Published in final edited form as:

Behav Brain Res. 2014 July 15; 268: 159–168. doi:10.1016/j.bbr.2014.03.044.

Partial inactivation of nucleus accumbens core decreases delay discounting in rats without affecting sensitivity to delay or magnitude

Travis M. Moschak, B.S. and Suzanne H. Mitchell, Ph.D.

Oregon Health & Science University

Abstract

Increased preference for smaller, sooner rewards (delay discounting) is associated with several behavioral disorders, including ADHD and substance use disorders. However, delay discounting is a complex cognitive process and the relationship is unclear between the pathophysiology of the disorders and the component processes underlying delay discounting, including sensitivity to reinforcer delay and sensitivity to reinforcer magnitude. To investigate these processes, male Long Evans rats were trained in one of three tasks measuring sensitivity to delay, sensitivity to magnitude, or both (typical delay discounting task). After learning the task, animals were implanted with bilateral cannulae into either the nucleus accumbens core (AcbC) or the lateral orbitofrontal cortex (IOFC), both of which have been implicated in delay discounting. Upon recovering from the surgery, a baclofen/muscimol cocktail was infused to temporarily inactivate each of these two regions and task performance was assessed. Unlike previous studies showing that lesions of the AcbC *increased* delay discounting, partial inactivation of the AcbC *decreased* delay discounting, although it had no effects on the tasks independently assessing either sensitivity to delay or magnitude. The effects of AcbC inactivation were larger in animals that had low levels of delay discounting at baseline. Inactivation of the IOFC had no effects on behavior in any task. These findings suggest that the AcbC may act to promote impulsive choice in individuals with low impulsivity. Furthermore, the data suggest that the AcbC is able to modulate delay and magnitude sensitivity together, but not either of the two in isolation.

Keywords

Delay discounting; Impulsive choice; intertemporal choice; Nucleus Accumbens; Orbitofrontal cortex; Rats

© 2014 Elsevier B.V. All rights reserved.

Correspondence: Travis Moschak, Department of Behavioral Neuroscience L470, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Portland, Oregon, U.S.A. 97239-3098, Fax: (503) 494-6877, Telephone: (503) 494-8464, moschakt@ohsu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Excessive impulsivity has been implicated in a number of behavioral disorders, including ADHD and substance use disorders (Winstanley et al., 2006; Crews & Boettiger, 2009). One measure of impulsivity associated with these disorders is the delay discounting task, in which an individual must choose between a smaller, sooner reinforcer and a larger, later reinforcer. Several pharmacological agents, including both treatments and drugs of abuse, affect delay discounting, and multiple brain regions are thought to mediate these effects (Pattij & Vanderschuren, 2008; Cardinal, 2006). Two brain regions that have been heavily implicated in both delay discounting and the disorders mentioned above are the nucleus accumbens core (AcbC) and the orbitofrontal cortex (OFC), (Winstanley et al., 2006; Crews & Boettiger, 2009).

The AcbC is thought to play a role in promoting choice of the larger, later reward, based on evidence that *lesioning* the AcbC *decreases* choice of the larger, later reward (Cardinal et al., 2001; Pothuizen et al., 2005; Bezzina et al., 2007; Bezzina et al., 2008; da Costa Araújo et al., 2009; Galtress & Kirkpatrick, 2010; but see Acheson et al., 2006). Conversely, the role of the OFC is less clear. Some studies have found that lesions increase delay discounting (Mobini et al., 2002; Kheramin et al., 2002; Kheramin et al., 2003; Rudebeck et al., 2006), one has found that lesions decrease discounting (Winstanley et al., 2004), and some have found no effect (Mariano et al., 2009; Finger et al., 2011; Abela et al., 2013; Jo et al., 2013). These discrepant findings have been suggested to be the result of procedural differences (Zeeb et al., 2010) and/or the regional specificity of the lesions (Mar et al., 2011).

Although both the AcbC and OFC have been implicated in discounting, it is unknown whether their role in discounting is mediated through sensitivity to reinforcer delay or sensitivity to reinforcer magnitude. Both delay and magnitude sensitivity are important components of delay discounting (Ho et al., 1999; Killeen, 2009; Logue et al., 1984), and determining the neurological underpinnings of these components may increase our understanding of discounting and its role in various behavioral disorders. Mathematical analysis of delay discounting data has suggested that the AcbC may modulate sensitivity to delay, while the OFC may modulate sensitivity to delay *and* magnitude (Kheramin et al., 2002; Bezzina et al., 2007; but see Galtress & Kirkpatrick, 2010). However, because these studies used a delay discounting task that manipulated delay and magnitude simultaneously, they were unable to *directly* measure sensitivity to delay or magnitude in isolation. To address this issue, a recent study by da Costa Araújo et al. (2010) developed a method to directly assess sensitivity to delay or magnitude using two separate tasks. In agreement with the previous studies, the authors found that rats exposed to the task requiring sensitivity to delay had increased neuronal activity (c-fos counts) in both the AcbC and OFC, but that rats exposed to the task requiring sensitivity to magnitude only had increased neuronal activity in the OFC. Nonetheless, because the authors did not directly manipulate either of the two brain regions, a direct causal role in sensitivity to delay and magnitude has not yet been established.

To determine the existence of such a role, we separately inactivated these regions in rats and measured their performance in a task measuring sensitivity to delay *and* magnitude (delay discounting), and in two tasks that separately measured sensitivity to delay *or* sensitivity to magnitude. We hypothesized that AcbC inactivation would increase delay discounting and increase sensitivity to delay, but have no effect on sensitivity to magnitude. Additionally, we hypothesized that OFC inactivation would impact each of the three measures, but we did not specify a direction for these effects due to the mixed nature of the OFC discounting literature.

We were also interested in the role that individual differences might play in these effects. Individual differences in delay discounting have been correlated with differences in medial prefrontal cortex physiology (Loos et al., 2010; Simon et al., 2013), a region that receives projections from the OFC and sends projections to the OFC and AcbC (Öngür & Price, 2000). Furthermore, a recent study by Zeeb et al. (2010) found that inactivation of the lateral OFC (lOFC) decreased delay discounting in animals that discounted delayed rewards more at baseline, but had no effect on animals that exhibited low levels of discounting at baseline. Therefore, we investigated whether individual differences in behavior would modulate the effects of inactivation on performance in any of the three tasks.

2. Methods

2.1 Subjects

Male Long Evans rats ($n = 96$) were purchased from Charles River Laboratories (Hollister, CA). Animals weighed approximately 275 – 300 g and were 8 – 9 weeks old upon arrival. Rats were housed two per cage in a temperature-controlled vivarium under a 12:12 light-dark cycle (lights on at 0600) at Oregon Health & Science University. All procedures were approved by the Institutional Animal Care and Use Committee and adhered to NIH guidelines. Animals were habituated to handling for a week and reduced to approximately 85% free-feeding body weight for the duration of the study. Animals were run in two separate cohorts ($n = 48$ each).

2.2 Apparatus

Eight operant chambers ($25 \times 32 \times 26$ cm; Med Associates, Inc., St. Albans, VT, USA) housed in sound-attenuating cabinets ($40 \times 64 \times 42$ cm) equipped with ventilation fans were used in this study. The front, back, and top walls of each chamber were composed of clear acrylic and the left and right walls were made of stainless steel. The left wall of each chamber contained a central houselight as well as left and right cue lights, non-retractable levers, and liquid receptacles capable of delivering sucrose (20 % w/v) from a computer-controlled pump. Between the receptacles was a nosepoke with a built-in cue light. The right wall contained a response clicker. The chamber had a grid floor with a metallic pan underneath containing bedding. All input/output was controlled by a computer using MED-PC (Med Associates, Inc.).

2.3 Procedure

2.3.1 Training—All animals underwent training in three distinct phases, and completed each phase after obtaining at least 45 reinforcers for two consecutive days. In Phase 1, animals completed training under a concurrent VT-120 s FR-1 schedule for each lever/feeder. In Phase 2, animals were required to nosepoke the center aperture before the FR-1 contingency would operate on either lever (the VT-120 s contingency was not continued). In Phase 3, animals were required to alternate their responding for either the left or right lever.

2.3.2 General Procedure (Fig. 1)—After completing training, animals were randomly assigned to one of three tasks: within sessions task (discounting task), adjusting delay task (delay task), or adjusting magnitude task (magnitude task). The within sessions discounting task was a modification of a procedure developed by Evenden & Ryan (1996). The delay and magnitude tasks were modified from those used by Moschak & Mitchell (2013) and originally developed by Mazur (1984) and da Costa Araújo et al. (2010). In these two tasks, high sensitivity to delay is measured as low adjusting delay, while high sensitivity to magnitude is measured as high adjusting magnitude (defined below). We have previously shown that both tasks are sensitive to acute manipulations (Moschak & Mitchell, 2013).

All tasks used the same basic structure. Each trial began with the illumination of the central aperture. If the rat poked its nose into the aperture, the light was extinguished and the lights above both levers were illuminated (free choice). If the animal pressed one of the levers, the lights were extinguished and the contingencies associated with that lever went into effect, followed by an intertrial interval (ITI). After two consecutive choices of the same lever, a forced trial occurred wherein only the light over the previously nonselected lever was illuminated and only responses on that lever resulted in a continuation of the trial. The ITI was adjusted to ensure that the duration from the lever press to the next trial was always 27 s. Animals performed in the task until 60 trials were completed or until 60 min had elapsed, whichever occurred first.

2.3.2.1 Within sessions task (Fig. 1a): All animals were assigned an immediate and a delayed reward lever. A press on the immediate reward lever resulted in immediate delivery of 50 μ l sucrose. A press on the delayed reward lever resulted in delivery of 150 μ l sucrose after a delay that increased after each block of 12 trials. The delay was set to 0, 2.5, 5, 10, and 20 s for each block.

2.3.2.2 Adjusting delay task (Fig. 1b): All animals were assigned a variable and an adjusting lever. A response on the variable lever resulted in the delivery of a liquid sucrose reinforcer (75 μ l) after 0 or 20 s (probability = .5). A response on the adjusting lever resulted in the delivery of 75 μ l of sucrose after t s, where t represents the adjusting delay. Variable lever choice decreased the adjusting delay by 10%, while adjusting lever choice increased the adjusting delay by 10%. Percentages were used instead of fixed values due to the psychophysical relationship between the actual length of the delay length and perceived length of the delay. The adjusting delay was 5 s at the start of each session, and could not go above 22 s or below 0.1 s.

2.3.2.3 Adjusting magnitude task (Fig. 1c): All animals were assigned a variable and an adjusting lever. A response on the variable lever yielded 0 μ l or 150 μ l of sucrose (probability = .5) delivered immediately. A response on the adjusting lever immediately yielded a sucrose reinforcer with a magnitude of m μ l, where m represents the adjusting magnitude. Variable lever choice increased the adjusting magnitude by 10%, while adjusting lever choice decreased the adjusting magnitude by 10%. The adjusting magnitude was 37.5 μ l at the start of each session, and could not go above 165 μ l or below 0.6 μ l.

Animals' behavior had to reach stability in the task both before and after surgery. To test stability, data from the most recent 10 sessions were split into two sets of 5, and the average was taken for each of these sets. The data for each task were further split into five 12-trial blocks to accommodate the different delays of the discounting task. Behavior was considered stable when a 2×5 ANOVA (Set $\times$ Block) indicated no significant difference between the first and second set of sessions across each block of 12 trials for the dependent measure of interest (i.e. adjusting delay, adjusting magnitude, choices of delayed lever). Animals required 52.7 ± 0.55 sessions to attain stability.

2.4 Surgery

Animals were anesthetized using 2–5% isoflurane during surgery and were administered subcutaneously 100,000 units of penicillin to prevent infection and 5 mg/kg of carprofen as an analgesic. Bilateral 21-gauge cannulae were inserted with coordinates of either +3.7 mm anteroposterior (AP) from bregma, ± 2.6 mm mediolateral (ML) from bregma, and -3.3 dorsoventral (DV) from the skull surface (IOFC) or +1.6 mm AP, ± 1.8 mm ML, and -3.3 DV (AcbC). Metal stylets extending 0.5 mm beyond the tip of the cannulae were inserted to prevent clogging. On test days, IOFC injectors extended 1 mm beyond the tip of the cannulae, while AcbC injectors extended 3.5 mm beyond the tip of the cannulae. Animals were given a minimum of 5 days recovery post-surgery before resuming behavioral testing.

2.5 Drugs

Baclofen and muscimol (Sigma-Aldrich, St. Louis, MO, USA) were both separately dissolved in 0.9% saline at a concentration of 0.5 g/mL and stored in frozen aliquots. On each injection day, baclofen and muscimol were thawed and mixed together. For IOFC injections, rats were given 125 ng / 125 ng of baclofen/muscimol in 0.5 μ l/hemisphere delivered over the course of 1 min 40 s (St. Onge & Floresco, 2010). Preliminary data showed that our initial dose of 75 ng / 75 ng baclofen/muscimol in the AcbC greatly reduced responding, so rats were instead given 6.409 ng / 0.342 ng of baclofen/muscimol in 0.3 μ l/hemisphere delivered over the course of 1 min (equivalent to the 0.03 nmol / 0.003 nmol used to successfully block reinstatement in McFarland & Kalivas, 2001). Injectors were left in the cannulae for 1 min following infusion to allow diffusion of the drugs.

Injections were administered on Tuesdays and Fridays. All animals initially received a saline injection to habituate them to the procedure. They then were administered 3 injections each of saline and baclofen/muscimol according to an alternating schedule (6 injections total). Half of the animals began with saline; the other half with baclofen/muscimol.

2.6 Histology

Following testing, animals were sacrificed by carbon dioxide exposure. Brains were removed and stored in 2% paraformaldehyde for 24 hours, followed by storage in 20% and then 30% sucrose/0.1% sodium azide. Sections (40 μ m) were sliced on a cryostat, mounted on slides, and subsequently stained with thionin. These were then examined under a microscope and injector tip placements were mapped onto a rat atlas (Paxinos & Watson, 1998; see Fig. 2).

2.7 Data Analysis

The dependent measures for each of the three tasks were number of choices of the delayed lever, the adjusting delay, and the adjusting magnitude. All data were averaged across the 3 injection sessions for each dose after removing the excluded data listed below. Data were subsequently organized into five 12-trial blocks to accommodate the different delays used in each 12-trial block in the discounting task. The data were analyzed with a 2×5 (Dose $\times$ Block) ANOVA to compare saline with baclofen/muscimol (there were no effects of saline itself on the dependent measures, $F_s < 1.99$, $p_s > 0.191$). Additionally, we divided animals into 'high' and low' groups to determine if baseline task performance modulated the effects of inactivation. To do this, we first averaged the data for each rat across the final 5 days before the injection phase began. Using this data, we calculated the average for all five blocks for each rat, and grouped animals according to a median split. The injection data were analyzed for Groups with a $2 \times 2 \times 5$ ANOVA (Group $\times$ Dose $\times$ Block). Lastly, to evaluate rats' ability to complete the task, we analyzed the number of trials completed in the task, latency data, and number of extraneous lever presses using a 3×2 ANOVA (Task $\times$ Dose), including data from sessions with uncompleted trials. Huynh-Feldt-corrected degrees of freedom were used wherever there were violations of sphericity and Bonferroni post hoc tests were used to compare effects at individual blocks.

2.8 Exclusions

Final group sizes were: AcbC: discounting task, $n = 12$; delay task, $n = 10$; magnitude task, $n = 10$. IOFC: discounting task, $n = 11$; delay task, $n = 12$; magnitude task, $n = 11$. Of the initial 96 animals, 10 animals died (discounting task, $n = 2$; delay task, $n = 3$; magnitude task, $n = 5$) and 16 animals were excluded due to poor cannulae placement (AcbC delay task, $n = 2$; IOFC discounting task, $n = 4$; IOFC delay task, $n = 9$; IOFC magnitude task, $n = 1$). The first cohort contained equal numbers of animals for each task and brain region ($n = 8$ each). However, since some groups in the first cohort lost disproportionate numbers of animals, the second cohort was uneven (AcbC discounting task = 6, AcbC delay task = 6, AcbC magnitude task = 6, IOFC discounting task = 12, IOFC delay task = 13, IOFC magnitude task = 5). Nonetheless, there were no significant differences in dependent variables between cohorts ($F_s < 3.30$, $p_s > 0.103$).

Sessions on which animals did not respond for all 60 free choice trials (AcbC: 18 injections, 10.1%; IOFC: 41 injections, 19.9%) were removed from the analysis of the primary dependent variables because the missing data for the final blocks of trials in those sessions rendered them incomparable to completed sessions. Four animals were removed from the primary analysis because they did not complete all 60 trials on any session (AcbC

discounting task, $n = 1$; IOFC discounting task, $n = 3$). It should be noted that only analyzing the first four blocks of 48 trials (so as to increase the number of sessions and animals included in the analysis) did not alter the results. For number of trials completed, response latency, and extraneous responses, we analyzed two data sets: One comprising data without incomplete sessions (identical to the method described above), and one comprising all data. The latter analysis is possible in this case because the data were not split into blocks.

3. Results

AcbC inactivation significantly increased choice of the large reinforcer at long delays (Dose $\times$ Delay: $F(2.43, 26.70) = 3.90$, $p = 0.026$; see Fig. 3a), suggesting that longer delays reduced reinforcer value less when the AcbC was inactivated than reductions seen following saline infusions. Furthermore, this effect was almost entirely driven by low discounters (Group $\times$ Dose: $F(1, 10) = 5.53$, $p = 0.041$; Group $\times$ Dose $\times$ Delay: $F(2.37, 23.71) = 3.17$, $p = 0.053$). Simple effects analyses show that low discounters strongly decreased discounting after inactivation of the AcbC (Dose $\times$ Delay: $F(4, 20) = 6.96$, $p = 0.001$) but that high discounters were unaffected (Dose $\times$ Delay: $F(4, 20) = 0.33$, $p = 0.856$; see Fig. 4a,b).

AcbC inactivation did not alter adjusting delay or adjusting magnitude ($F(1, 9) = 0.68$, $p = 0.432$; $F(1, 9) = 0.01$, $p = 0.910$; see Fig. 3).

IOFC inactivation did not alter delay discounting, adjusting delay, or adjusting magnitude (Discounting: $F(1, 10) = 2.00$, $p = 0.188$; Adjusting Delay: $F(1, 10) = 0.00$, $p = 0.960$; Adjusting Magnitude: $F(1, 10) = 0.01$, $p = 0.918$; see Figs. 3,5).

AcbC inactivation did not affect the number of trials completed in the tasks (saline: 58.12 ± 0.67 trials completed, baclofen/muscimol: 58.37 ± 0.92 trials completed; $F(1, 31) = 0.10$, $p = 0.756$). In contrast, IOFC inactivation decreased the number of trials completed in the tasks (saline: 58.89 ± 0.40 trials completed, baclofen/muscimol: 54.28 ± 1.46 trials completed; $F(1, 34) = 12.07$, $p = 0.001$). This was likely caused by an increase in both nosepoke latency (saline: 5.35 ± 0.65 s, baclofen/muscimol: 7.72 ± 0.90 s; $F(1, 34) = 7.72$, $p = 0.009$) and lever press latency (saline: 3.24 ± 0.53 s, baclofen/muscimol: 7.23 ± 2.14 s; $F(1, 34) = 5.19$, $p = 0.029$). These effects on latency were diminished when only including data in which animals completed all 60 trials, although there was still a strong trend towards an increase in lever press latency (nosepoke latency, saline: 4.59 ± 0.61 s, baclofen/muscimol: 5.45 ± 0.61 s; $F(1, 30) = 1.43$, $p = 0.241$; lever press latency, saline: 2.75 ± 0.26 s, baclofen/muscimol: 3.43 ± 0.42 s; $F(1, 30) = 3.81$, $p = 0.060$). A similar increase in response latency was seen by Zeeb et al. (2010), although to a lesser degree.

Additionally, both AcbC and IOFC inactivation increased the number of extraneous lever presses performed throughout the session, although this effect was much stronger after IOFC inactivation (AcbC: saline: 64.31 ± 7.94 presses, baclofen/muscimol: 79.64 ± 9.05 presses; $F(1, 31) = 5.72$, $p = 0.023$; IOFC: saline: 65.61 ± 8.81 presses, baclofen/muscimol: 110.42 ± 12.74 presses; $F(1, 34) = 23.76$, $p < 0.001$; there was no bias in this effect for one lever over the other for any group: $F_s < 2.00$, $p_s > 0.187$). Notably, this effect persisted even when only including data in which animals completed all 60 trials (AcbC: saline: 63.22 ± 7.86 presses,

baclofen/muscimol: 80.48 ± 9.31 presses; $F(1,30) = 6.88$, $p = 0.014$; IOFC: saline: 67.04 ± 9.39 presses, baclofen/muscimol: 118.41 ± 14.47 presses; $F(1,31) = 15.22$, $p < 0.001$). This suggests that the increase in response latency in IOFC rats is unlikely to be due to a decrease in motor function or motivation. Indeed, ability of IOFC inactivation to increase extraneous responses, but decrease the number of trials completed is very similar to that seen after lesion of the entire OFC (Chudasama et al., 2003).

4. Discussion

Our primary finding of interest was that inactivation of the AcbC decreased delay discounting, suggesting that the AcbC normally acts to promote impulsive choice. This result was surprising, given that the majority of studies have found that lesions of the AcbC *increase* delay discounting (Cardinal et al., 2001; Pothuizen et al., 2005; Bezzina et al., 2007; da Costa Araújo et al., 2009; Galtres & Kirkpatrick, 2010; but see Acheson et al., 2006 which lesioned the entire Acb, including shell). There are several possible explanations for this finding. One notable difference between our study and these studies was our use of reversible inactivations rather than lesions. Importantly, permanent loss of the AcbC may lead to compensation from other brain regions. Indeed, while AcbC lesions result in locomotor hyperactivity (Maldonado-Irizarry & Kelley, 1995; Parkinson et al., 1999), AcbC inactivations result in locomotor *hypo*activity (Fuchs et al., 2004; Ghods-Sharifi & Floresco, 2010). However, it is also important to note that the low dose used likely only resulted in a *partial* inactivation of the AcbC. Thus, it may be that “partial lesions” would have led to results similar to ours. Additionally, different GABA receptor subtypes have varying degrees of affinity for GABA agonists (Farrant & Nusser, 2005). Therefore, it may be that the low dose preferentially activated a subset of these receptors, which may in turn have yielded a different firing pattern than would be expected from a pure inactivation. Finally, it is possible that the AcbC inactivations also inactivated the nucleus accumbens shell. This may be particularly relevant considering the fact the lesion of the entire Acb (core + shell) has also been shown to decrease delay discounting (Acheson et al., 2006).

Although our results are at odds with the lesion studies, they do have some support in the human literature. Early imaging studies have suggested that increased ventral striatal activity is associated with choosing the immediate reward (McClure et al., 2004, 2007). However, subsequent studies have suggested that ventral striatal activity is associated with the overall subjective value of the reward, be it delayed or not (Kable & Glimcher, 2007; Sripada et al., 2010). It may be that partially inactivating the AcbC decreases animals’ ability to identify the subjective value of the reward, causing them to continue to choose the large reinforcer. However, if this were the case we would expect a similar perseverative result in the other two tasks. Thus, at present our data seem to favor the former account over the latter.

AcbC inactivation significantly decreased delay discounting in a task requiring *both* sensitivity to delay and reinforcer magnitude, but it did not modulate sensitivity to delay or reinforcer magnitude in isolation. This suggests that manipulating the AcbC only affects behavior that synthesizes both delay and magnitude. Such a conclusion appears to conflict with previous results, which suggest that the AcbC is involved in sensitivity to delay *per se* (Kheramin et al., 2002; Bezzina et al., 2007; da Costa Araújo et al., 2010). However, the

studies by Kheramin et al. (2002) and Bezzina et al. (2007) used tasks that manipulated both delay *and* magnitude, and had no explicit measure of delay sensitivity in isolation. On the other hand, the study by da Costa Araújo et al. (2010) used a task that independently assessed sensitivity to delay, but did not directly manipulate the AcbC. Although the authors found that exposure to the adjusting delay task increased c-fos counts in the AcbC, this does not establish a causal link between neuronal activity and behavioral output. Furthermore, there is some evidence that neurons differentially encode delay and magnitude depending on the task used. Roesch & Bryden (2011) noted that, when using tasks that *independently* manipulated delay and magnitude, many individual neurons in the ventral striatum encoded either delay or magnitude, but few encoded both. Conversely, in a study by Cai et al. (2011), wherein *both* delay and magnitude were manipulated within the same task, ventral striatal neurons that encoded the delay of the chosen reward were also highly likely to encode the magnitude of that reward. Thus, the results suggest that the firing patterns of neurons in the ventral striatum depended on whether the task included *both* delay and magnitude or only one of the two.

However, it is also possible that the procedural differences between the adjusting tasks and the discounting task were enough to obscure an effect on sensitivity to delay or magnitude in isolation. There were two main elements of the adjusting tasks that differed from the discounting task: the choice-dependent titrating design and the probabilistic component. However, there is little evidence that either of these would confound our experiment. A study using a titrating task found that lesions of the AcbC increased discounting (da Costa Araújo et al., 2009), so it seems unlikely that the titrating design would block an effect of AcbC inactivation. Additionally, although AcbC lesions have been shown to affect probability discounting (Cardinal & Howes, 2005), the evidence is mixed (Acheson et al., 2006) and inactivations of the AcbC have found no effect (Stopper & Floresco, 2011). However, one additional difference between the tasks is that the discounting task shifts bias from the delayed lever towards the immediate lever, while the adjusting tasks shift bias towards indifference between the two levers. More research would be required to investigate the role this might play in differentiating the two types of tasks.

In addition to the general effect on discounting, we found that inactivation of the AcbC predominantly decreased delay discounting in animals that had a low level of discounting to begin with. This may suggest that lower discounters have different AcbC physiology. Such individual differences in the physiological makeup of the nucleus accumbens have been associated with individual differences in a number of different behaviors, including both behavioral inhibition and the self-administration of cocaine (Dalley et al., 2007). Indeed, one study found that low discounters had more evoked dopamine released in the AcbC than high discounters (Diergaarde et al., 2008), although other studies found no relationship between levels of discounting and dopamine-related proteins in the AcbC (Loos et al., 2010; Simon et al., 2013). Nonetheless, it is also possible that the AcbC is either regulating or being regulated by another brain region that plays a more direct role in the individual differences seen in discounting. For example, the two aforementioned studies did find a relationship between delay discounting and levels of dopamine-related proteins in the medial prefrontal

cortex (Loos et al., 2010; Simon et al., 2013), which sends projections to the AcbC (Öngür & Price, 2000).

Inactivation of the IOFC did not affect delay discounting, which is in agreement with other studies using inactivation (Churchwell et al., 2009; Zeeb et al., 2010). However, we also did not see a specific decrease in delay discounting for animals with high baseline discounting, as was reported in Zeeb et al. (2010). Nonetheless, the effect in Zeeb et al.'s study was fairly small and occurred primarily at the 45 s time interval. Thus, a larger sample size and longer delays may have been required to replicate the effect. It may also be possible the inactivations also inactivated the medial OFC, which may have negated the effect because medial OFC lesions *increase* delay discounting (Mar et al., 2011).

In conclusion, we found that, contrary to most lesion studies, AcbC inactivation decreased delay discounting. In addition, we found that the AcbC did not modulate sensitivity to delay or magnitude when the two were independently assessed, which may indicate that the AcbC only modulates behavior synthesizing both delay *and* magnitude. We also found that the effect of AcbC inactivation on discounting was greatest in animals with low levels of discounting at baseline, and we believe that future studies should examine the role that the AcbC and its efferent/afferent projections play in these individual differences. Lastly, we found that the IOFC does not appear to play a role in any of the aforementioned processes. In total, these findings suggest a complex role for the AcbC in discounting, and support existing evidence that temporary inactivation of the IOFC has no effect on discounting.

Acknowledgments

TMM and SHM designed the study, TMM collected and analyzed the data, and TMM and SHM prepared the manuscript. The authors would like to thank Wesley Wenzel, Janelle Payano-Sosa, Katrina Bettencourt, Mary Ann Reeves, Christie Pizzimenti and Robbie Mills for technical assistance.

TMM was supported by NIH grant F31AA020741 and an N.L. Tartar Fellowship. SHM was supported by NIH grant R01 DA027580 and Portland Alcohol Research Center grant P60 AA10760.

References

- Abela AR, Chudasama Y. Dissociable contributions of the ventral hippocampus and orbitofrontal cortex to decision-making with a delayed or uncertain outcome. *European Journal of Neuroscience*. 2013; 37:640–647. [PubMed: 23190048]
- Acheson A, Farrar AM, Patak M, Hausknecht KA, Kieres AK, Choi S, de Wit H, Richards JB. Nucleus accumbens lesions decrease sensitivity to rapid changes in the delay to reinforcement. *Behavioural brain research*. 2006; 173:217–228. [PubMed: 16884790]
- Basar K, Sesia T, Groenewegen H, Steinbusch HW, Visser-Vandewalle V, Temel Y. Nucleus accumbens and impulsivity. *Progress in neurobiology*. 2010; 92:533–557. [PubMed: 20831892]
- Bezzina G, Body S, Cheung TH, Hampson CL, Bradshaw CM, Szabadi E, Anderson IM, Deakin JF. Effect of disconnecting the orbital prefrontal cortex from the nucleus accumbens core on inter-temporal choice behaviour: a quantitative analysis. *Behav. Brain Res*. 2008; 191:272–279. [PubMed: 18472170]
- Bezzina G, Cheung TH, Asgari K, Hampson CL, Body S, Bradshaw CM, Szabadi E, Deakin JF, Anderson IM. Effects of quinolinic acid-induced lesions of the nucleus accumbens core on inter-temporal choice: a quantitative analysis. *Psychopharmacology (Berl.)*. 2007; 195:71–84. [PubMed: 17659381]

- Cai X, Kim S, Lee D. Heterogeneous coding of temporally discounted values in the dorsal and ventral striatum during intertemporal choice. *Neuron*. 2011; 69:170–182. [PubMed: 21220107]
- Cardinal RN. Neural systems implicated in delayed and probabilistic reinforcement. *Neural Networks*. 2006; 19:1277–1301. [PubMed: 16938431]
- Cardinal RN, Howes NJ. Effects of lesions of the nucleus accumbens core on choice between small certain rewards and large uncertain rewards in rats. *BMC Neurosci*. 2005; 6:37. [PubMed: 15921529]
- Cardinal RN, Pennicott DR, Sugathapala CL, Robbins TW, Everitt BJ. Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science (New York, N.Y.)*. 2001; 292:2499–2501.
- Chudasama Y, Robbins TW. Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. *The Journal of neuroscience*. 2003; 23:8771–8780. [PubMed: 14507977]
- Churchwell JC, Morris AM, Heurtelou NM, Kesner RP. Interactions between the prefrontal cortex and amygdala during delay discounting and reversal. *Behavioral neuroscience*. 2009; 123:1185–1196. [PubMed: 20001103]
- Crews FT, Boettiger CA. Impulsivity, frontal lobes and risk for addiction. *Pharmacol. Biochem. Behav*. 2009; 93:237–247. [PubMed: 19410598]
- da Costa Araujo S, Body S, Hampson CL, Langley RW, Deakin JF, Anderson IM, Bradshaw CM, Szabadi E. Effects of lesions of the nucleus accumbens core on inter-temporal choice: further observations with an adjusting-delay procedure. *Behavioural brain research*. 2009; 202:272–277. [PubMed: 19463712]
- da Costa Araujo S, Body S, Valencia Torres L, Olarte Sanchez CM, Bak VK, Deakin JF, Anderson IM, Bradshaw CM, Szabadi E. Choice between reinforcer delays versus choice between reinforcer magnitudes: differential Fos expression in the orbital prefrontal cortex and nucleus accumbens core. *Behavioural brain research*. 2010; 213:269–277. [PubMed: 20570596]
- Dalley JW, Fryer TD, Brichard L, Robinson ES, Theobald DE, Laane K, Pena Y, Murphy ER, Shah Y, Probst K, Abakumova I, Aigbirhio FI, Richards HK, Hong Y, Baron JC, Everitt BJ, Robbins TW. Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *Science*. 2007; 315:1267–1270. [PubMed: 17332411]
- Diernaarde L, Pattij T, Poortvliet I, Hogenboom F, de Vries W, Schoffelmeer AN, De Vries TJ. Impulsive choice and impulsive action predict vulnerability to distinct stages of nicotine seeking in rats. *Biological psychiatry*. 2008; 63:301–308. [PubMed: 17884016]
- Evenden JL. Varieties of impulsivity. *Psychopharmacology (Berl.)*. 1999; 146:348–361. [PubMed: 10550486]
- Evenden JL, Ryan CN. The pharmacology of impulsive behaviour in rats: The effects of drugs on response choice with varying delays of reinforcement. *Psychopharmacology (Berl.)*. 1996; 128:161–170. [PubMed: 8956377]
- Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABAA receptors. *Nature Reviews Neuroscience*. 2005; 6(3):215–229.
- Finger BC, Deacon RMJ, Burns P, Campbell TG. The effects of lesions of the hippocampus or orbitofrontal cortex on an operant box based intertemporal choice task in mice. *Journal of Neuroscience, Psychology, and Economics*. 2011; 4:217–234.
- Fuchs RA, Evans KA, Parker MC, See RE. Differential involvement of the core and shell subregions of the nucleus accumbens in conditioned cue-induced reinstatement of cocaine seeking in rats. *Psychopharmacology*. 2004; 176(3–4):459–465. [PubMed: 15138757]
- Galtress T, Kirkpatrick K. The role of the nucleus accumbens core in impulsive choice, timing, and reward processing. *Behav. Neurosci*. 2010; 124:26–43. [PubMed: 20141278]
- Ho MY, Mobini S, Chiang TJ, Bradshaw CM, Szabadi E. Theory and method in the quantitative analysis of "impulsive choice" behaviour: Implications for psychopharmacology. *Psychopharmacology (Berl.)*. 1999; 146:362–372. [PubMed: 10550487]
- Jo S, Kim KU, Lee D, Jung MW. Effect of orbitofrontal cortex lesions on temporal discounting in rats. *Behavioural brain research*. 2013; 245:22–28. [PubMed: 23434604]

- Kable JW, Glimcher PW. The neural correlates of subjective value during intertemporal choice. *Nat. Neurosci.* 2007; 10:1625–1633. [PubMed: 17982449]
- Kheramin S, Body S, Ho M, Velazquez-Martinez D, Bradshaw C, Szabadi E, Deakin J, Anderson I. Role of the orbital prefrontal cortex in choice between delayed and uncertain reinforcers: A quantitative analysis. *Behavioural processes.* 2003; 64:239–250. [PubMed: 14580695]
- Kheramin S, Body S, Mobini S, Ho MY, Velazquez-Martinez DN, Bradshaw CM, Szabadi E, Deakin JFW, Anderson IM. Effects of quinolinic acid-induced lesions of the orbital prefrontal cortex on inter-temporal choice: A quantitative analysis. *Psychopharmacology (Berl.)*. 2002; 165:9–17. [PubMed: 12474113]
- Killeen PR. An additive-utility model of delay discounting. *Psychol. Rev.* 2009; 116:602–619. [PubMed: 19618989]
- Logue AW, Rodriguez ML, Pena-Correal TE, Mauro BC. Choice in a self-control paradigm: Quantification of experience-based differences. *J. Exp. Anal. Behav.* 1984; 41:53–67. [PubMed: 16812358]
- Loos M, Pattij T, Janssen MC, COUNOTTE DS, Schoffelman AN, Smit AB, Spijker S, van Gaalen MM. Dopamine receptor D1/D5 gene expression in the medial prefrontal cortex predicts impulsive choice in rats. *Cerebral Cortex.* 2010; 20:1064–1070. [PubMed: 19690230]
- Maldonado-Irizarry CS, Kelley AE. Excitotoxic lesions of the core and shell subregions of the nucleus accumbens differentially disrupt body weight regulation and motor activity in rat. *Brain Research Bulletin.* 1995; 38(6):551–559. [PubMed: 8590077]
- Mar AC, Walker AL, Theobald DE, Eagle DM, Robbins TW. Dissociable effects of lesions to orbitofrontal cortex subregions on impulsive choice in the rat. *J. Neurosci.* 2011; 31:6398–6404. [PubMed: 21525280]
- Mariano TY, Bannerman DM, McHugh SB, Preston TJ, Rudebeck PH, Rudebeck SR, Rawlins JN, Walton ME, Rushworth MF, Baxter MG, Campbell TG. Impulsive choice in hippocampal but not orbitofrontal cortex-lesioned rats on a nonspatial decision-making maze task. *European Journal of Neuroscience.* 2009; 30:472–484. [PubMed: 19656177]
- Mazur JE. Tests of an equivalence rule for fixed and variable delays. *J. Exp. Psychol. Anim. Behav. Process.* 1984; 10:426–436.
- McClure EB, Ericson KM, Laibson DI, Loewenstein G, Cohen JD. Time discounting for primary rewards. *J. Neurosci.* 2007; 27:5796–5804. [PubMed: 17522323]
- McClure SM, Laibson DI, Loewenstein G, Cohen JD. Separate neural systems value immediate and delayed monetary rewards. *Science.* 2004; 306:503–507. [PubMed: 15486304]
- McFarland K, Kalivas PW. The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *J. Neurosci.* 2001; 21:8655–8663. [PubMed: 11606653]
- Mobini S, Body S, Ho MY, Bradshaw CM, Szabadi E, Deakin JF, Anderson IM. Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. *Psychopharmacology (Berl.)*. 2002; 160:290–298. [PubMed: 11889498]
- Moschak TM, Mitchell SH. Sensitivity to reinforcer delay predicts ethanol's suppressant effects, but itself is unaffected by ethanol. *Drug Alcohol Depend.* 2013; 132:22–28. [PubMed: 23910798]
- Öngür D, Price JL. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cerebral Cortex.* 2000; 10:206–219. [PubMed: 10731217]
- Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ. Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by d-amphetamine. *The Journal of neuroscience.* 1999; 19(6):2401–2411. [PubMed: 10066290]
- Pattij T, Vanderschuren LJ. The neuropharmacology of impulsive behaviour. *Trends Pharmacol. Sci.* 2008; 29:192–199. [PubMed: 18304658]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 3rd edn.. San Diego: Academic Press; 1998. (1998).
- Pothuizen HH, Jongen-Relo AL, Feldon J, Yee BK. Double dissociation of the effects of selective nucleus accumbens core and shell lesions on impulsive-choice behaviour and salience learning in rats. *Eur. J. Neurosci.* 2005; 22:2605–2616. [PubMed: 16307603]

- Roesch MR, Bryden DW. Impact of size and delay on neural activity in the rat limbic corticostriatal system. *Front. Neurosci.* 2011; 5:130. [PubMed: 22363252]
- Rudebeck PH, Walton ME, Smyth AN, Bannerman DM, Rushworth MFS. Separate neural pathways process different decision costs. *Nat. Neurosci.* 2006:1161–1168. [PubMed: 16921368]
- Simon NW, Beas BS, Montgomery KS, Haberman RP, Bizon JL, Setlow B. Prefrontal cortical-striatal dopamine receptor mRNA expression predicts distinct forms of impulsivity. *The European Journal of Neuroscience.* 2013
- Sripada CS, Gonzalez R, Luan Phan K, Liberzon I. The neural correlates of intertemporal decision-making: Contributions of subjective value, stimulus type, and trait impulsivity. *Hum. Brain Mapp.* 2010
- St. Onge JR, Floresco SB. Prefrontal cortical contribution to risk-based decision making. *Cereb Cortex.* 2010; 20:1816–1828. [PubMed: 19892787]
- Stopper CM, Floresco SB. Contributions of the nucleus accumbens and its subregions to different aspects of risk-based decision making. *Cogn. Affect. Behav. Ne.* 2011; 11:97–112.
- Winstanley CA, Eagle DM, Robbins TW. Behavioral models of impulsivity in relation to ADHD: Translation between clinical and preclinical studies. *Clin. Psychol. Rev.* 2006; 26:379–395. [PubMed: 16504359]
- Winstanley CA, Theobald DE, Cardinal RN, Robbins TW. Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. *J. Neurosci.* 2004; 24:4718–4722. [PubMed: 15152031]
- Zeeb FD, Floresco SB, Winstanley CA. Contributions of the orbitofrontal cortex to impulsive choice: interactions with basal levels of impulsivity, dopamine signalling, and reward-related cues. *Psychopharmacology.* 2010; 211:87–98. [PubMed: 20428999]

Highlights

Inactivation of accumbens core decreased delay discounting.

The effect of accumbens core inactivation depended on baseline levels of discounting.

Inactivation of lateral orbitofrontal cortex did not affect discounting.

Inactivation of accumbens or orbitofrontal cortex did not alter sensitivity to delay or magnitude.

A) Delay Discounting Task

B) Adjusting Delay Task

C) Adjusting Magnitude Task

Figure 1.

Task schematics. A) The within sessions delay discounting task. Animals chose between an immediate, small reward and a large, delayed reward. The length of the delay increased every 12 trials (0, 2.5, 5, 10, 20 s). B) The adjusting delay task. Animals chose between a medium reward after an adjusting delay, or a medium reward after either no delay or a 20 s delay ($p = 0.5$ for either). C) The adjusting magnitude task. Animals chose between an immediate, adjusting reward, or an immediate large reward / no reward ($p = 0.5$ for either).

Figure 2.

A) Placement of injector tips in the AcbC. B) Placement of injector tips in the IOFC.

Figure 3.

Inactivation of the AcbC decreased delay discounting (a), but had no effect on sensitivity to delay or magnitude (b, c). Inactivation of the IOFC did not affect any measure (d, e, f). * $p < 0.05$

Figure 4.

AcbC animals with few choices of the large reward (high discounting), low adjusting delay (high sensitivity to delay), and low adjusting magnitude (low sensitivity to magnitude) at baseline are on the left, and their counterparts are on the right. Inactivation of the AcbC decreased delay discounting in animals with high choices of the large reward (low discounting) (d), but not in animals with low choices of the large reward (high discounting) (a). No other effects were seen (b, c, e, f). It should be noted that animals with low adjusting delay at baseline actually had higher adjusting delay after saline than animals with high

adjusting delay at baseline (b, e). This effect was entirely driven by one animal that had a very large adjusting delay after saline. However, removal of this animal from the analysis did not affect the results. * $p < 0.05$

Figure 5.

IOFC animals with few choices of the large reward (high discounting), low adjusting delay (high sensitivity to delay), and low adjusting magnitude (low sensitivity to magnitude) are on the left, and their counterparts are on the right. No effects of inactivation were seen (a, b, c, d, e, f).