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Functional Interactions Between the Nucleus Tractus Solitarius (NTS) and Nucleus Accumbens Shell in Modulating Memory for Arousing Experiences

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Abstract

The shell division of the nucleus accumbens receives noradrenergic input from neurons in the nucleus of the solitary tract (NTS) that transmit information regarding fluctuations in peripheral hormonal and autonomic activity. Accumbens shell neurons also receive converging inputs from limbic areas such as the hippocampus and amygdala that process newly acquired information. However, few studies have explored whether peripheral information regarding changes in emotional arousal contributes to memory processing in the accumbens. The beneficial effects on memory produced by emotional arousal and the corresponding activation of NTS neurons may be mediated through influences on neuronal activity in the accumbens shell during memory encoding. To explore this putative relationship, Experiment 1 examined interactions between the NTS and the accumbens shell in modulating memory for responses acquired after footshock training in a water-motivated inhibitory avoidance task. Memory for the noxious shock was significantly improved by posttraining excitation of noradrenergic NTS neurons. The enhanced retention produced by activating NTS neurons was attenuated by suppressing neuronal activity in the accumbens shell with bupivacaine (0.25%/0.5 μ l). Experiment 2 examined the direct involvement of accumbens shell noradrenergic activation in the modulation of memory for psychologically arousing events such as a reduction in perceived reward value. Noradrenergic activation of the accumbens shell with phenylephrine (1.0 μ g/0.5 μ l) produced an enhancement in memory for the frustrating experience relative to control injections as evidenced by runway performance on an extended seven-day retention test. These findings demonstrate a functional relationship between NTS neurons and the accumbens shell in modulating memory following physiological arousal and identifies a role of norepinephrine in modulating synaptic activity in the accumbens shell to facilitate this process.

INTRODUCTION

Emotionally laden experiences have a unique characteristic of creating vivid representations of the contextual and episodic features of stimuli that collectively comprise new learning events. Neural representations created by exposure to emotional events are attributed to the physiological consequences these experiences produce on peripheral hormonal secretion and the corresponding release of specific neurotransmitters that activate limbic brain regions. Traditionally, these areas have included the amygdala and hippocampus, both of which have well established roles in encoding and processing memory for emotional events into long term

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storage (Cahill, et al., 1996; Canli, Zhao, Brewer, Gabrieli, & Cahill, 2000; Diamond, Park, Campbell, & Woodson, 2005; Joels, et al., 2004; McGaugh 2004; Phelps, 2004; Richter-Levin & Akirav, 2003; van Stegeren, Everaerd, Cahill, McGaugh, & Gooren, 1998; Vianna, Coitinho, & Izquierdo, 2004).

Recent findings suggest that the shell division of the nucleus accumbens may play an equally important role in integrating and consolidating representations of new experiences that are initially processed by both the amygdala and hippocampus (Reynolds & Berridge, 2002; Seamans & Phillips, 1994; Setlow, Roozendaal, & McGaugh, 2000). This view derives from anatomical findings demonstrating that the accumbens shell receives neural input from the basolateral nucleus of the amygdala concerning the affective components of experiences (Mogenson, Jones, & Yim, 1980; Petrovich, Risold, & Swanson, 1996), projections from the ventral subiculum region of the hippocampus regarding contextual features from the environment (Groenewegen, Vermeulen-Van der Zee, Kortschot, & Witter, 1987; Meredith, Wouterlood, & Pattiselanno, 1990) and reward related components of learning experiences from the ventral tegmental area (Nauta, Smith, Faull, & Domesick, 1978).

In addition to receiving vital limbic input from the amygdala and hippocampus, the shell region of the accumbens receives a dense supply of noradrenergic terminals exclusively from brainstem neurons in the A2 region of the nucleus of the solitary tract (NTS; Delfs, Zhu, Druhan, & Aston-Jones, 1998). Although the locus coeruleus (LC) and NTS both project to the accumbens (Brog, Salypongse, Deutch, & Zahm, 1993), only the projections from A2 neurons of the NTS release norepinephrine as a neurotransmitter in the accumbens shell (Delfs, et al., 1998). Presently, the phenotype of LC terminals innervating the accumbens has not been identified. The projection of norepinephrine containing neurons from the NTS to the shell may play an important modulatory function during memory formation in that these cells receive vital input from the periphery regarding heightened physiological states in response to emotionally arousing events (Dev & Philip, 1996; Gabriel & Seller, 1970; Paton, 1998a; Paton, 1998b). The A2 neurons within the caudal NTS then convey this information to limbic regions involved in the memory storage process (Castle, Comoli, & Loewy, 2005; Fallon, Koziell, & Moore, 1978; Petrov, Krukoff, & Jhamandas, 1993; Ricardo & Koh, 1978). For example, activation of the norepinephrine releasing A2 neurons by local infusion of glutamate (Miyashita & Williams, 2002) or noradrenergic agonists (Clayton & Williams, 2000; Miyashita & Williams, 2003) enhance memory in spatial, discrimination or emotionally arousing learning tasks and cause a significant increase in norepinephrine output within the amygdala. Moreover, the facilitation in memory produced by exciting NTS A2 neurons with glutamate is attenuated by blocking noradrenergic receptors in the basolateral amygdala (Miyashita & Williams, 2002). In that NTS noradrenergic neurons convey crucial information regarding peripheral arousal to limbic structures, it is possible that the beneficial effects on memory produced by NTS activation may be mediated not only by activating noradrenergic receptors in the amygdala, but by similar influences within the accumbens shell.

Currently, no behavioral studies have examined whether the mnemonic consequences of increasing neural output in the NTS in response to peripheral arousal induced by traditional training stimuli such as footshock or in response to heightened emotional states such as frustration, may be mediated by interactions between the NTS and accumbens shell. To address this shortcoming, Experiment 1 examined interactions between the NTS and the accumbens shell in modulating memory for responses acquired during footshock training in a modified inhibitory avoidance task. The functional interaction between the accumbens shell and NTS in encoding and storing the emotional footshock experience was assessed on a retention test given 24 hr post-training. Experiment 2 examined the consequences of direct activation of noradrenergic receptors within the accumbens shell in modulating memory for an arousing event produced by presenting large reductions in the magnitude of the expected quantity of

reinforcements. The reduction in perceived reward value creates a psychological state of frustration and arousal that is manifested by increased secretion of peripheral hormones and decreased levels of motivation and behavioral responding (Amsel, 1962; Mitchell & Flaherty, 1998; Salinas & Gold, 2005; Salinas & McGaugh, 1996). The mnemonic effects of activating noradrenergic receptors within the accumbens after emotional frustration induced by reward reduction was assessed by measuring the latency to run the length of the alley and consume the new quantity of one reward pellet on an extended long-term retention test given seven days later.

The present studies were developed to examine whether norepinephrine release in the accumbens shell, produced indirectly by activating NTS neurons or directly by infusion of the noradrenergic agonist phenylephrine, modulates memory for emotionally arousing learning experiences. The learning tasks employed in Experiments 1 and 2 were selected because they each contain appetitive/reward and aversive components. Instrumental responses are emitted to obtain water rewards in the inhibitory avoidance task (Exp. 1) or food in the straight alley (Exp. 2). The most significant factor to distinguish the two learning conditions is the nature of the manipulations used to produce “learning” and corresponding responses of avoidance to either the food or water. In Exp. 1, a physical footshock was employed to produce avoidance responses to the reward whereas similar avoidance behavior to the food in Exp. 2 was produced by simply violating the subject’s expectation regarding the quantity of expected rewards. Despite the use of two separate experimental manipulations, the outcome of both situations resulted in the acquisition of a new response of avoidance to stimuli that previously generated strong approach behavior. Based upon these findings, it is somewhat clear that learning induced by application of physical noxious stimuli or psychologically stressful conditions can be modulated into memory more effectively by increasing neuronal activity in either the NTS (Exp. 1), the accumbens shell (Exp. 2) or between both the NTS and accumbens shell after learning.

METHODS

Subjects

Sixty-two male Sprague-Dawley rats (275-300 g) obtained from Charles River Laboratories (Wilmington, MA) were used in Experiment 1 ($n = 36$) and Experiment 2 ($n = 26$). The rats were individually housed in polypropylene cages with corncob bedding and maintained on a standard 12:12 hour light-dark cycle with lights on at 7:00 A.M. Food and water were available ad libitum during the seven day adaptation period to the vivarium. In Experiment 1, seven rats were excluded from the study due to incorrect cannula placement ($n=5$) or outlying scores beyond two standard deviations away from the group mean ($n=2$). Only one animal was excluded from Experiment 2 due to incorrect cannula placement. After these corrections, data from 29 animals were analyzed for Experiment 1 and 25 animals for Experiment 2.

Surgery

Each rat received an injection of atropine sulfate (0.1 mg/kg i.p., American Pharmaceutical Partners, Inc., Schaumburg, IL) and was then anesthetized with sodium pentobarbital (50 mg/kg, i.p., Abbot Laboratories, North Chicago, IL). For the subjects in Experiment 1, a midline scalp incision was made and 15 mm long extra thin wall stainless steel guide cannulae (25 gauge, Small Parts, Miami Lakes, FL) were implanted bilaterally 2 mm above both the NTS (AP -13.3, ML $\pm$ 1.0 from bregma, DV -5.6 from skull surface) and shell region of the nucleus accumbens (AP +0.7, ML $\pm$ 1.0 from bregma, DV -5.4 from skull surface). In Experiment 2, identical guide cannulae were implanted bilaterally above the nucleus accumbens shell. All coordinates were adapted from the atlas of Paxinos and Watson (1986). The guide cannulae and two skull screws for anchoring were affixed to the skull with dental cement and the scalp

was closed with sutures. Stylets (15 mm, 00 insect dissection pins) were then inserted into the injection cannulae to prevent occlusion. Penicillin (0.1 ml i.m., Fort Dodge Animal Health, Fort Dodge, IA) was administered immediately after surgery along with the analgesic, buprenex (0.05 ml s.c., Hospira, Inc., Lake Forrest, IL). The rats remained in a temperature-controlled chamber for at least one-hour following surgery and were given seven days to recover before initiating food or water deprivation procedures and behavioral training.

EXPERIMENT 1

Water-Motivated Inhibitory Avoidance Task

Apparatus—A trough-shaped, two compartment rectangular apparatus (91 cm long, 21 cm wide at the top and 6.4 cm wide at the bottom) with a hinged lid was used to train the rats in a water-motivated inhibitory avoidance task. A sliding metal door (14.5 cm) separated a neutral and dark compartment. The neutral compartment was constructed of white opaque Plexiglas (31 cm long) and brightly illuminated by a 60 watt light located directly above the compartment. The dark compartment was constructed of stainless steel plates (60 cm long). A curved stainless steel water spout connected to a 30-cc plastic syringe containing water was placed 1 cm above the floor at the end of the dark compartment.

Training—One-week after surgery, rats were placed on a water restriction schedule with access to water for twenty minutes a day in addition to water consumed during behavioral training. Body weights were monitored daily to insure that weights did not deviate below 10% of their ad-lib feeding weights throughout the experiment. Animals were habituated by placing each in the inhibitory avoidance apparatus for 300 sec. During this time, they were given the opportunity to cross between the white and dark compartment (shown in Figure 1a and 1b) and to explore the drinking spout.

For the next six days of training each rat was placed in the dark compartment facing the retractable door that separated the dark from the illuminated compartment. The metal door was lowered to 2/3 of its length (i.e. creating a 4 cm hurdle). A timer was started and the following measures were recorded until the completion of the trial: a) latency to begin drinking, b) total amount of time spent drinking, c) total amount of time spent in the dark compartment and d) total amount of time spent in the white illuminated compartment. Each training day consisted of one trial lasting 120 seconds.

On Day 7 (i.e. experimental day), each rat was placed in the dark compartment as before however, a 0.35 mA electrical footshock was administered once the rat initiated a lick toward the water spout. The shock remained on until the animal escaped from the dark compartment by crossing over the 4 cm high hurdle into the illuminated neutral compartment. Each animal was retained in the neutral compartment for 30 sec with the door two-thirds open, the door was then raised and the animals remained in the neutral compartment for an additional 30 sec. Hence, the animals were allowed 60 sec to learn that the white illuminated compartment was safe relative to the dark compartment where footshock was just experienced. Each animal was then removed from the apparatus and given a microinfusion of either phosphate buffered saline (PBS) or bupivacaine (0.25% per 0.5 μ l) into the nucleus accumbens shell. The accumbens injections were then followed by bilateral infusions of either PBS or glutamate (50ng/0.5 μ l) into the NTS. The concentration of glutamate used in this study was selected from a range of doses previously shown to significantly improve retention under these behavioral conditions when infused into the NTS posttraining (Miyashita & Williams, 2002).

Microinjection Procedure—Each rat was restrained by hand in the experimenter's lap, the stylets were removed and 17 mm, 30 gauge injection needles were inserted bilaterally into the accumbens guide cannulae followed approximately two minutes later by bilateral injections

into the NTS. The tip of the injection needles extended 2 mm beyond the base of the guide cannulae. The needles were connected to 10 μ l Hamilton syringes by PE-20 (polyethylene) tubing. An automated syringe pump (Sage-Orion, Boston MA) delivered 0.5 μ l PBS, glutamate (50ng; Sigma Aldrich, St. Louis, MO) or bupivacaine (0.25%; Hospira, Inc., Lake Forrest, IL) over 60 sec. Rats were randomly assigned to one of four groups that received PBS into both the accumbens shell and NTS (PBS/PBS: $n = 7$), PBS into the accumbens and glutamate into the NTS (PBS/glutamate: $n = 8$), bupivacaine in the accumbens and PBS in the NTS (bupivacaine/PBS: $n = 8$), or microinjections of bupivacaine in the accumbens and glutamate in the NTS (bupivacaine/glutamate: $n = 6$). The injection needles were left in place for an additional 60 sec following infusions to ensure complete delivery of the drugs and the stylets were then reinserted into the cannulae.

Retention Test—Retention of the footshock experience in the dark compartment was assessed 24 hr later and consisted of two phases. During Phase 1, the rats were placed in the dark compartment facing the partially lowered metal door and given 60 sec to enter the neutral compartment or alternatively, to initiate the first lick from the water spout. If the rat entered the neutral compartment, the metal door was raised and the rat remained in the neutral compartment for 30 sec. Phase 2 of retention began after this 30 sec period. Those that did not enter the dark compartment after 60 sec were removed and placed in the neutral compartment with the metal door raised for 30 sec. During Phase 2, the metal door was lowered and the time spent avoiding the dark compartment, latency to drink from the spout, total time spent drinking and the total amount of time spent in the neutral compartment was recorded over a period of 300 sec. Figures 1a and 1b depict the separate measures recorded for Phase 1 and Phase 2 of the retention test, respectively.

Statistical Analysis—The behavioral measures from the water-motivated inhibitory avoidance task are expressed as mean $\pm$ standard errors (SE). Between-group comparisons for the behaviors measured during retention testing were made with a two-way analysis of variance (ANOVA; NTS $\times$ nucleus accumbens shell injections) followed by post hoc tests. Comparisons for the total amount of time spent in the neutral compartment on the last two days of training before footshock (Day 5 and Day 6), on the shock day (Day 7) and on the retention test (Day 8) were made with a two-way repeated measures ANOVA followed by a post-hoc test. Outlying scores (i.e., those scores that were two standard deviations away from the mean of a group) were excluded from statistical analysis. No more than one score was excluded from each experimental group (1 PBS/PBS and 1 PBS/glutamate).

Histology—Rats were deeply anesthetized with a euthanasia solution and perfused intracardially with 0.9% saline followed by 10% formalin to verify microinjection cannulae placement. The brains were stored in a 10% formalin and 12% sucrose solution until sectioned on a vibratome. Sections were cut 60 μ m thick, mounted on glass slides, subbed with chromium-aluminum and stained with cresyl violet. The location of the cannulae and injection needle tips were verified by examining enlarged projections of the slides. In Experiment 1, 5 animals were excluded from statistical analysis due to incorrect placement of at least one of the four bilaterally implanted cannulae (1 PBS/PBS, 2 PBS/glutamate, 1 bupivacaine/PBS and 1 bupivacaine/glutamate). The location of injection needle tips for both the accumbens shell and NTS in Experiment 1 are displayed in Figures 2a and 2b.

RESULTS FOR EXPERIMENT 1

The first study determined whether the enhancement in memory produced by activating NTS neurons after learning is mediated in part by influences on the shell division of the nucleus accumbens. We examined this hypothesis by suppressing neuronal activity in the accumbens approximately two minutes before activating NTS neurons with the excitatory amino acid

glutamate (50ng/0.5 μ l). The training procedures employed in this study may initiate the development of representations in memory for both the environment where the surprising footshock was experienced and the specific response the subject emitted before this shock was delivered (i.e. a licking response). Given this possibility, several measures were taken to assess each type of memory. Two-way factorial ANOVA's verified that there were no statistical differences between treatment groups on the measures recorded prior to the experimental treatment or on the day of footshock delivery.

PHASE 1 OF RETENTION TESTING

Latency to Emit an Active Avoidance Response or Drink from the Spout—During Phase 1 of the retention test each animal was placed in the dark compartment facing the lowered metal door. As depicted in Figure 1a & b, the latency to lick the water spout (measured from the very beginning of the retention test until the end of the 60 sec test) or alternatively to enter the safe/neutral compartment was measured. A two-way ANOVA on the mean seconds to exit the dark and enter the neutral-illuminated compartment revealed no significant interaction between the treatment groups, $F(1, 25) = 1.66, p = \text{ns}$ (PBS/PBS 39.8 ± 8.9 , PBS/glutamate 42.9 ± 7.5 , bupivacaine/PBS 58.2 ± 1.2 , bupivacaine/glutamate 41.4 ± 11.8). However, during this initial phase of retention testing an overall ANOVA indicated a significant main effect of NTS and accumbens injections for the difference in the latency to drink from the spout, $F(1, 25) = 7.05, p < 0.05$ and $F(1, 25) = 36.47, p < 0.01$ (NTS and accumbens respectively). As shown in Figure 3a the latency to lick from the spout where footshock was delivered 24 hr earlier was significantly shorter in animals given bupivacaine (0.25%) in the accumbens shell and PBS infusions into the NTS. Although there were main effects in the latency to first approach and lick from the drinking spout, Figure 3b demonstrates that there were no significant main effects for the total amount of time any treatment group spent drinking, $F(1, 25) = 1.70, p = \text{ns}$ and $F(1, 25) = 0.89, p = \text{ns}$; NTS and accumbens respectively). This time was brief and did not vary statistically between the groups. This second analysis indicates that although the initial latency to drink from the spout was shorter in animals injected with bupivacaine in the accumbens shell, animals in these two groups did not differ from any of the remaining treatment groups in the total amount of time spent drinking from the spout during the first phase of retention testing.

PHASE 2 OF RETENTION TESTING

Contextual Memory: Time Spent Avoiding the Shock / Dark Compartment—Each animal began Phase 2 of the retention test in the neutral compartment as shown in Figure 1b. After 30 sec, the metal door separating the neutral-illuminated and shock (dark) compartment was lowered and the latency to enter the dark compartment was recorded. If memory of the context where footshock was experienced 24 hrs earlier was retained, then latencies to exit the neutral area and reenter the section of the maze where the footshock occurred on Day 7 of training should be extended. A two-way ANOVA indicated a significant interaction between NTS and accumbens treatments for the latency to enter the shock compartment, $F(1, 25) = 15.59, p < 0.01$. As shown in Figure 4, animals given PBS into the accumbens shell followed by microinjections of glutamate within the NTS spent significantly more time in the neutral compartment before entering the context in which footshock was administered 24 hours previously. Blocking neuronal activity in the accumbens with bupivacaine attenuated the memory enhancing actions of exciting NTS neurons with glutamate.

A two-way ANOVA also indicated significant interactions in the percentage of animals within each group to enter the dark compartment, $F(1, 25) = 4.33, p < 0.05$. Post-hoc tests revealed that significantly fewer animals in the group given PBS into the accumbens and glutamate (50ng/0.5 μ l) into the NTS entered the dark compartment relative to all other treatment groups ($p < 0.05$; see Figure 5). This high level of responding to avoid entry into the dark compartment

was not observed in the group given an identical dose of glutamate after the accumbens was functionally inactivated by bupivacaine.

During Phase 2 of the retention test, the door separating the neutral and shock compartment remained open. Consequently, animals could freely move between both compartments. As such, time spent in the illuminated compartment provides an indirect measurement of the total time animals spent avoiding the shock compartment. A repeated measures ANOVA revealed a significant Day $\times$ NTS $\times$ Accumbens interaction between the groups on this measure, $F(3, 75) = 10.08$, $p < 0.01$. As depicted in Figure 6, all subjects spent the majority of time in the dark compartment containing the water-spout during the last two days of training (D5 and D6). However, after footshock training on Day 7 (Shock), animals given PBS in the accumbens and glutamate in the NTS spent significantly more time in the neutral compartment compared to all other treatment groups. More importantly, the extended period of time displayed by the PBS/glutamate group in the neutral compartment was not observed in animals given an identical dose of glutamate into the NTS after the accumbens was functionally inactivated by bupivacaine injection (bupivacaine/glutamate).

Response Specific Memory: Latency to First Lick the Spout—Given the possibility of both contextual and response based memory formation in this task, two additional measures were recorded to assess response-specific memory. If the last behavior emitted before initiation of the arousing footshock (approaching the spout to drink) was retained in memory, then subjects should take significantly longer to initiate contact with the spout and spend less time drinking from the spout. To address this view, the time required to initially drink from the spout was recorded from the start of the 300 sec period allotted for Phase 2 of retention testing. A two-way ANOVA revealed a significant interaction between NTS and accumbens injections, $F(1, 25) = 16.84$, $p < 0.01$ (see Figure 7). The group given PBS/glutamate required a significantly longer period of time to initiate drinking from the spout relative to PBS/PBS controls ($p < 0.01$) or animals given bupivacaine in the accumbens and PBS in the NTS ($p < 0.01$). However, this effect was blocked by inactivating the accumbens shell. Unlike the PBS/glutamate animals, subjects given bupivacaine into the shell before intra-NTS infusion of glutamate (50 ng/ μ l; bupivacaine/glutamate) exhibited significantly shorter latencies to drink from the water spout ($p < .01$) during Phase 2 of retention testing.

The amount of time each animal spent drinking after initial contact was made with the water spout served as an additional measure of retention for the response emitted immediately before the delivery of footshock. As shown in Figure 8, a two-way repeated measures ANOVA revealed significant differences in the mean time spent drinking from the spout where footshock occurred 24 hours earlier for the last day of training (D6) compared to Phase 2 of the retention test, $F(1, 25) = 22.22$, $p < 0.01$. The mean time animals spent drinking on Day 6 did not differ between the groups. However, on the day of retention testing, PBS/glutamate animals spent significantly less time drinking from the spout relative to PBS/PBS controls ($p < 0.01$) or the bupivacaine/PBS group ($p < 0.01$). Without accumbens shell functioning, animals in the bupivacaine/glutamate group did not display the high level of avoidance observed in the PBS/glutamate ($p < 0.01$).

EXPERIMENT 2

The objectives of the second experiment were two-fold. This study first examined whether reducing the quantity of expected rewards produces emotional changes that are manifested by decreases in motivation and behavioral responding. The second goal was to determine whether retention of the emotional experience involving reward reduction is improved by increasing noradrenergic drive within the nucleus accumbens shell by direct injections of phenylephrine.

Straight Alley Food Reward Task

Apparatus—The training apparatus consisted of a 72 inch long straight alley that was 15 inches in height, 10 inches wide and constructed of wood (see Figure 9). The apparatus included a start compartment (6 inches long) that was blocked by a sliding door connected to a digital timer. The alley also included three hurdles (5cm high) that were placed 2, 12 and 24 inches from the start box to ensure that a standard level of effort and motivation develops to obtain the 10 pellet food reward. The goal cup containing the food rewards was 1 inch wide and placed at the very end of the 72 inch long wooden alley.

Training—Rats were placed on a weight maintenance schedule seven days before training to reduce weights to 15% below ad-lib feeding weights. Each animal was pre-exposed for two days to sucrose pellets (45 mg; Research Diets, Inc, New Brunswick, NJ) that would be used for rewards throughout the study. Following pre-exposure, animals were habituated to the maze for five minutes of free exploration and then returned to their home cage and given 20 of the 45 mg sucrose pellets.

After habituation, all animals were randomly assigned to groups that would be given a large reward of 10 pellets or a small reward of 1 sucrose pellet on each of the six daily trials. Training began by placing each rat in the start box shown in Figure 9 facing away from the closed sliding door. The door was opened and the rat was given 120 sec to jump over the three hurdles to reach and consume the sucrose pellet rewards placed in the food cup at the end of the six foot long alley. As soon as the rat consumed the reward the trial ended and the rat was removed from the alley and placed in a holding cage for an inter-trial interval (ITI) of 30 sec. Six training trials were given on each of the 10 days of training and the latency to consume the food reward was measured using a digital timer. At the end of day 10, animals were assigned to either a “shift” or “non-shift” group and as shown in Figure 11, there were no differences between these two groups in the latency to run the length of the alley and retrieve the food pellets prior to the change in reward magnitude.

The experimental or “SHIFT” day in which emotional frustration was produced by the reduction in reward magnitude occurred on Day 11. On this day, animals assigned to the “non-shift” group continued to receive the same level of rewards given from the beginning of training. In contrast, those in the “shift group” received only 1, as opposed to 10 food pellets on each of the 6 training trials. Each rat was removed from the alley after the sixth trial and those in the “non-shifted” groups received intra-accumbens infusions of 0.5 μ l of PBS (non-shift large = 6; non-shift small = 7). Animals assigned to the “shifted group” received intra-accumbens infusion of either PBS (Shift-PBS; $n = 5$) or the alpha 1-noradrenergic agonist phenylephrine hydrochloride (Shift-Phenylephrine; 1.0 μ g/0.5 μ l; $n = 7$) according to the procedures described in the Microinjection section. The concentration of phenylephrine hydrochloride was selected from a range of doses (1.0, 5.0, or 10 μ g/0.5 μ l) previously shown to affect memory when infused into either the nucleus accumbens shell (Roosendaal & Cools, 1994) or NTS (Miyashita & Williams, 2003).

Retention Test—After the shift in reward quantity and accumbens injection, the animals were returned to the vivarium, maintained on at 15% weight maintenance schedule and left undisturbed during the seven day interval between reward shift and retention testing. The extended interval was imposed to examine whether memory of the frustrating experience of reward reduction is encoded more effectively by activating the accumbens shell following training since it is known that retention for this type of change in reward contingency decays in control animals after a one-week delay but remains intact in groups administered memory modulating treatments (Chen & Williams, 2006; Salinas & Gold, 2005; Salinas, Williams, & McGaugh, 1996). The retention test involved six consecutive trials in which the same quantity

of reward was given as on the shift day. The latency to run the length of the alley and cross the three hurdles to consume the 10 pellets (non-shift groups) or the reduced quantity of 1 pellet (shifted groups) was recorded.

Statistical Analysis—Behavioral measures from the straight alley task are expressed as mean $\pm$ SE. A one-way analysis of variance (ANOVA) followed by post hoc tests were used to detect differences in the latency to run the length of the maze and consume the food rewards between shifted PBS and phenylephrine groups as well as the non-shifted controls. Repeated measure ANOVA's were used to analyze differences in the latency to consume the reward between treatment groups during the training days, the shift day, and the first trial of the retention test.

Histology—Histological procedures were the same as those described in the previous histology section for Experiment 1. In Experiment 2, one animal was excluded from statistical analysis (Shift-Phenylephrine) due to incorrect placement of guide cannulae. The location of injection needle tips in this study is displayed in Figure 10.

RESULTS FOR EXPERIMENT 2

Effects of Reducing the Magnitude of Expected Rewards

All animals performed in a similar fashion on the days prior to shift, therefore Figure 11 depicts the latency to run the length of the alley and consume the food reward on the last day of training (Day 10) and on the day of shift in reward magnitude. There were no between group differences on Day 10 and the average latency to consume the food pellet(s) ranged between 2.6 ± 0.16 (Shift-PBS) and 3.3 ± 0.34 seconds (Non-shift, Small reward) $F(3, 21) = 0.95, p > 0.05$.

On Day 11 however, decreasing the expected value in reward from 10 pellets to only 1 caused a significant overall change in the runway performance of the shifted groups as revealed by a one way ANOVA $F(3, 21) = 8.44, p < 0.01$. Post-hoc analysis revealed that animals in the Shift-PBS group took significantly longer to cross the three hurdles and run the length of the six foot long alley to consume the new quantity of 1 reward pellet compared to the two non-shifted groups ($p < 0.01$ for both groups). Similarly, animals in the Shift-Phenylephrine group took significantly longer to traverse the alley to consume the reduced reward compared to both nonshifted groups ($p < 0.01$ for both groups).

Individual subjects were assigned to the separate shift groups after all animals completed training on Day 10. This was implemented to ensure that no differences in performance existed between the two groups prior to the experimental manipulation involving the shift in reward quantity. Although there were no differences in runway latencies after Day 10 of training (i.e. 2.5 sec for the Shift-PBS and 2.8 for the Shift-Phenylephrine groups), the latency to consume the reduced reward following shift was not consistent across the two shifted groups on Day 11. For example, although both shifted groups (i.e. Shift-PBS and Shift-Phenylephrine) took significantly longer to reach the end of the alley and consume the rewards relative to non-shifted groups, the latency to emit this response was longer in the group that would later receive intraaccumbens infusion of PBS compared to those designated to receive infusion of the alpha-1 noradrenergic agonist phenylephrine (i.e. Shift-Phenylephrine). This difference approached, but did not reach statistical significance ($p = .08$) and may be interpreted to suggest that subjects in the Shift-PBS group may have experienced a greater emotional reaction to the reduction in reward quantity relative to rats in the Shift-Phenylephrine group.

Effects of Noradrenergic Activation of the Accumbens Shell on Long Term Retention

The retention test was delayed for seven days to assess how well the representation of the reduction in reward quantity was encoded into long-term memory. A comparison of the mean runway latencies recorded during the six trials given during the delayed retention test is shown in Figure 12a. Repeated measures ANOVA on the six test trials demonstrated significant group differences across the trials ($F(3, 21) = 18.4, p < 0.01$). Individual comparisons between all treatment groups on each of the test trials demonstrated significantly longer latencies in the Shift-Phenylephrine group relative to all other groups on all trials with the exception of the second and last trial ($p < 0.05$).

Post-hoc comparisons indicate that average latencies to consume the new reward of only 1 food pellet over all 6 trials of the retention test was significantly longer in the shifted group given posttraining infusions of phenylephrine (1.0 $\mu\text{g}/0.5 \mu\text{l}$) into the accumbens shell (Shift-Phenylephrine) relative to the group that received PBS injections (Shift-PBS) after also experiencing a decrease in reward quantity ($p < .05$). Although the Shift-PBS group took a longer period of time to reach the end of the alley and consume the food pellets on the shift day, this behavioral reaction was not observed after the long interval between training and retention testing (Figure 12b). The increased latency to consume pellets in the shifted groups on Day 11 is interpreted to reflect frustration and the corresponding reduction in motivation associated with the decrease in expected reward quantity. If this emotional experience is retained in memory, comparable delays in runway latencies are expected even when subjects are returned to the apparatus after extended periods such as that imposed by the seven day training-retention interval. This prediction was evident in the Shift-Phenylephrine group in that runway latencies were not different between the day of Shift (6.1 sec) and retention testing (7.0 sec). In contrast, a repeated measures ANOVA comparing runway latencies between the day of shift (11.7 sec) and retention test (3.5 sec) in the Shift-PBS group showed that the decreased latencies recorded on the retention test approached significance ($p = .06$).

This finding is consistent with previous results demonstrating a decay in memory for changes in reward contingencies after a seven day training-retention interval (Salinas & Gold, 2005; Salinas et al., 1996). In contrast to the performance observed in controls, the mean runway latencies to consume the pellets over six retention trials in the Shift-Phenylephrine group was significantly longer than the Nonshift small, $p < 0.01$ or Nonshift large, $p < 0.01$ groups suggesting that the encoding and storage of new emotional experiences are strengthened by activating noradrenergic receptors in the accumbens shell after learning.

This view is further substantiated by other measures recorded during retention testing. For example, the first trial of retention testing provides the most accurate assessment of long term memory for the reduction in reward quantity since all trials occurring after trial 1 (i.e. trials 2-6) represent relearning as the animals are continually updated of the change in reward contingencies from 10 pellets to only 1. Consistent with the view, Figure 12a illustrates that only those subjects given intraaccumbens infusions of the noradrenergic agonist phenylephrine (Shift-Phenylephrine) after the shift on Day 11 of training displayed significantly longer runway latencies on the first trial of the extended retention test given seven days later, $F(3, 21) = 6.75, p < 0.01$. This enhancement in retention was not observed in the control group (Shift-PBS) that experienced the same reduction in reward quantity on Day 11 of the shift in reward contingencies.

DISCUSSION

Despite a substantial number of studies documenting roles for the amygdala and hippocampus in encoding and processing memory for emotional events into long term storage (Cahill, et al., 1996; Canli, et al., 2000; Diamond, et al., 2005; Joels, et al., 2005; McGaugh 2004; Phelps,

2004; Richter-Levin & Akirav, 2003; van Stegeren, et al., 1998; Vianna, et al., 2004), the role of the accumbens shell which receives input from both areas (French & Totterdell, 2003; Groenewegen, et al., 1987; Meredith, et al., 1990; Mogenson, et al., 1980; Petrovich, et al., 1996;) as well as peripheral information from the NTS (Delfs, et al., 1998) has yet to be explored. The results from this study reveal a functional relationship between the NTS and nucleus accumbens shell in modulating memory following physiological arousal and suggests a role of norepinephrine in regulating synaptic activity in the accumbens shell to facilitate this mnemonic process.

Findings emerging from Experiment 1 are two-fold. First, the results confirm previous findings (Miyashita & Williams, 2002) demonstrating that intra-NTS infusion of glutamate, the transmitter that is naturally released onto these neurons by ascending fibers of the vagus nerve (Allchin, Batten, McWilliam, Vaughan, 1994), improve memory for emotionally arousing learning experiences. These findings illustrate how neural impulses from the vagus nerve that represent changes in peripheral physiological functioning, influence synaptic activity in the brain during the initial encoding and storage of emotionally arousing events into memory. Glutamatergic activation of NTS neurons in the present study produced a robust improvement in memory that was evident by several independent measures of retention performance. For instance, the percentage of animals to enter the dark compartment where footshock was given 24 hr earlier was significantly lower in those given intra-NTS injections of glutamate relative to all other experimental or control groups (Figure 5). Interestingly, the small percentage of animals in the glutamate group that eventually entered the previously shocked context took significantly longer to emit this response than all other groups (Figure 4). The most convincing evidence indicating that associations were formed between the water spout and footshock was displayed by the resistance of animals in the glutamate group to drink from the water spout. Animals given intra-NTS infusion of glutamate not only required a significantly longer period of time to initiate the first lick at the water spout as shown in Figure 7, but they also spent a significantly smaller fraction of time drinking from the spout than all other experimental or control groups (Figure 8).

The second and perhaps most important result to emerge from Experiment 1 is the finding of a functional interaction between activated NTS neurons and their influences on the shell division of the nucleus accumbens during memory processing. The actions intra-NTS glutamate infusions had in enhancing performance on behavioral measures that reflect adequate memory was effectively suppressed by inactivation of the nucleus accumbens shell. Retention performance of the group given accumbens infusions of the long acting local anesthetic bupivacaine (Barsa, Batra, Fink, & Sumi, 1982; Thomas, Behbehani, Coyle, & Denson, 1986) prior to intra-NTS infusion of glutamate was reduced to that of animals given control injections of PBS. It is also important to note that functional inactivation of accumbens shell neurons after learning did not significantly diminish performance. However, as shown in Figure 3a, animals given this treatment after learning did approach and lick from the spout significantly faster than all other groups on the initial retention test in Phase 1. This finding can easily be misinterpreted to suggest that reversible inactivation of the accumbens shell alone “impairs” processes associated with memory storage using this paradigm. However, review of Figure 3b demonstrates that although subjects in the bupivacaine/PBS group approached and initially licked the spout sooner than all other groups, there were no differences in the actual amount of time spent drinking from the spout (i.e. less than 10 sec) during the 60 sec. test in Phase 1 of retention testing. Another measure that could be misinterpreted is the finding that there were no group differences in the latencies to exhibit an active avoidance response to exit the dark compartment and enter the safe neutral compartment during the same phase of retention testing. The failure to observe any significant between group differences on this measure may be attributed to the fact that animals in this study were given only a single footshock on Day 7 of training. Studies have shown that multiple pairings of at least four to five escapable footshocks

are necessary before animals consistently perform active avoidance responses (Myhrer, 1975). One caveat in interpreting the current findings is that bupivacaine suppresses activity in affected neurons as well as fibers passing through the accumbens shell. Therefore, changes in memory produced by suppressing the accumbens shell after NTS excitation with glutamate may also be mediated by influences on fibers innervating this structure from areas such as the amygdala or ventral tegmentum. The current experimental design does permit clarification on whether the attenuation was due to direct inactivation of accumbens neurons or by suppression of these afferent fibers.

Increased peripheral activity has been shown to stimulate glutamate release from the terminals of vagal afferent fibers onto NTS neurons (Allchin, et al., 1994; Sumal, Blessing, Joh, Reis, & Pickel, 1983). Miyashita and Williams (2002) showed that direct activation of NTS neurons with glutamate dose dependently improves memory for the same water-motivated inhibitory avoidance task as that used in the present study. Results from the current study confirm previous results showing that glutamatergic transmission between ascending fibers of the vagus nerve and NTS neurons play an important role in mediating changes in memory following peripheral activation by emotionally arousing events (Miyashita & Williams, 2002). These findings are extended however, by showing that along with the amygdala, the nucleus accumbens is another area in the brain where changes in peripheral autonomic functioning plays an important role in modulating memory.

Although peripheral physiological arousal, increased vagal transmission and the resulting activation of the NTS affects memory by influencing norepinephrine output in the amygdala and hippocampus (Hassert, et al., 2004; Miyashita & Williams, 2003; Miyashita & Williams, 2004), there are no known direct projections from the vagus/NTS complex to either of these structures. However, the NTS provides a direct noradrenergic innervation to the nucleus accumbens shell (Delfs et al., 1998). These findings suggest that changes in physiological arousal and NTS activity may influence memory not only through indirect actions on the amygdala and hippocampus, but through direct noradrenergic activation of the nucleus accumbens as well. Thus, it is possible that the beneficial effects on memory that are produced by activating NTS neurons following emotional experiences may be mediated by activation of noradrenergic receptors in the accumbens shell. This view is substantiated by findings showing that increasing discharge along ascending fibers of the vagus nerve with electrical stimulation potentiates neuronal firing in the accumbens shell (Mehendale, Xie, Haung, Guan, & Yuan, 2004). In addition, similar changes in the firing properties of accumbens neurons are observed following either electrical or glutamatergic activation of neurons in the NTS (Kirouac & Ciriello, 1997).

Because peripheral arousal and the corresponding activation of NTS neurons may influence memory through activation of noradrenergic receptors in the accumbens, Experiment 2 addressed the question of whether direct activation of these receptors in the shell region affects memory for emotional experiences. In this study, emotional arousal was induced by altering the quantity of rewards that were normally expected by subjects in the shifted groups. Reduction in an expected level of reinforcement produces heightened levels of peripheral physiological arousal as well as psychological frustration (Crespi, 1942; Goldman, Coover, & Levine, 1973; Levine, Goldman, & Coover, 1972). An abrupt reduction in an expected reward magnitude produces behavioral displays of frustration, reduced motivation and reluctance to consume the reduced reward. This view is supported by the observation that both groups that were shifted from 10 pellets to 1 pellet on Day 11 took significantly longer to traverse the alley and consume the new quantity of reward compared to the non-shifted animals (Figure 11).

To assess the possible contribution of norepinephrine activation of the accumbens in modulating memory for this form of psychologically-induced emotional arousal, subjects were

given intra-accumbens infusions of either PBS (Shift-PBS) or the alpha-1 noradrenergic agonist, phenylephrine (Shift-Phenylephrine) immediately following the last trial on Day 11 of the change in reward contingencies. Consistent with the findings of a previous study (Salinas et al., 1996), memory for this emotional experience was not retained in shifted control animals (Shift-PBS) for the duration of the seven day training-retention interval. The runway latencies of this group returned to pre-shift levels and were not different from those of nonshifted subjects that continued to receive 10 food pellets (Figure 12b). This natural decay in memory over time was however prevented by activation of accumbens noradrenergic receptors with phenylephrine after training on Day 11 with the reduced reward quantity. Of particular interest is the finding that even seven days following the change in reward contingencies, the Shift-Phenylephrine group continued to take a significantly longer period of time than all other groups to cross the three hurdles and run the length of the six foot alley to consume the reduced reward of a single pellet. This finding demonstrates that activation of noradrenergic receptors in the accumbens shell influences synaptic changes that are necessary at the time of encoding to facilitate long term memory for emotional events. In light of anatomical findings demonstrating that noradrenergic innervation of the accumbens shell is derived exclusively from terminals originating in the NTS but not the LC (Delfs, et al. 1998), these findings indirectly demonstrate that activation of the NTS-A2 neurons play a key role in modulating accumbens activity by releasing norepinephrine in response to heightened states of emotional arousal.

Traditionally, a great deal of attention has been devoted to understanding the contribution of the nucleus accumbens in forming associations between rewarding stimuli and increases in motivated behaviors. The functional role of the accumbens is now being broadened to also contribute significantly in processing memory for responses acquired in fear conditioning and contextual discrimination learning tasks. Lesions of accumbens shell neurons disrupt memory for pavlovian conditioned responses as evidenced by attenuated freezing to a context in which footshock was administered. Similar impairments in memory are observed in delayed working memory learning tasks (Jongen-Relo, Kaufmann & Feldon, 2003). The findings emerging from Experiment 1 of the current study extend these results by demonstrating that activation of NTS neurons, which send noradrenergic input to the accumbens shell improves contextual memory. This view is supported by the finding that a significantly larger percentage of animals in the PBS/glutamate group avoided entry into the context in which an aversive footshock was experienced relative to all other treatment groups in this study. Peripheral inputs conveyed by brainstem neurons may be critical in signaling information encoded at the time of footshock as being extremely significant. Without proper accumbens shell functioning, representations of the contextual attributes in which arousing events transpire may not be strengthened by the excitatory input provided to this nucleus by NTS neurons that are also activated by peripheral arousal.

The accumbens is a recipient of highly processed information. In particular, the shell division receives input regarding decision related activities from the prefrontal cortex (French & Totterdell, 2002; Sesack & Pickel, 1992), affective components of experiences from the basolateral amygdala (Mogenson, et al., 1980; Petrovich, et al., 1996), contextual features comprising the environment from the hippocampus (Groenewegen, et al., 1987; Meredith, et al., 1990) and peripheral information in response to heightened states of physiological arousal from the vagus nerve and NTS (Delfs, et al., 1998; Kirouac & Ciriello, 1997; Mehendale, et al., 2004). It is important to note that the projections from the basolateral amygdala and hippocampus converge monosynaptically onto projection neurons within the caudomedial part of the accumbens shell (French & Totterdell, 2003). Interestingly, the caudomedial accumbens shell also represents the site of termination for the majority of noradrenergic terminals that originate from the NTS (Delfs, et al. 1998). This arrangement of synaptic inputs provides the foundation for understanding how information from the NTS regarding physiological arousal

may ultimately modulate the processing of emotional and contextual attributes of newly learned experiences within the accumbens shell.

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A) Responses Recorded in Phase 1 of the Retention Test:

1. Latency to escape into the neutral compartment
2. Latency to first lick the spout
3. Amount of time spent drinking from the spout

B) Responses Recorded in Phase 2 of the Retention Test:

1. Time spent in neutral to avoid the dark compartment
2. Latency to enter the dark compartment
3. Percentage of animals to enter the dark compartment
4. Latency to first lick the spout
5. Amount of time spent drinking

Figure 1.

A. Overhead view of the inhibitory avoidance apparatus along with the measures recorded during the first Phase of retention testing. Subjects were placed in the dark compartment facing the sliding door and the (1) latency to either escape into the neutral compartment or alternatively (2) lick the spout at the end of the dark compartment was recorded. The total amount of time each animal spent drinking during the 60 sec test in Phase 1 was also assessed (3).

Figure 1B. Overhead view of the inhibitory avoidance apparatus and a description of the sequence of responses recorded to assess memory during Phase 2 of retention testing. The subjects were placed in the neutral compartment and the following behaviors were recorded: (1) time spent avoiding the dark compartment, (2) latency to enter the shock compartment, (3)

percentage of animals to enter the dark compartment, (4) time required to initiate contact and lick from the waterspout and (5) total time spent drinking from the spout once initial contact was made.

Figure 2.

A. Location of injection needle tip placements in the nucleus accumbens shell overlaid onto a representative photomicrograph from animals trained and tested in Experiment 1.

Abbreviations and symbols: _ = correct cannula and needle tip placements, x = incorrect cannula and needle tip placements, AC = anterior commissure, CPu = caudate putamen and NAC-core = nucleus accumbens core.

Figure 2B. Location of needle tip placements in the nucleus of the solitary tract (NTS) overlaid onto a representative photomicrograph from animals trained and tested in Experiment 1.

Abbreviations: _ = correct cannula and needle tip placements, x = incorrect cannula and needle tip placements, 4V = fourth ventricle, ECu = ext cuneate nucleus and Sp5n = spinal trigeminal nucleus

Figure 3.

A. Mean (+ SE) latency to lick from the water spout during Phase 1 of retention testing. The group given bupivacaine (0.25%) into the accumbens shell and PBS into the NTS (Bupivacaine/PBS) took significantly less time to first lick the spout than all other treatment groups. Asterisks indicate a significant difference between groups ($*p < 0.05$).

Figure 3B. Mean (+ SE) amount of time spent drinking from the water spout during Phase 1 of retention testing. Although the latency to first lick the spout varied between treatment groups, the amount of time spent drinking was brief (i.e. less than 10 sec) and did not differ between any of the experimental groups.

Figure 4.

Mean (+ SE) latency to enter the dark compartment in Phase 2 retention testing. Glutamatergic activation of NTS neurons following PBS injections in the accumbens shell improved memory as assessed by the increased time it took animals in this group to enter the context in which an arousing footshock had been delivered 24 hr previously. This enhancement in memory is attenuated in animals given the same dose of glutamate in the NTS but without nucleus accumbens functioning (Glutamate/Bupivacaine). The later animals did not differ from controls (PBS/PBS) or those animals just given bupivacaine into the accumbens shell (Bupivacaine/PBS). Asterisks indicate significantly different performance from all groups (** $p < 0.01$).

Figure 5.

Mean (+ SE) percentage of animals to enter the dark compartment during Phase 2 of Retention testing. A significantly smaller percentage of animals (i.e. 37.5%) in the group given PBS into the accumbens and glutamate (50 ng/0.5 ul) into the NTS (PBS/glutamate) entered the dark compartment relative to PBS control groups ($p < 0.05$) or the group given an identical dose of glutamate after the accumbens was functionally inactivated with 0.25% of the local anesthetic bupivacaine (i.e. Bupivacaine/Glutamate: 83.7%; $p < .05$ vs PBS/Glutamate).

Figure 6.

Mean (+ SE) time spent in the neutral compartment on the last two days of training (day 5 and 6), the day of footshock delivery (Shock) and while avoiding entry into the dark compartment during retention testing (Phase 2). The amount of time spent in the neutral compartment did not differ between any of the treatment groups until after footshock delivery. As shown during Phase 2 of the Retention test, subjects in the PBS/glutamate group spent significantly more time in the neutral compartment avoiding entry into the dark section of the apparatus than any other treatment group ($*p < 0.05$). The enhancement in avoidance responding produced by NTS infusion of glutamate was attenuated by inactivation of the accumbens shell. Note that the time spent in the neutral compartment did not differ between PBS/PBS controls and those given bupivacaine into the accumbens prior to intra-NTS infusion of the same memory enhancing dose of glutamate.

Figure 7.

Mean (+ SE) latency to first lick the spout in Phase 2 retention testing. Animals in the PBS/ glutamate group took significantly longer to enter the dark compartment and begin drinking relative to controls (PBS/PBS) or the group given 0.25% bupivacaine in the NAC-shell (bupivacaine/PBS). The significant avoidance of the water spout produced by posttraining infusion of glutamate (50 ng/0.5 ul) into the NTS was attenuated by functional inactivation of the accumbens shell with 0.25% bupivacaine (PBS/Glutamate vs Bupivacaine/Glutamate ** $p < 0.01$).

Figure 8.

Mean (+ SE) amount of time spent drinking from the water spout during the last day of training prior to footshock (D6) and Phase 2 of retention testing on day 8. Animals in the PBS/Glutamate group spent appreciably less time drinking from the spout compared to all other treatment groups. Asterisks indicate a significant differences between the PBS/GLUT group (** $p < 0.01$) and each of the remaining treatment groups. There were no differences between the PBS/PBS controls and Bupivacaine/PBS (BUPIV/PBS) or Bupivacaine/Glutamate (BUPIV/GLUT) groups on this measure.

Figure 9.

The straight alley apparatus included a start compartment (6 inches long) that was blocked by a sliding door connected to a digital timer. The alley also included three hurdles (5cm high) that were placed 2, 12 and 24 inches from the start box to ensure that a standard level of effort and motivation develops to obtain the 10 pellet food reward. The goal cup containing the food rewards was 1 inch wide and placed at the very end of the 72 inch long wooden alley. Six training trials were given on each of the 10 days of training and the latency to consume the food reward was measured using a digital timer.

Figure 10.

Location of injection needle tip placements in the nucleus accumbens shell overlaid onto a representative photomicrograph from animals trained and tested in Experiment 1.

Abbreviations and symbols: _ = correct cannula and needle tip placements, x = incorrect cannula and needle tip placements, AC = anterior commissure, CPu = caudate putamen and NAC-core = nucleus accumbens core.

Figure 11.

Mean (+ SE) latency to run the length of the 6-foot alley to consume food rewards on the last day of training (Day 10) and the day of “shift” in reward magnitude. With the exception of the Nonshift Small group which always received 1 reward pellet per trial, all other groups received a reward of 10 pellets after running to the end of the alley. The number of rewards given on the “Shift” day to animals in the Shift-PBS or Shift-Phenylephrine groups were reduced from 10 pellets to only 1 on each of the six training trials. This manipulation caused a significant increase in the latency for the Shift-PBS or Shift-Phenylephrine group to run the length of the 6-foot alley and consume the reduced reward relative to both Nonshifted groups (** $p < 0.01$).

Figure 12.

A. Mean (+ SE) latency to consume the reduced quantity of reward during the six trials of the retention test administered 7 days following the Shift in reward magnitude. On trial 1, animals in the group given posttraining intra-accumbens infusion of the norepinephrine agonist phenylephrine (1.0 ug/0.5 ul; Shift-Phenylephrine) took significantly longer to run the length of the alley to consume the reduced reward of 1 pellet than any other treatment group (** $p < 0.01$). In contrast, animals given the control PBS solution into the accumbens shell (Shift-PBS) following the reduction in reward quantity displayed latencies to consume the pellets that were not different from those of groups that continued to receive 10 pellets (Nonshift Large) or those that always received only one food pellet reward (Nonshift Small). Animals in the Shift-

Phenylephrine group continued to display high latency to consume the food reward over all other trials with the exception of trial 2 and 6.

Figure 12B. Mean (+ SE) latency to run the length of the 6-foot alley to consume food rewards on the “shift” day and retention test given seven days later. The latency to traverse the alley and consume the reduced quantity of food rewards remained significantly elevated only in the group given a posttraining intra-accumbens infusion of the norepinephrine agonist phenylephrine (1.0 ug/0.5 ul; Shift-Phenylephrine). The latency to consume pellets in this group was significantly longer than those of the group that also received a reduction in reward magnitude (i.e. Shift-PBS, $p < .01$) or the two non-shifted groups ($p < 0.01$).