DISSOCIABLE INVOLVEMENT OF THE NUCLEUS ACCUMBENS SUBREGIONS IN EFFORT-BASED DECISION MAKING

by

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ABSTRACT

Animals routinely engage in cost-benefit analysis, choosing between different courses of actions with potentially greater response costs that may lead to greater rewards. Previous research has shown that the preference to exert more physical effort to obtain a larger magnitude of reward is mediated by a complex neural circuit including the anterior cingulate, basolateral amygdala, and mesoaccumbens dopamine system. Past studies investigating the neural basis of effort-based decision making have utilized a T-maze task whereby rats have had to choose between climbing a barrier in one arm to obtain a high reward (HR), or retrieve a low reward (LR) from an arm with no barrier. Destruction of dopamine terminals in the nucleus accumbens (NAc) has been shown to reduce the preference to work harder to obtain a larger reward. Yet, the role of the different subregions of the NAc on this form of decision making is not very clear. The present study investigated the contributions of the NAc core and shell in effort-based decision making using an automated procedure conducted in an operant chamber. The task consisted of 4 discrete blocks of 10 trials. A response on one lever delivered an LR immediately (2 reward pellets), whereas responding on the other lever delivered an HR (4 pellets) after a fixed ratio of presses, which increased with each block (2, 5, 10, or 20). Inactivation of the NAc core, but not shell, via infusion of $GABA_{A/B}$ agonists muscimol/baclofen (75 ng each) reduced the preference for animals to exert greater effort to obtain the HR. In order to control for the greater delay from initiation of response to delivery of reward in the HR compared to LR condition, we conducted a subsequent experiment that equalized the delay. Inactivation of the core, but not shell reduced the preference for the HR. Therefore the NAc core, but not the shell, is part of a neural circuit that mediates effort-based decision making. Additionally the contributions by the NAc core to this form of decision making are distinct from those involving delay-based decisions.

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LIST OF ABBREVIATIONS

6-hydroxy-dopamine (6-OHDA)
Basolateral amygdala (BLA)
Dopamine (DA)
Higher magnitude reward (HR)
Lower Magnitude reward (LR)
Nucleus accumbens (NAc)
Prefrontal cortex (PFC)

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DEDICATION

I dedicate this work to my parents, Behrooz and Parvin, and my brother Roozbeh.

INTRODUCTION

The nucleus accumbens (NAc), a region within the anteroventral extent of the striatum, has been implicated in processes related to reinforcement (Salamone, 1996; Nicola, 2007) and rewarding aspects of natural rewards such as food as well as drugs of abuse (Koob, 1992). Lesions of this structure disrupt learning about conditioned stimuli associated with either drug or food rewards (Salamone, Cousins, Snyder, 1997; Salamone, Correa, Farrar, Mingote, 2007). Furthermore, this nucleus has been implicated in facilitating certain executive functions related to behavioral flexibility (Floresco, Ghods-Sharifi, Vexelman and Magyar, 2006). The NAc also aids goal directed behavior guided by short term memory, and inactivation of this region leads to disruptions in foraging strategies (Whishaw and Kornelsen, 1993; Floresco, Seamans and Phillips, 1997). The multiple roles that NAc plays in different forms of behavior can be attributed to its anatomical connectivity, as it receives input from different prefrontal cortical regions, such as the orbitofrontal and the medial regions of the prefrontal cortex (Chiba, Kayahara and Nakano, 2001; Reynolds and Zahm, 2005), as well as from limbic regions such as the hippocampus and the basolateral amygdala (BLA) (Zahm, 1999; Kelley, 2004).

The NAc can be divided into two biochemically and anatomically distinct regions with unique functional roles: the core and the shell (Zahm and Brog, 1992). The core region of this nucleus appears to mediate evaluative processes that allow animals to encode incentive values in goal-directed actions and the instrumental outcome, while the shell seems to be more involved in mediating stimulus-reward associations to guide goal-directed actions (Corbit, Muir, Balleine, 2001). Furthermore, inactivation of the NAc core severely impairs the maintenance of a new strategy rule whereas the shell appears to play a facilitative role in learning about the irrelevance of environmental stimuli (Weiner and Feldon, 1997; Floresco et al., 2006). In addition, the shell region of the NAc has been shown to have more of a role in unconditioned behaviors (such as

appetitive and fearful behaviors) (Faure, Reynolds, Richard, Berridge, 2008). Based on anatomical and behavioral findings, it has been suggested that the NAc region of the ventral striatum serves as the "limbic-motor-interface", and therefore plays a critical role in determining response priorities, particularly in ambiguous situations (Mogenson, Jones, Yim, 1980; Floresco, 2007). It was recently reported that a patient with damage to the NAc displayed poor cognitive abilities and showed symptoms similar to that of patients with frontal lobe syndrome (Mateen and Jesephs, 2008). The authors of this report hypothesize that a lesion to the NAc appears to be similar to lesions of the frontal lobes, and report that the patient seems to suffer, amongst other symptoms, of "inattention and poor abstraction" (Mateen and Josephs, 2008). It seems, therefore, that in humans as well as in animals the NAc facilitates behaviors mediated by the frontal lobes.

Human and animal studies have implicated the NAc in certain forms of cost-benefit decision making mediated by the frontal lobes, where subjects choose between different rewards associated with different costs. Responding to a growing interest in the neurobiological basis of decision making in humans, researchers have devised a number of tasks to identify brain regions mediating different aspects of these processes. Most tasks designed to quantify decision making take advantage of the economical underpinnings embedded in everyday cost-benefit judgments. In these tasks, participants choose between options with varying response costs, where a choice of one option associated with a higher cost generally ends in a more valuable reward. Imaging studies have shown that a network consisting of the amygdala, prefrontal cortex (PFC) and the ventral striatum becomes active when participants are engaged in these types of tasks (Daw, O'Doherty, Dayan, Seymour, Dolan, 2006). The extent with which these areas guide decisions toward the most beneficial outcome differs based on the task at hand. For example, some decision making tasks have participant choose between safe/less rewarding and risky/more

rewarding options. In one such task, the Iowa Gambling Task, participants choose between four decks of cards, where one pair of decks provides low immediate reward but high overall gain, and the other two decks result in high immediate gain with high overall loss (Damasio, 1994; Bechara, Damasio, Damasio and Lee, 1999). It is well established that patients with damage or lesions to specific regions of the PFC perform poorly on such decision-making tasks (Bechara el al., 1999; Fellows and Farah 2005). Additionally, functional imaging studies of normal participants have shown activation of the frontostriatal circuits when participants are engaged in these forms of decision making (Fukui, Murai, Fukuyama, Hayashi, Hanakawa, 2005; Akitsuki, Sugiura, Watanabe, Yamashita, Sassa, Awata, Matsuoka, Maeda, Matsue, Fukuda, Kawashima 2003).

Though human functional imaging studies have shed light on the neurobiological correlates of some types of decision making, the specific role of these distinct regions and the degree with which they guide behavior remains unclear. To address these issues, animal tests of decision making have been devised to clarify the role of each brain region. However, one obstacle in attempting to model decision making in animals is related to the issue of reward and punishment. As previously mentioned, human neuropsychological tests tend to use real or hypothetical monetary rewards as the motivator, and when punishment is necessary, reward is taken away. In animal models however, food is typically used as the motivating factor, making it impossible to take the food away once it has been consumed. Yet, another aspect of decision making that can be assessed in both humans and animals entails choosing between smaller immediate rewards or larger rewards delivered sometime in the future. In the case of animal studies, particularly those using rodents, the 'better' reward consists of a higher magnitude of a food reward that is delivered sometime in the future. As the delay to the larger reward increases, there is a decrease in the preference, or a "discounting", for the larger reward (Cardinal,

Pennicott, Sugathapala, Robbins, Everitt, 2001). Costs associated with a reward can be varied in a number of ways such as increasing the delay prior to the delivery of the larger reward (Cardinal et al., 2001). In this instance a "cost" to the larger magnitude reward is implemented by having the reward delivered after a delay, which increases over time. This type of task activates similar brain regions as seen with risk-based decision making when participants face a choice. The medial frontal cortex (McClure, Laibson, Loewenstein, Cohen, 2004; Kable and Glimcher, 2007), the striatum (Hariri, Brown, Williamson, Flory, de Wit, Manuck, 2006), the orbital frontal cortex and the posterior cingulate cortex show increased activity when faced with these types of decisions (McClure et al., 2004; Hariri et al., 2006; Kable and Glimcher, 2007). In animal models of delay based decision making, it has been shown that the NAc plays a particularly crucial role in mediating these types of judgments. Specifically, lesions to the core but not the shell region of this nucleus have been implicated in making animals more impulsive, biasing choice towards the immediate but smaller reward (Cardinal et al., 2001; Pothuizen, Jongen-Rêlo, Feldon and Yee, 2005).

Another way to vary response cost is to increase the physical effort output required to retrieve the larger magnitude reward. One of the first of these studies employed a T-maze task initially designed by Salamone (1994) where rats had to choose between two arms with different reward sizes; one that was easily accessible containing a lower magnitude reward (LR) and the other with a higher magnitude reward (HR), which could be obtained after scaling a barrier which blocked the arm (Salamone, Cousins, Bucher, 1994). Dopamine (DA) depletion via 6-hydroxy-dopamine (6-OHDA) lesions within the NAc of rats trained prior to surgery caused a dramatic decrease in the preference to exert greater effort to retrieve the larger reward (Salamone et al., 1994). Subsequent studies by this group used a concurrent choice task in which the rats chose between pressing a lever on an FR schedule for preferred food, or consuming a readily

available chow present within the operant chamber. These studies also revealed that dopamine lesions of the NAc in rats reduced the preference to work harder for the preferred food (Salamone et al., 1994; Salamone, Steinprei, McCullough, Smith, Grebel, Mahan, 1991; Salamone and Correa, 2002). More recent studies, using modified versions of the T-maze task have shown that a neural circuit consisting of the BLA, anterior cingulate cortex (ACC) and the NAc appear to mediate this form of decision making (Salamone et al., 1994; Walton, Bannerman, Alterescu, Rushworth, 2003; Floresco and Ghods-Sharifi, 2007). In a new study looking at the effects of mental effort on reward processing, Botvinick, Huffstetler and McCuire (2009) have confirmed that similar neural circuits appear to be mediating effort-based decision making in humans. Recent work in our laboratory has developed a novel, automated effort discounting task conducted in an operant chamber (Floresco, Tse, Ghods-Sharifi, 2008a). In this task, one lever is assigned to be the HR, delivering four pellets after a fixed ratio of presses that increase over four discrete blocks of trials throughout the session (2, 5, 10, 20 presses). Another lever, the LR, delivers two pellets immediately after one press. The use of this task allows for assessment of a number of behavioral variables including choice behavior, response latencies, and rates of instrumental responding. Using this task, we recently showed that decreasing DA activity via systemic injections of the broad spectrum DA antagonist flupenthixol or increasing DA release with amphetamine, increases effort discounting, reducing the rats' preference to work harder for a larger reward (Floresco et al., 2008a).

It is important to note that while an animal is exerting physical effort to receive a "better" reward, it typically incurs a delay to obtain that reward as it takes some time for a rat to complete pressing a lever a number of times, or climb a barrier to receive the HR (Floresco et al., 2008a). This aspect of effort-based decision making has not been accounted for in prior studies. Given that previous research has shown that the NAc, BLA and the DA systems all contribute to both

effort-based and delay based decision making (Cardinal et al., 2001; Cheung and Cardinal, 2005; Winstanly, Theobald, Cardinal, and Robbins, 2004), it is unclear whether the effects of manipulation of these systems on effort component of a task are due to a reduced preference to work harder for a larger reward, or a reduced tolerance for delayed rewards. Thus, a key modification of the effort discounting task described above can be employed to allow us to investigate these different types of costs independently of each other. By adding a delay to the delivery of the LR equivalent to the average time it takes the rats to emit multiple presses of the HR lever, the delay to reward is effectively equalized across both response options. Using this equivalent delay procedure, we observed that disruptions of the DA systems via flupenthixol or amphetamine continued to be effective at reducing the preference for the HR independently of the effects of delay (Floresco et al., 2008a). This indicates that disruptions in DA transmission alter effort-related judgment independent of the effects of these manipulations on delay-based decision making.

Employing 6-OHDA lesions of DA terminals, the above mentioned studies have shown that the NAc DA systems are involved in effort-based decision making. However, these lesions have been large in size, encompassing both the core and the shell (Sokolowski and Salamone, 1998). Interestingly, to date, no studies have looked at cell body inactivations of different subregions of the NAc on this form of decision making. Additionally, the decreased preference for the HR following disruptions in NAc core functioning on delay-based decision making tasks (Floresco et al, 2008a) warrants a closer look at the role of this economical component of decision making in effort-related judgments. The following study used reversible inactivation of the core and shell regions of the NAc on the automated effort-discounting task described earlier. We were particularly interested in parsing out the effects of delay versus effort discounting. Two groups of rats were trained on this task and received reversible lesions via microinfusion of

$GABA_{A/B}$ agonists muscimol and baclofen in either the NAc core or shell. Our findings indicate that the NAc core, but not the shell, seems to mediate effort related functions, independently of its role in delay-based decision making.

MATERIALS AND METHODS

Animals

Male Long Evans rats (Charles River Laboratories, Montreal, Canada) were used in this study. Upon arrival, rats were group housed for one week prior to being individually housed. Once single-housed, rats were food restricted to maintain ~85% of their free feeding weight, but had *ad-libitum* access to water. At the start of behavioral training rats weighed between 275g and 300g. The colony room was maintained on a 12:12hr light-dark cycle, and all testing took place during the light cycle. Experimentation and animal treatments were in accordance of the Canadian Council of Animal Care and the Animal Care Committee of the University of British Columbia.

Apparatus

Eight operant chambers (30.5 x 24 x 21 cm; Med-Associates, St. Albans, VT., USA) enclosed in sound-attenuating boxes were used. Fans embedded in the boxes provided ventilation and masked extraneous noise. Each chamber was fitted with two retractable levers. Located between the two levers was a food receptacle in which food reinforcement (45 mg; Bioserv, Frenchtown, NJ) was delivered by a pellet dispenser. A single 100-mA house light, located in the top-center of the wall opposite the levers, illuminated the chamber at the start of each trial, and would extinguish at the end of each trial. Four infrared photobeams were mounted on the sides of each chamber 3 cm above the grid floor, with an additional photobeam in the food receptacle. Locomotor activity was indexed by the number of photobeam breaks that occurred during a

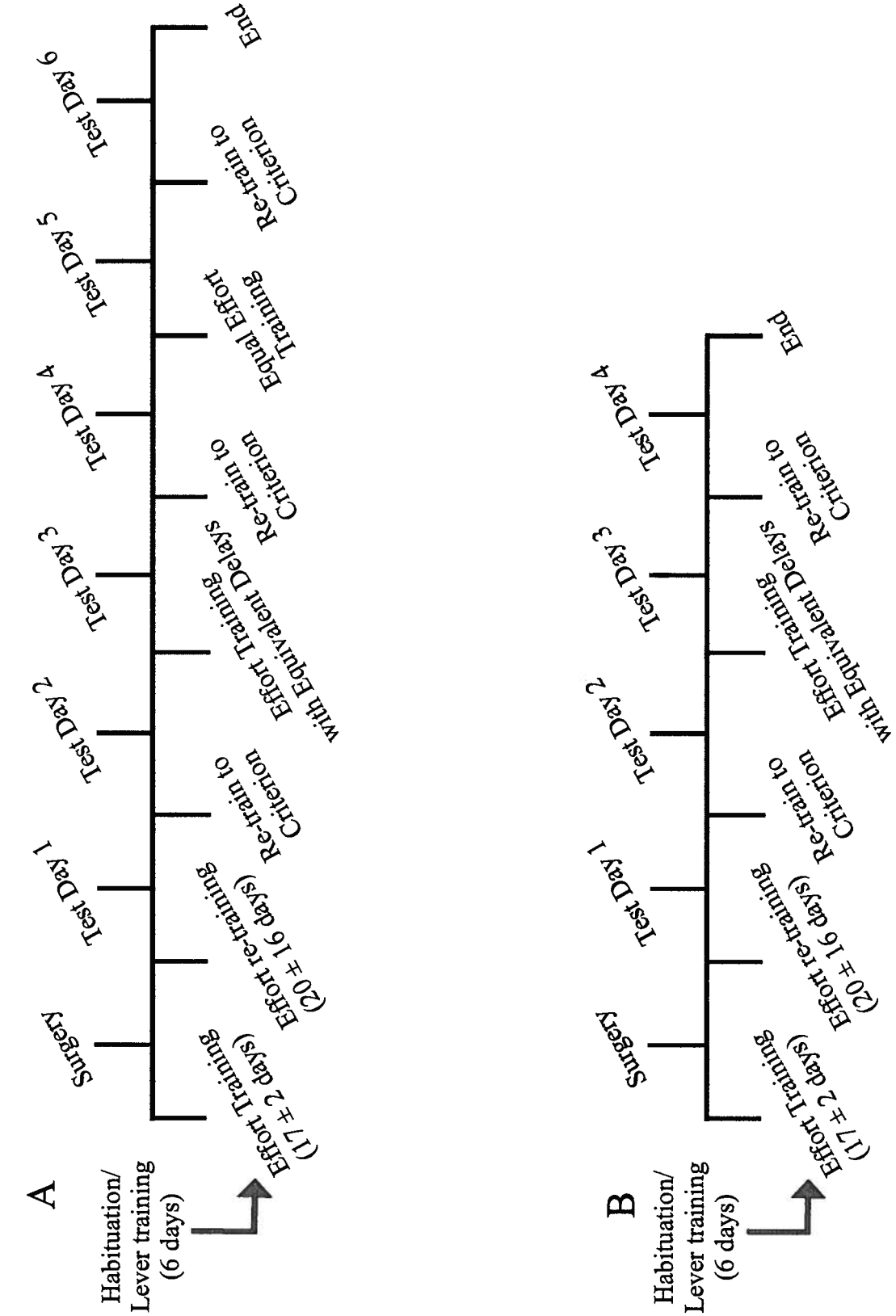
session. All experimental data were recorded by an IBM personal computer connected to the chambers via an interface.

Lever-Pressing Training

A summary of the training protocol is diagramed in Figure 1. Our initial training protocols were adapted from Cardinal et al. (Cardinal, Robbins, Everitt, 2000). To familiarize the rats with the novel food, each rat received ~20 reward pellets in their home cage one day prior to initial exposure to the operant chamber. On the first day of training, 2-3 crushed pellets were placed in the food cup and on the active lever before animals were introduced to the chamber. Rats were trained under a fixed-ratio 1 schedule to a criterion of 50 presses in a 30 min session (one session per day), first for one lever, then the other (counterbalanced left/right between subjects). This phase of the training was completed in two consecutive days. On following days, training occurred on a simplified version of the full task. In this task, rats were randomly presented with one of the two levers over 90 training trials, whereby one press on the lever delivered a single sugar pellet. Prior to the commencement of the trial, the levers were retracted and the houselight was off. Every 40 s, the houselight would illuminate and one of the two levers would be inserted into the chamber. Omissions were scored when the rat failed to respond to the extended lever within 25 s, whereby the lever would retract and the chamber would darken. However, a response on the extended lever would result in the retraction of the lever, and the immediate delivery of a single sugar pellet. The houselight remained illuminated for another 4 s. For each pair of trials, the left or the right lever would be presented only once, and the order within the pair of trials was random. Before moving on to the full task, rat had to achieve a criterion of 80 or more successful trials (i.e.; ≤ 10 omissions) over at least four training sessions.

Figure 1. Timeline depicting habituation, training, surgery, and testing procedures for the (A) NAc core group and (B) NAc shell group.

Figure 1


A
Habituation/
Lever training
(6 days)
Effort Training
(17 ± 2 days)
Surgery
Test Day 1
Effort re-training
(20 ± 16 days)
Re-train to
Criterion
Test Day 2
Effort Training
with Equivalent Delays
Re-train to
Criterion
Test Day 3
Equal Effort
Training
Re-train to
Criterion
Test Day 4
Test Day 5
Test Day 6
End
B
Habituation/
Lever training
(6 days)
Effort Training
(17 ± 2 days)
Surgery
Test Day 1
Effort re-training
(20 ± 16 days)
Re-train to
Criterion
Test Day 2
Effort Training
with Equivalent Delays
Re-train to
Criterion
Test Day 3
Test Day 4
End

Decision Making Tasks

Initially, rats were trained on the Effort Discounting task described below, receiving 5-6 daily training sessions per week. After showing stable performance for three consecutive days, rats were assigned to the NAc core or the NAc shell groups, and were implanted with cannula (see Surgery).

Effort Discounting. Figures 2 A and B diagram the basic procedures in this task. Each day, animals received one 32 min session that consisted of 48 discrete trials, separated into 4 blocks. Each block of trials began with two forced-choice trials. On these trials, only one of the two levers was randomly presented. During the next ten trials, both levers were presented and the animal had a free choice between the two levers. Throughout the intertrial state, the chamber was in darkness, and both levers were retracted. At 40 s intervals, a new trial began by the illumination of the houselight, followed by the extension of one or both levers 3 s later. One lever was designated as the Low Reward (LR) lever, and the other lever was designated as the High Reward (HR) lever. These levers were counterbalanced (left/right) between animals, and remained constant for that animal for the duration of the experiment. Once the levers were presented, the animal had to make a response within 25 s; a failure to do so counted as an omission, and the chamber was reset to the intertrial state. A single press of the LR lever resulted in the retraction of both levers and the immediate delivery of two pellets. However, after the first response on the HR lever, the LR lever was immediately retracted, and the HR lever remained inserted in the chamber until the required fixed ratio (FR) of presses were completed. The FR requirement for the HR lever increased within the session (described below). Upon completion of the FR requirement for the HR, the HR lever retracted, four pellets were immediately delivered 0.5 s apart, and the houselight remained on for another 4 s after the delivery of the last pellet. The chamber then darkened, and was reset to the intertrial state.

Figure 2. Schematic of the decision making task used. (A) The format of a single free choice trial on the effort discounting task. Cost/benefit contingencies associated with responding on either the low-reward (LR) or high-reward (HR) lever on the (B) effort discounting (left), (C) effort discounting with equivalent delays or (D) equal effort tasks.

Figure 2

A
House-light ON
3 sec
Both levers extended
Choose HR Lever
LR lever retracts
No choice in 25 sec -or-
Failure to complete FR on HR lever in 25 sec
Press HR lever 2-20 times
4 pellets delivered
4 sec
Houselight OFF (ITI state)
Both levers retract (Omission)
Choose LR Lever
Both levers retract
2 pellets delivered
4 sec
40 sec
B
Effort Discounting
1 press = 2 pellets (immediate)
2-20 presses = 4 pellets (immediate)
LR lever
HR lever
C
Effort Discounting w/ equivalent delays
1 press = 2 pellets (delayed 0.5-7 s)
2-20 presses = 4 pellets (immediate)
LR lever
HR lever
D
Equal Effort
1 press = 2 pellets (immediate)
1 press = 4 pellets (immediate)
LR lever
HR lever

The fixed ratio of lever presses required to obtain the HR increased over the four blocks of trials, beginning with 2 presses, then 5, 10, and finally 20 presses respectively. If a rat failed to complete the FR within 25 s of choosing the HR, the lever retracted without delivery of any food, and the chamber was reset to the intertrial state. However, the animal's choice was still incorporated into the data analysis. In addition, the amount of time taken for a rat to initiate a lever press, and the time it took the rat to finish pressing the lever once a choice was made were also recorded.

Following surgery, they were once again retrained on this task until a stable criterion was reached prior to being tested, retested, or moved into the next phase of the experiment. For this and all subsequent tasks, training continued until rats as a group 1) chose the HR lever during the first trial block (fixed-ratio 2) on at least 70% of successful trials, and 2) demonstrated stable baseline levels of choice. Stable baseline performance was determined using a similar procedure to that described by Winstanley, Theobald, Cardinal, Robbins (2004). Briefly, data from three consecutive sessions were analyzed with a repeated-measures ANOVA with two within-subjects factors (Training Day and Trial Block). If the effect of Trial Block was significant at the $P<0.05$ level but there was no main effect of Training Day or Training Day x Trial Block interaction, animals were judged to have achieved stable baseline levels of performance. On the following day, rats received either saline or inactivation microinfusion treatments (see Microinfusion). Rats were then retrained on the task daily until a stable level of choice was again demonstrated, at which point they were administered their second test day. This procedure was repeated until rats in both groups received each of their designated infusion treatment (Figure 1).

Effort Discounting with Equivalent Delays: Once training and drug tests using the Effort Discounting procedure were complete, rats were then retrained on a variant of the Effort Discounting task. This task was identical to the Effort Discounting task, with one exception.

Rats still received two pellets after a single press on the LR lever, yet a delay was imposed prior to reward delivery (Figure 2C). More specifically, the delay imposed prior to the delivery of the LR was equivalent to the average time it took the rats to complete the ratio of presses on the HR in the Effort Discounting procedure (~0.5-7 s). This delay increased across trial blocks and was calculated based on the average time it took all rats within each group to press the lever 2, 5, 10, and 20 times during the last three days of training on the Effort Discounting task. Thus the amount of time it took to finish the ratio on the HR was equivalent to that of the delay imposed on the LR on each block of trials; for instance, if a rat required 7 s to press the HR lever 20 times during the last block, a single press on the LR lever during this block would deliver the two pellets after a 7 s delay. Drug tests and retraining were conducted in identical manner as that of the Effort Discounting procedure.

Equivalent Effort: A priori we had determined that if inactivation of either subregions of the NAc altered effort discounting, rats in that group would be retrained on a simpler reward magnitude discrimination task. This would confirm whether or not the obtained results were due to alterations in satiety or an inability to discriminate between larger and smaller rewards. This procedure was similar to the Effort Discounting procedure with one exception. One press on the LR lever immediately delivered two reward pellets. Likewise, one press on the HR lever resulted in the delivery of four pellets immediately. That is, across four blocks of trials, the rats only had to choose between a larger and a smaller reward. Drug tests and retraining were conducted in an identical manner as that of the Effort Discounting and Effort Discounting with Equivalent Delay procedures.

Surgery

Rats were anesthetized with 100 mg/kg of ketamine hydrochloride and 7 mg/kg xylazine and implanted with bilateral 23 gauge stainless-steel guide cannulae. Rats in the NAc core group

were implanted with a pair of cannulae in the core region of this nucleus (flat skull, from bregma: anterior-posterior [AP] = +1.6 mm, medial-lateral [ML] = ±1.8 mm, and dorsal-ventral [DV] = 6.8 mm from dura). Rats in the NAc shell group were implanted with a pair of cannulae in the shell region of this nucleus (flat skull, from bregma: AP = +1.3 mm, ML = ±0.9 mm from bregma, and DV = –6.0 mm from dura) (Paxinos and Watson 1998). Rats were given seven days to recover from their surgery during which time they were handled daily for five minutes.

Microinfusion and Experimental Design

A within-subjects design was used in these experiments. Inactivation of either region was achieved by microinfusion of a drug cocktail containing the $GABA_A$ agonist muscimol (mus; Sigma-Aldrich Canada, Oakville, Ontario, Canada), and $GABA_B$ agonist baclofen (bac; Sigma-Aldrich).

Drugs were dissolved in physiological saline and were protected from light. Each drug was separately mixed at a concentration of 500ng/µl, and was then combined in equal volumes, making the final concentration of each compound in solution 250 ng/µl. A volume of 0.3 µl was infused, making the final dose of both baclofen and muscimol 75 ng per side. Infusions of these compounds at these volumes have been used in our laboratory previously to reveal dissociable effects on behavior when administered in the NAc core or NAc shell (Floresco et al., 2006; Floresco, McLaughlin, Haluk, 2008b).

Infusion of drug or saline was counterbalanced across rats over the two test days. Thirty gauge injection cannulas which extended 0.8 mm past the end of the guide cannulas were used. Infusion of either Bac/Mus or saline were administered using a microsyringe pump (model 341; Sage Instruments, Cambridge, MA), and 0.3 µl was delivered into the region of interest over 48s. Once complete, injection cannulas were left in place for an additional 1 min to allow for

diffusion. After the infusion, rats were returned to their home cage, where they remained for an additional 10 min period before behavioral testing.

Histology

Upon completion of the experiment, rats were euthanized in a carbon dioxide chamber. Brains were removed and fixed in a 4% formalin solution. Brains were then frozen and sliced at 50 μm sections, mounted and stained with Cresyl Violet, and placements were verified with reference to the neuroanatomical atlas of Paxinos and Watson (1998).

Data Analyses

The key dependent measure of interest was the proportion of HR lever choices, factoring trial omissions. This was calculated by dividing the total number HR choices by the total number of successful trials. For each set of tests (drugs and saline), these data were analyzed with separate two-way, repeated-measures ANOVAs, with Treatment and Trial Block as two within-subjects factors. The latency to select a lever was analyzed similarly. The rate of lever pressing was obtained by dividing the ratio of required presses in each block by the average time it took to complete pressing the lever in that block. These values were then averaged for each treatment. The number of trial omissions and the locomotor activity data (i.e., photobeam breaks) were analyzed with one-way repeated-measures ANOVAs.

RESULTS

Initial training. Prior to surgery, all rats displayed sensitivity to increasing effort requirements, and reached a stable baseline of performance on choice behavior after an average of 17± 2 days of training on the effort-discounting task. At this point, rats were choosing the HR lever on approximately 75% of trials during the first block, discounting the HR over the ensuing blocks of trials as the necessary ratio of presses increased.

NAc core Inactivation via $GABA_{A/B}$ Agonist

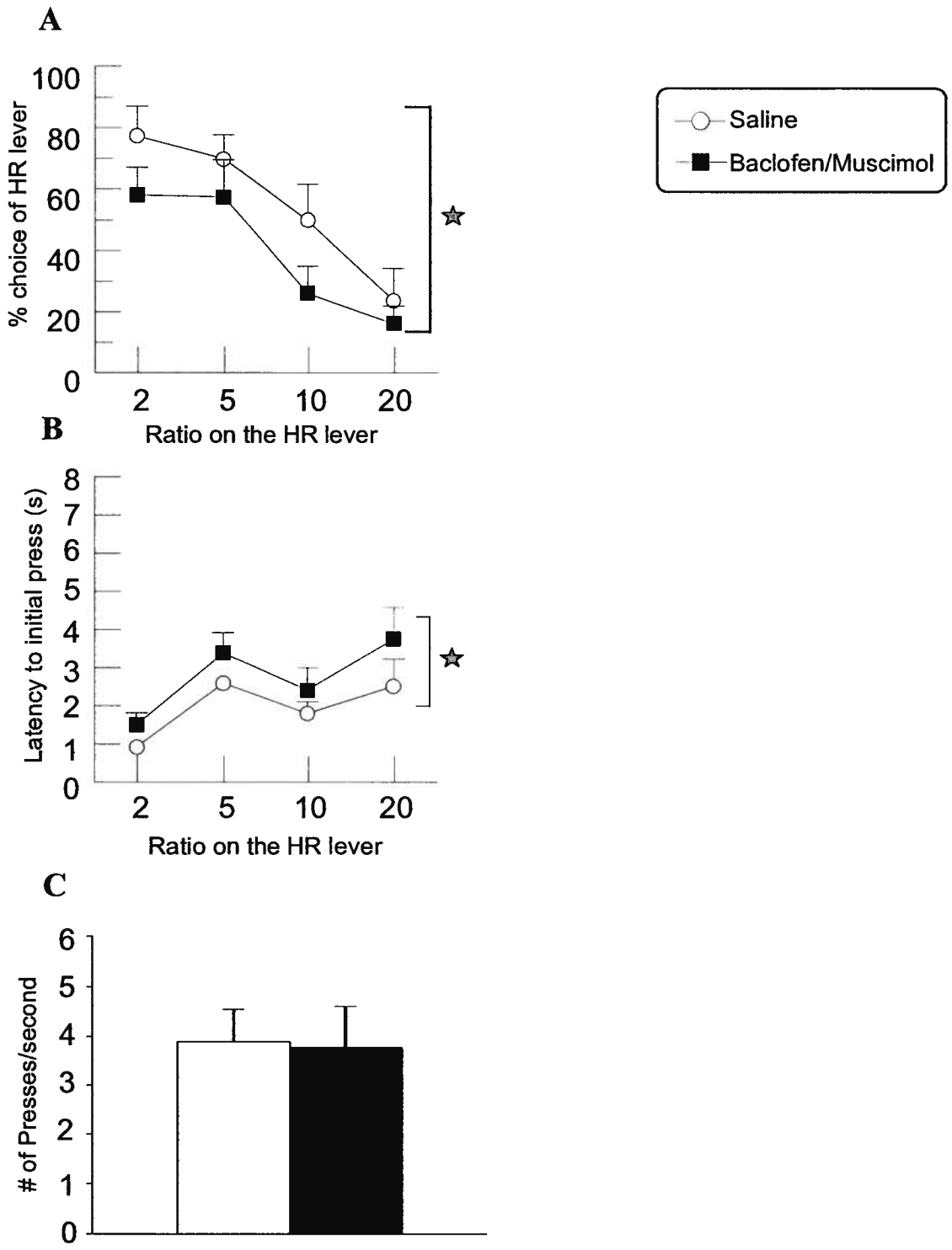
Effort discounting. Initially, ten rats were trained on the effort discounting task. Two rats were removed from data analysis due to inaccurate cannula implantation. Rats were retrained on the effort task one week after recovery from surgery. Once stable choice was achieved, rats received their first microinfusion tests entailing counterbalanced treatment of saline or $GABA_{A/B}$ agonists Bac/Mus. Inactivation of the NAc core decreased the preference to work harder, evidenced by a decrease in selection of the HR to receive the larger reward. Analysis of the choice behavior showed a significant main effect of Treatment ($F(1, 7) = 5.820$, $P < 0.05$), a significant main effect of Trial Block ($F(3, 21) = 20.645$, $P < 0.001$), but no significant Treatment × Block interaction ($F(3, 21) = 0.542$, NS). As shown in Figure 3A, inactivation of the NAc core resulted in an overall decrease in the number of choices directed towards the HR lever on free-choice trials. Moreover, this effect was apparent during the 1st block of trials, and continued over the session.

Analysis of the latency to initiate lever press revealed a significant main effect of Treatment ($F(1, 7) = 5.678$, $P < 0.05$), but no significant Treatment × Block interaction ($F(3, 21) = 0.321$, NS). As can be observed in Figure 3B, inactivation of the NAc core resulted in a moderate, but significant increase ($P < 0.05$) in the time it took the animals to make a choice between the HR and LR lever. As the session progressed, and the effort requirements increased, the response latencies also increased, which is indicated by a significant main effect of Trial Block ($F(3, 21) = 5.216$, $P < 0.05$). Figure 3C shows the rate of lever pressing after saline and inactivation treatments. Inactivation of the NAc core did not alter the rate of lever pressing, indicating that once a choice was made, rats pressed the HR lever just as quickly as they did on the saline test day ($t(7) = 0.205$, NS). Finally, there were no significant differences in omissions on trials between the two treatment conditions (mean of 0.130±0.125 on saline, and 1.88±1.35 on

Figure 3. Effort discounting task: Inactivation of the NAc core disrupts choice behavior when rats have to choose between the LR and the HR. As seen in (A) there is an overall decrease in the preference for the HR when rats receive an inactivation to the NAc core via $GABA_{A/B}$ agonist Muscimol/Baclofen, when compared to their performance on saline. (B) Inactivation of the NAc core leads to a significant increase in the latency to initiate lever press. This increase is likely not due to motor deficits, since the number of presses per second remains unchanged for both saline and drug treatments (C).

Figure 3

NAc Core - Effort Discounting



drug test days; t(7) = 1.416, NS). Thus, inactivation of the NAc core decreases preference for rats to work harder to obtain a larger reward, but did so without altering the rates of responding.

Effort discounting with equivalent delays. The findings mentioned above seem to indicate that inactivation of the NAc core reduces the preference to work harder to obtain the larger reward. However, it is also possible that this effect may be due to a decreased tolerance for a delayed reward. While a rat is pressing the HR lever a number of times, as mentioned previously, it is also incurring a delay to the reward. It has been shown that excitotoxic lesions of the NAc core reduce the preference of rats for higher magnitude rewards which are accompanied by increasing delays (Cardinal et al., 2001; Pothuizen et al., 2005). To further clarify this issue, rats were subsequently trained on the effort with equivalent delays task. After their last test day on the effort discounting task, rats continued training on the same task for an additional three days pending stable performance. Once stability criterion was established, the average latency to complete the fixed ratio of HR presses was calculated across the 4 trial blocks. These values (0.4, 1.7, 2.8, and 6.5 seconds) were used as the delay to reward delivery after a single press on the LR lever. One rat was removed from data analysis, because of an irreparable blockage in one of the implanted cannula, thus decreasing the number of rats to seven for the remainder of the experiment. Rats were then trained on this modified version of the task until they reached stability criterion as before. Once performance was stable, rats received a second round of counterbalanced saline or drug treatment. Under these conditions, inactivation of the NAc core reduced the preference to exert more effort for the HR, and increased LR lever pressing. A significant decrease in the proportion of choices of the HR lever across all trial blocks was seen when the relative delay to the delivery of the reinforcer for the HR and LR were equal. Analysis of these data revealed a significant main effect of Treatment ($F(1, 6) = 6.692$, $P < 0.05$), a significant main effect of Trial Block ($F(3, 18) = 27.459$, $P < 0.001$), but no Treatment × Trial

Block interaction ($F(3, 18) = 0.222$, NS) (Figure 4A). Further, under these conditions the latency to initiate a choice was not affected by inactivation of NAc core (Figure 4B; Treatment ($F(1, 6) = 2.160$, NS); Treatment × Trial Block ($F(3, 18) = 0.299$, NS)). Compared to saline treatment, there were no differences in the rates of lever pressing after NAc core inactivation (Figure 4C; $t(6) = 1.65$, NS). Again, there were no significant differences between the two groups in terms of omissions (mean of 2.86±2.09 on saline, and 1.57±0.571 on drug test days; $t(6) = 0.765$, NS). Thus the effect of NAc core inactivation on effort-based decision making appears to be independent of the delays to reward that are incurred when rats select the higher effort response option.

Equivalent Effort. To ensure that the decreased preference for the HR induced by inactivation of the NAc core was not due to the inability to distinguish between larger and smaller rewards, or alterations in satiety, rats were subsequently trained on a modified task to control for these effects. Rats were trained for 5 days on a task where one press on the LR or the HR lever immediately delivered two or four pellets respectively. Figure 5 shows the number of choices on the HR lever with saline as well as Bac/Mus treatment on the equivalent effort task. We found that while there was a small decrease between the second and third block, overall, there were no significant main effects of Treatment, or Treatment × Trial Block on the preference for the HR ($F(1, 6) = 3.654$, NS and $F(3, 18) = 0.332$, NS respectively). There was a slight increase in omissions when the core region was inactivated, however, t-test showed that the difference between the two groups were not significant (mean of 0.0 omissions on saline, and 5.0±0.36 omissions when inactivated, with a $t(5) = 1.00$, NS). Together, the above data suggest that the NAc core mediates effort-based decision making regardless of its influence on delay based decision making. In addition, the decrease in preference to work harder for a larger reward

Figure 4. Effort discounting with equivalent delays task: Inactivation of the NAc core disrupts choice behavior when rats have to choose between the LR and the HR when the delay to reward is equal on both levers. (A) There is an overall decrease in the preference for the HR when NAc core is inactivated via $GABA_{A/B}$ agonist Muscimol/Baclofen, compared to their performance on saline. (B) Inactivation of the NAc core does not change the latency to initiate lever press, or affect the rate of press (C).

Figure 4

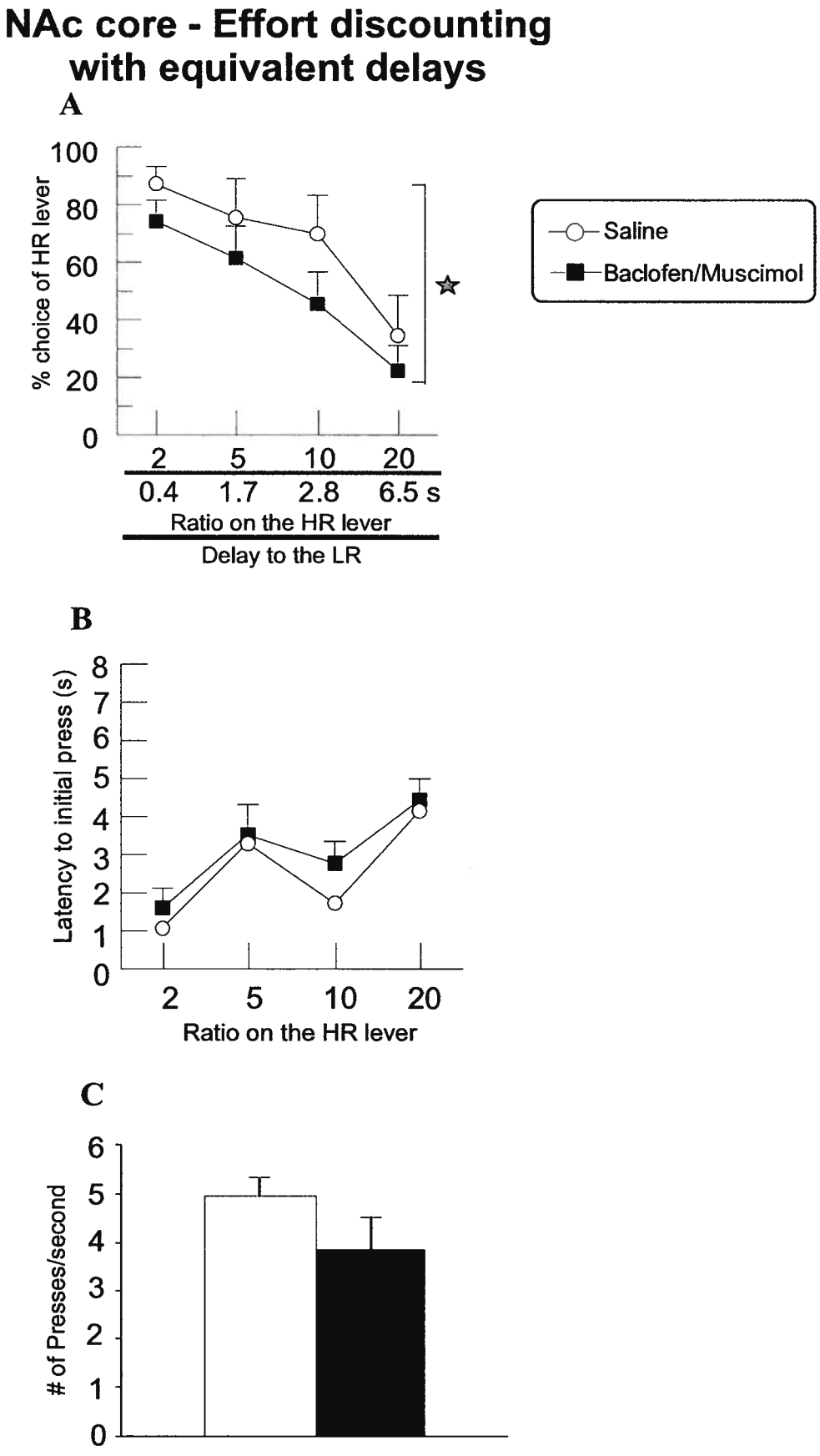
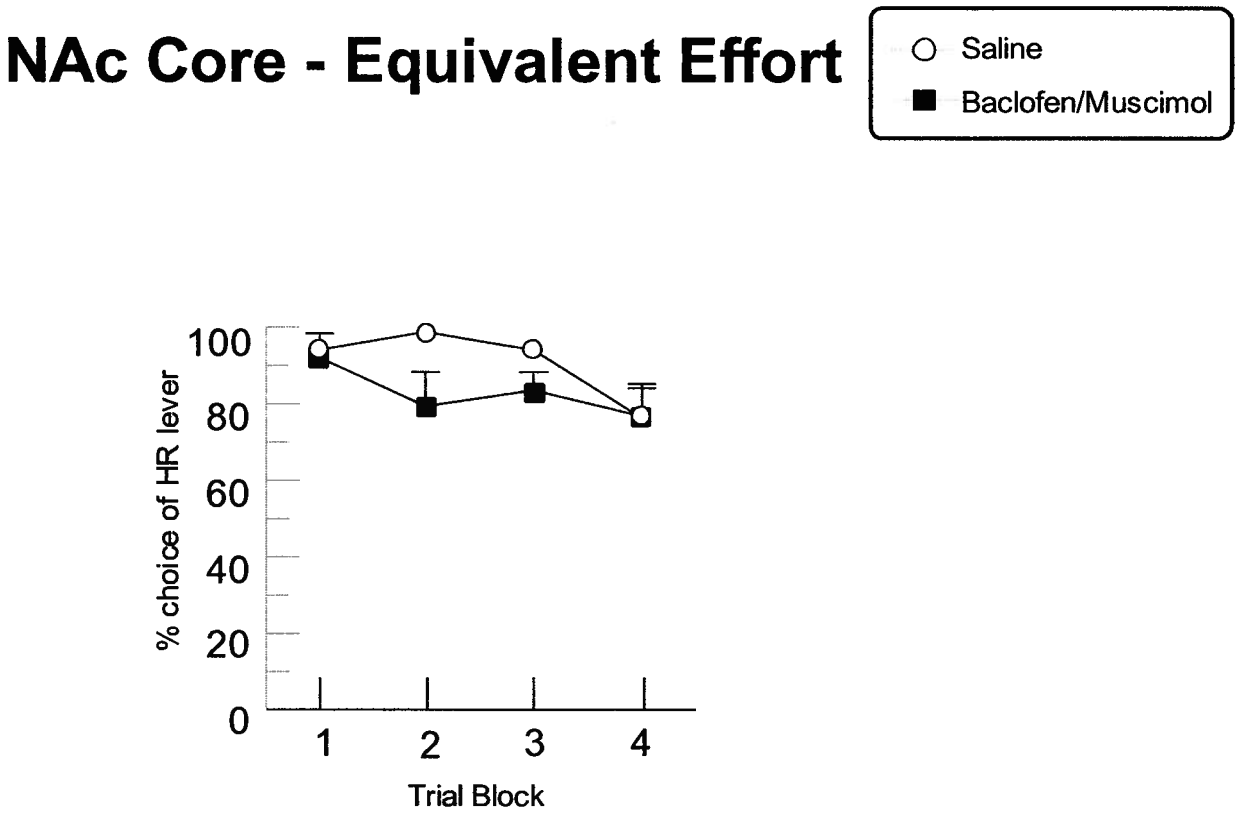

NAc core - Effort discounting with equivalent delays
A
100
80
60
40
20
0
% choice of HR lever
2
5
10
20
0.4
1.7
2.8
6.5 s
Ratio on the HR lever
Delay to the LR
Saline
Baclofen/Muscimol
B
Latency to initial press (s)
8
7
6
5
4
3
2
1
0
2
5
10
20
Ratio on the HR lever
C
# of Presses/second
6
5
4
3
2
1
0

Figure 5. Equivalent Effort Task. Inactivation of the Nac core did not significantly alter choice behavior when rats only had to press a lever once on the HR or the LR lever for the respective reward size, indicating that inactivation of this region did not affect memory or appetite.

Figure 5


NAc Core - Equivalent Effort
Saline
Baclofen/Muscimol
% choice of HR lever
100
80
60
40
20
0
1
2
3
4
Trial Block

induced by inactivation of the NAc core, is not due to an inability to discriminate between larger and smaller rewards, or changes in satiety.

NAc shell Inactivation via GABAA/B Agonist

Effort discounting. Initially, 8 rats were trained on this task. One rat was removed from the data analysis due to inaccurate cannula implantation. As opposed to what was observed after inactivation of the NAc core, analysis of the choice behavior showed no significant main effects of Treatment ($F(1, 6) = 0.014$, NS) or Treatment × Trial Block ($F(1, 6) = 0.371$, NS) in the NAc shell group (Figure 6A). As evident in Figure 6B, the latency to initiate a lever press was not altered by the inactivation of the NAc shell, and analysis of the data revealed no significant changes on Treatment or Treatment × Trial Block ($F(1, 6) = 0.771$, NS, and $F(1, 6) = 0.310$, NS, respectively). The rates of lever pressing was comparable across treatment days (Figure 6C; $t(6) = 0.89$, NS). There were no differences on the omission rates (mean of 0.0 omissions for both treatments). Thus, inactivation of the NAc shell does not affect effort-based decision making.

Effort discounting with equivalent delays. As with rats in the NAc core group, we trained the rats on the effort discounting with equivalent delays. One rat in this group developed seizures after infusion of Bac/Mus, reducing the number of rats in this group to 6. We found that inactivation of this region did not significantly alter the preference for the HR ($F(1, 6) = 3.895$, NS) (Figure 7A). There were no significant differences in the latency to initiate a response (Figure 7B; $F(1, 6) = 0.001$, NS), nor were there any changes in the rate of lever pressing once a choice had been made (Figure 7C; $t(5) = 0.152$, NS). Omission rates remained similar for both saline and drug treatments (mean of 1.67±1.12 and 0.833±0.477 respectively, with $t(5) = 0.667$, NS). Though not significant, there was a slight increase in the preference for the HR when the shell was inactivated. After further inspection of the individual data, we noticed that 3 rats

Figure 6. Effort discounting task: Inactivation of the NAc shell did not alter choice behavior when rats had to choose between the LR and the HR. (A) There was no significant difference in the preference for the HR when rats receive an inactivation to the NAc shell via $GABA_{A/B}$ agonist Muscimol/Baclofen in comparison with saline test days. Further, inactivation of the NAc shell did not alter the (B) latency to initiate lever press or (C) rate of lever press.

Figure 6

NAc Shell - Effort Discounting

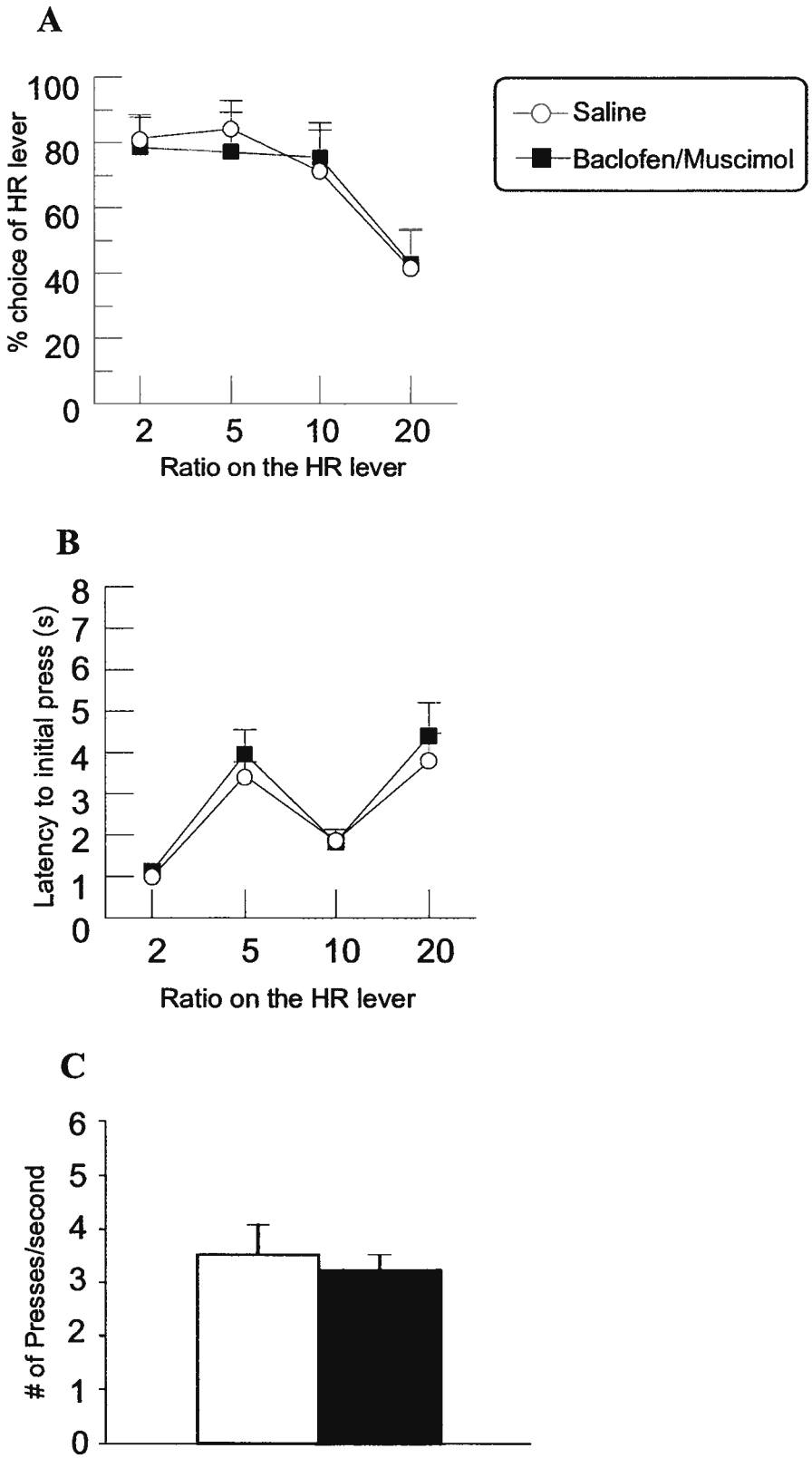
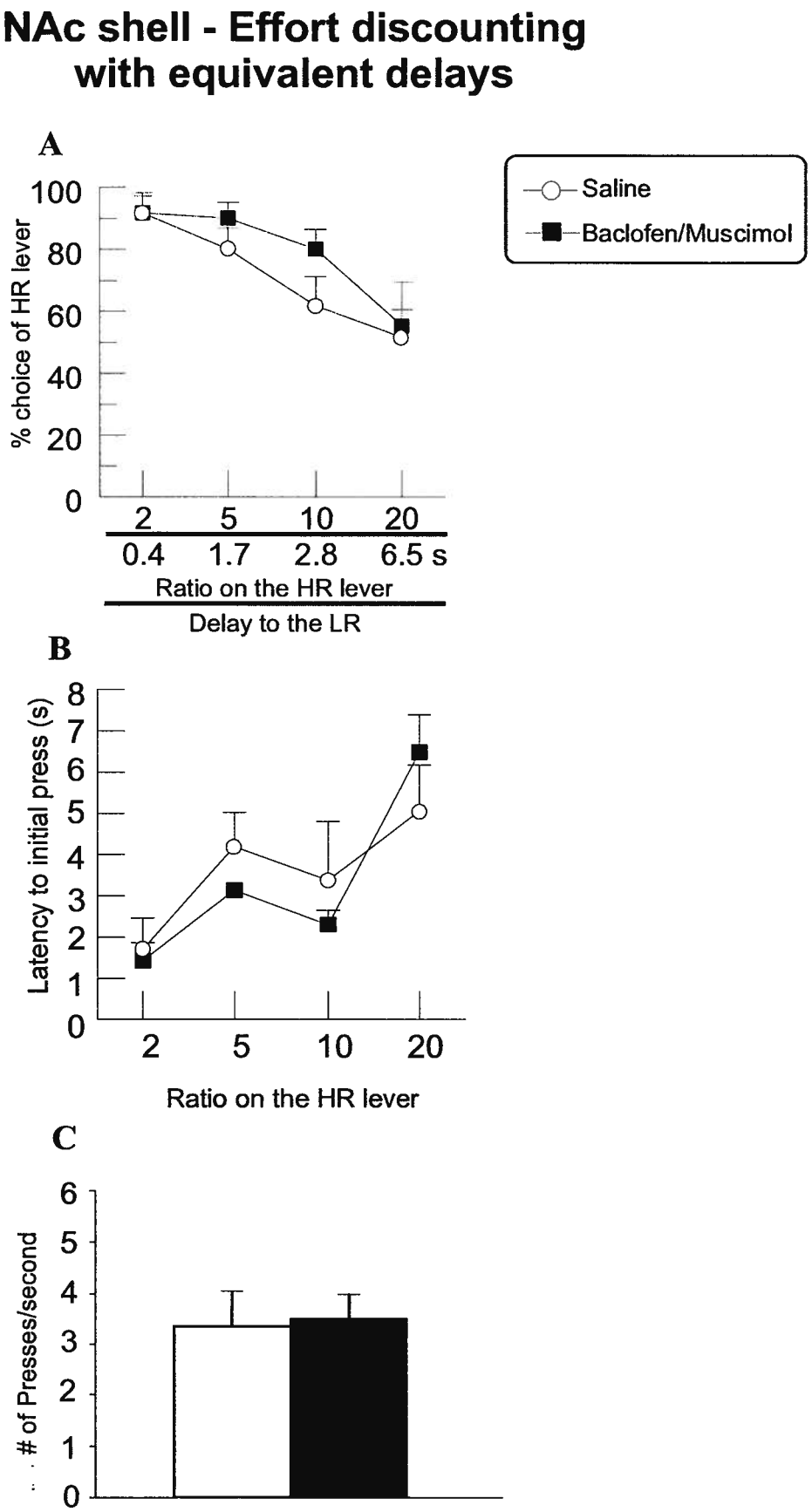


Figure 7. Effort discounting with equivalent delays task: Inactivation of the NAc shell did not alter choice behavior for choosing between the LR and the HR when the delay to reward was equal on both levers. (A) There was no change in the preference for the HR, (B) the latency to initiate a lever press, or the ratio of presses on both saline and inactivation test days (C).

Figure 7


NAc shell - Effort discounting with equivalent delays
A
Saline
Baclofen/Muscimol
% choice of HR lever
100
80
60
40
20
0
2
5
10
20
0.4
1.7
2.8
6.5 s
Ratio on the HR lever
Delay to the LR
B
Latency to initial press (s)
8
7
6
5
4
3
2
1
0
2
5
10
20
Ratio on the HR lever
C
# of Presses/second
6
5
4
3
2
1
0

increased their preference for the HR, while 2 rats made a comparable number of HR choices for both treatments. Only one rat displayed a reduced preference for the HR after inactivation treatment relative to saline.

Histology

NAc core. Locations of all acceptable infusions are displayed in Figure 8A. As noted above, the data from two rats in this group were excluded from the analyses due inaccurate placements. The placement for these two rats were either asymmetrical in the medial/lateral plane, or were located dorsal or posterior to the NAc core. In these rats, the proportion of choice of the HR lever after infusions of baclofen/muscimol (69 +/- 22%) was comparable to that after saline infusions (76+/-23%). Thus, our findings that alterations in effort-based decision making in the NAc core group after bilateral infusion of Bac/Mus are primarily due to inactivation of this site.

NAc shell. Figure 8B illustrates the placement of all acceptable infusions in the NAc shell group. The rats that was removed from data analysis received infusions that were ventral to the shell region. Infusions of baclofen/muscimol did not reduce the preference for the HR lever (43 +/- 22%) when compared to saline infusion (40 +/- 22%).

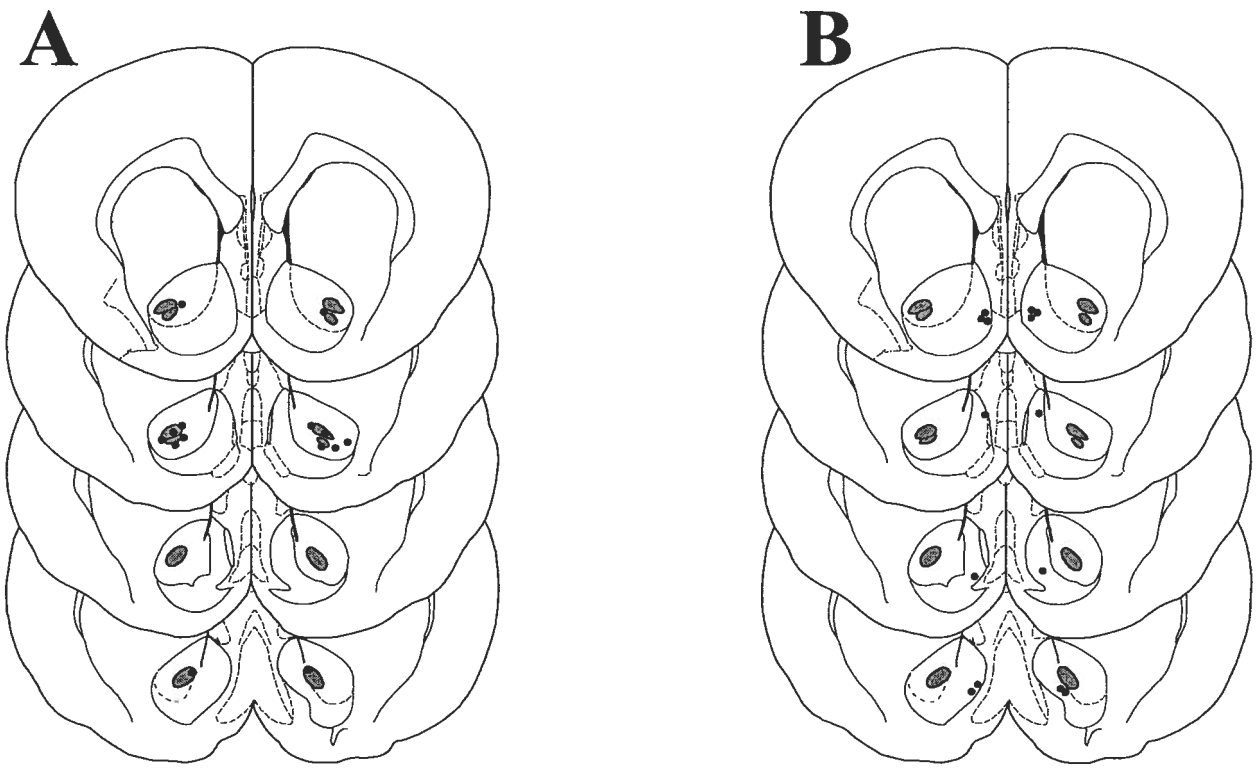
DISCUSSION

Our findings indicate that inactivation of the NAc core, but not the shell, reduces the preference for larger rewards associated with a greater effort cost. Furthermore, the effects of core inactivation do not appear related to an intolerance for delays to reward delivery incurred when rats were required to press a lever multiple times to obtain the larger magnitude reward. Inactivation of the NAc core reduced the preference for the HR when a delay equal to the time it takes the rats to complete pressing the HR was implemented on the LR lever, leading the rats to choose the smaller, delayed reward option more often. These reductions do not appear to be

Figure 8. Location of infusions for all rats in the (A) NAc Core group, and (B) NAc Shell group.

Figure 8

Histology



NAc Core **NAc Shell**

attributable to alterations in motor response or changes in satiety, since inactivation of this region did not alter the rates of lever pressing, nor did it disrupt choice behavior when the relative response cost of the HR and LR were equal.

A role for the NAc core in effort-based decision making

The finding that inactivation of the NAc core reduced preference for the larger, more costly reward is in keeping with other studies investigating the role of this nucleus in other forms of cost-benefit decision making. Cardinal and colleagues (2001) reported that excitotoxic lesions to the NAc core reduced the preference for larger delayed rewards. Likewise, Botvinick et al. (2009), have shown that in humans, the core region of this nucleus (in addition to other regions involved in decision making) appears to mediate effort-based decision making when the cost to the more beneficial reward was greater mental effort. Animal studies that have investigated the role of the NAc in facilitating effort-related decisions have shown that manipulations of this region interfere with certain forms of choice behavior. In particular, disrupting DA activity in the NAc decreases preference for the larger rewards associated with a greater effort cost using a variety of different paradigms. For example, on a t-maze based task where one arm of the maze contains a scalable barrier leading to a higher density of food, local infusions of DA antagonists into the NAc or 6-OHDA lesion of the DA terminals in this nucleus reduced choice to exert more effort to obtain the larger reward (Cousins, Atherton, Turner, Salamone, 1996; Salamone et al. 1994). Similar findings have been observed using a concurrent choice task. Here, rats have a choice between pressing a lever multiple times to receive a preferred food or consuming less-preferred lab chow that is freely available. Under these conditions, DA antagonism or lesions to the DA terminals within the NAc reduce lever pressing and increase chow consumption (Salamone et al. 1991; Cousins, Sokolowski, Salamone, 1993; Cousins and Salamone 1994; Sokolowski and Salamone 1998; Nowend, Arizzi, Carlson, and Salamone, 2001). In most of

these studies, the specific subregion of the NAc that mediated these effects was not investigated directly, yet, two studies did attempt to distinguish the roles of the core and shell (Sokolowski and Salamone, 1998; Nowend et al., 2001). These studies used a concurrent choice paradigm in combination with either DA lesions of the NAc (Sokolowski and Salamone, 1998) or local D1/D2 antagonism (Nowend et al., 2001). The results of these studies showed that there appeared to be a dissociable role for the subregions of NAc in effort-based decision making. While their findings supported a role for the core region in effort-based decision making, Sokolowski and Salamone (1998) reported substantial loss of DA in the shell region, making it difficult to conclude that their effects were due solely to reduced DA activity in the core. Similarly, infusions of antagonists into either the core or shell resulted in reduced lever pressing for the preferred food, however the reduction of lever press following shell infusions were not as robust as that following core infusions (Nowend et al., 2001). Furthermore, the infusion volumes used in this study were substantially larger than the ones employed here (0.5 µl per side, as opposed to 0.3 µl in the present study), and may thus have diffused from the shell region to the core. By utilizing smaller infusion volumes of GABA agonists that have been effective at dissociating the roles of these different subregions of the NAc in other forms of behavior (McFarland, Davidge, Lapish, Kalivas, 2004; Floresco et al., 2006, 2008a), the present results indicate that neural activity (not just DA activity) in the core, but not the shell subregion of the NAc appears to be of primary importance in mediating effort-related decisions.

The fact that dopaminergic manipulations or inactivation of the NAc core reduces the preference for the "better" but more costly reward contrasts with other studies investigating the contribution of the NAc to other forms of decision making entailing delay-related costs. For example, as mentioned earlier, excitotoxic lesions to the NAc core increased delay discounting whereby rats were less likely to chose a larger, delayed reward (Cardinal et al., 2001). In a

recent study, we showed that systemic injections of amphetamine induced dose-dependent effects on effort- and delay-based decision making (Floresco et al., 2008). At a lower dose there was an increase in willingness to work harder or wait longer for a larger reward – an effect likely attributable to an increased DA transmission. However, Winstanley and colleagues (2005) reported that 6-OHDA lesions of DA terminals in the NAc did not alter delay-based decision making using a paradigm similar to that used by Cardinal et al. (2001) (Winstanley, Theobald, Dalley, Robbins, 2005). In addition, depletion of DA in the NAc did not interfere with the ability of amphetamine to reduce impulsive choice, indicating that although intrinsic neurons of the NAc mediate choice behavior related to delay discounting, DA transmission in this nucleus does not (Winstanley et al., 2005). As such, it is evident that the neural mechanisms through which the NAc core contributes to effort- and delay-based decision making differ. While both delay and effort-based decision making require intact NAc functioning, mesoaccumbens DA does not seem to be required for normal delay-based decision making, but is an integral component of effort-based decision making. Our experiment, using an equivalent delays procedure, provides further evidence for the dissociation of the neural mechanisms underlying delay- and effort-related decision making.

It is important to highlight the fact that under most conditions, response options that require greater effort to obtain a reward are typically confounded with a delay to reward delivery from the point that the animal makes a choice to the completion of the effort requirement. As noted above, lesions or inactivation of the NAc interfere with both delay and effort-based decision making (Cardinal et al., 2001; Salamone et al., 1994). Thus, it is difficult to parse out whether effects on effort-discounting observed here are due to a reduced preference to work harder to obtain a larger reward, or a reduced tolerance for delays to reward incurred when the animal was required to press a lever multiple times to obtain the HR. In the present study, we

introduced a control that consisted of equalizing delays to either reward. Our equivalent delays procedure permitted a selective evaluation of the role of the NAc in effort-related decisions without the interference of delay costs. In comparison to the standard effort discounting task, when animals were retrained on the equivalent delays procedure, there was a noticeable increase in the preference for the HR lever across all trial blocks; an effect that has been reported previously (Floresco et al., 2008a). There are two possible explanations for the increased preference for the HR that occurs under these conditions. First, it could be that the additional training resulted in the rats becoming more efficient at pressing the HR lever, making it easier for them to obtain the HR. However, rats had received close to 30 days of training on the original discounting task, and received less than 10 days of training with the equivalent delays procedure. Furthermore, rates of responding (or the number of presses/s) on the HR lever did not differ significantly between the two tasks, rendering this explanation unlikely. That is, rats were responding as robustly on the HR lever on the standard effort discounting task as they did on the effort with equivalent delays task when treated with saline ($F(3,18) = 0.388$, NS). An alternative and perhaps more viable account for these results is that by equalizing the delay costs across both response options, this manipulation effectively makes selection of the HR lever less costly when compared to the standard task, leading to an increased choice of the HR lever. Simply put, when the relative delay cost was equalized on both levers, rats showed an increased preference to work harder for a larger reward rather than just wait for a smaller reward.

Despite the finding that delays to reward delivery intertwined with high effort response options do play a role in biasing choice behavior, inactivation of the NAc core was still effective at reducing the preference for the HR lever when rats were tested on the equivalent delays procedure. This is a key finding in that it demonstrates that the effect of NAc core inactivations on effort discounting are not simply due to a reduced tolerance of the delays to reward delivery

associated with increasing effort requirements. This finding, when viewed in light of the broader literature on the ventral striatum and decision making, suggest that the NAc plays a prominent role in multiple forms of cost-benefit decision making, allowing animals to evaluate the cost to potential rewards, regardless of the type of cost (effort, delay, etc.).

The NAc core, and in particular, DA transmission in this nucleus has been implicated in motivational processes (Salamone et al., 1997). For example it was shown that NAc DA depletions reduced lever pressing on FR5 schedule whereas FR1 schedules were not altered (Mingote, Weber, Ishiwari, Correa, Salamone, 2005). Indeed, the decreased lever responding seems to be directly related to the ratio of lever press requirements (Hamill, Trevitt, Nowend, Carlson, Salamone, 1999). The NAc has also been implicated in different forms of discriminative learning. Cell body lesions or DA receptor blockade in the NAc core have been shown to reduce responding for conditioned stimuli associated with reward in Pavlovian and instrumental approach tasks (Parkinson, Willoughby, Robbins, Everitt, 2000; Di Ciano, Cardinal, Cowell, Little, Everitt, 2001; Cardinal, Parkinson, Everitt, 2002; Floresco et al., 2008b). Therefore, it may be argued that the disruptions in effort discounting reported here are attributable to either a reduction in motivation or an impaired ability to discriminate between the two levers associated with different magnitudes of reward. However, several lines of evidence dispute this notion. Even though inactivation of the NAc core reduced choice of the HR lever, when animals did select this lever, their rates of lever pressing on the HR lever did not differ between treatments since rats responded as robustly as they did after saline infusion. Likewise, inactivation of the NAc did not increase the number of trial omissions. On the other hand, if these treatments merely disrupted the ability to distinguish between the levers, one would expect that rats would choose both levers equally and at random over the duration of the session. This was not the case as rats continued to display a prominent discounting curve after infusion of

GABA agonists into the NAc. More important however, is the finding that NAc inactivation did not reduce the choice of the HR on the Equivalent Effort task, indicating that rats were able to distinguish between larger and smaller rewards. This latter finding is in keeping with other studies that have employed either DA or cell body lesions of the NAc (e.g. Salamone et al. 1991; Cousins et al. 1993; Cousins and Salamone 1994; Sokolowski and Salamone 1998; Cardinal et al., 2001). This indicates therefore that the role of the NAc in different forms of decision making does not seem to be limited to judgments about larger/smaller rewards. Rather the contribution of this nucleus to choice behavior appears to be more prominent under conditions that require integration of information about both differential costs and reward magnitudes that may be associated with different courses of action.

One important measure on behavioral tasks involving conflicting response options is response latency. In the standard effort-discounting task, infusion of GABA agonists into the NAc resulted in rats taking more time to choose between the two options. However, when tested with the equivalent delays procedure, NAc inactivations were no longer effective in altering response latencies. This lack of effect on the latter task may merely imply that with additional training, the effect on response latencies is diminished. However, a more intriguing explanation is that when the relative delay cost associated with the HR was reduced, animals were confronted with a relatively easier choice compared to the standard effort discounting task. In other words, the reduction of response conflict resulted in the elimination of a response latency effect. This would suggest that the ability of the NAc core to modulate choice latencies may be dependent on the relative difficultly of the type of decision animals are required to make.

NAc shell is not important for effort-based decision making.

Previous studies have indicated that the shell region of the nucleus accumbens is involved in functions such as associative learning, stress-induced reinstatement of drugs, cue-induced reinstatement of food seeking behavior (McFarland et al., 2004; Floresco et al., 2008b), and learning about the irrelevance of stimuli (latent inhibition) (Weiner and Feldon, 1997). Other studies have indicated that this region does not substantially contribute to cost-benefit decision making (e.g.; Pothuizen et al., 2005; Sokowloski and Salamone, 1998). The results from the present study suggests that neural activity within this region of the NAc does not make an essential contribution to decision making when animals overcome effort-related costs to obtain larger rewards. Inactivation of this region did not alter choice behavior using the standard effort discounting task or on effort with an equivalent delays procedure. The lack of effect of shell inactivation is in keeping with previous studies investigating the role of DA in this nucleus. Sokolowski and Salamone (1998) reported that DA lesions of the shell region did not appear to decrease the preference to work harder to receive a preferred food; however as mentioned earlier they could not make a firm conclusion due to the large size of their lesion.

It is interesting to point out that although NAc shell inactivations had no effect on preference for the HR in the standard effort-discounting task, there was a slight increase in the selection of the HR in the effort discounting with equivalent delays. In this regard, it is of interest to note that the 3 rats that showed an increase in the preference for the HR when this region was inactivated, received infusions in the more rostral area of the shell. (Reynolds and Berridge, 2002; Faure et al., 2008). Therefore, this increase in the willingness to exert more effort to obtain a larger reward may have been the result of increased desire for food, similar to that observed by Reynolds, and Berridge, and Faure and colleagues (2002 and 2008 respectively). However, given the lack of statistical significance between the treatment

conditions, this conclusion may be premature. Future studies investigating the role of the NAc shell in cost-benefit decision making need to delve further into how this region may be mediating this form of decision-making.

CONCLUSION

The current findings show a functional dissociation between the NAc core and shell in effort-based decision making. Inactivation of the NAc core using the $GABA_{A/B}$ agonist Bac/Mus reduces the preference to work harder for a larger magnitude reward, whereas the same treatment within the shell region does not alter choice behavior. Furthermore, the contributions of the NAc core in this form of decision making are independent of its role in delay-based decision making. Thus it seems that when the NAc receives input from other cortical and limbic structures, the core likely acts as relay point where information regarding competing costs converges, and is then filtered into appropriate regions to bias the direction of behavior.

It is apparent that the contribution of the NAc to different forms of decision making and choice behavior is critically dependent on inputs from different cortical and limbic regions in the frontal and temporal lobes (Salamone et al., 2007). Indeed, given its location in the brain as well as the efferent and afferent connections to corticolimbic and motor effector regions, it is suggested that the NAc serves as a "limbic-motor interface," (Mogenson et al., 1980; Nicola, 2007; Floresco, 2007). Two such inputs that have been shown to play a role in effort-related decisions are the ACC (Walton, Bannerman, Rushworth, 2002; Schweimer and Hauber, 2005), and the BLA (Floresco and Ghods-Sharifi, 2007), as lesions or inactivation of these regions reduce preference for larger rewards associated with a greater effort cost, in a manner similar to inactivation of the NAc. In addition, disconnection between the BLA and ACC also disrupted effort-based decision making, indicated that the integration of effort-related costs is mediated by this circuit (Floresco and Ghods-Sharifi, 2007). In other forms of cost/benefit decision making,

different regions of the prefrontal cortex play a role depending on the types of costs involved. For example, studies of risk-based decision making entailing a choice between small, certain and large, uncertain rewards have shown that in rats the prelimbic region of the medial PFC appears to facilitate choice (St. Onge and Floresco, 2008). On the other hand, the orbitofrontal cortex seems to be critical in mediating decisions in delay discounting tasks (e.g.: Mobini et al., 2002; Winstanley et al., 2004; Zeeb, Floresco and Winstanley, 2007). Thus, it is apparent that different forms of cost/benefit evaluations are mediated by distinct cortico-limbic circuitry. However, the present data, in addition to previous findings, indicate that the NAc plays a fundamental role in integrating information processed by these different circuits in order to bias choice towards options that yield more favorable rewards.

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