wald Value	Significance Value
Responses	-.114	.045	6.303	.012
Reinforcements	.119	.043	7.681	.006
Efficiency	.130	.049	7.104	.008

Results of the Binary regression for the number of responses, reinforcements, and DRL task efficiency suggest a systematic difference between the two groups therefore, analysis of the 9 animals should be viewed with caution.