# MESOLIMBIC DOPAMINERGIC MODULATION OF CUE-GUIDED RISK-REWARD DECISION MAKING

by

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# Abstract

In naturalistic decision making, we are often presented with options that provide a small reward with a high degree of certainty, or riskier options with a larger reward at the cost of higher uncertainty. Human models of decision making often provide participants with external cues to guide subsequent decisions, however in rodent models of decision making it is common for the animal to develop an internal model of decision making in response to previous choice outcomes. To bridge the gap between human and rodent models of externally guided decision making, our lab has developed the “blackjack task”, where animals listen for an external auditory cue which signals the probability of a larger, riskier reward being delivered with favourable odds (50%) or unfavourable odds (12.5%) in comparison to a smaller reward delivered with 100% certainty. Both human and rodent studies have verified the importance of dopamine (DA) transmission in nucleus accumbens (NAc) and its core and shell subregions in the modulation of such risk-reward decision making. Here, we further clarify this role by delivering intracranial infusions of D1 or D2 antagonists directly into the NAc core or shell. It was found that D2 but surprisingly not D1 receptors modulate risk-reward decision making differentially in the NAc core versus NAc shell. D2 blockade in the NAc core blunted risky choice, while D2 blockade in the NAc shell heightened risky choice. These data contribute to the understanding that externally guided risk-reward decision making is affected differently by DA receptors, and that this effect is also subregion dependent.

# Lay Summary

When faced with a choice, it can be better to play it safe or go for the risky option. We make these choices based on clues from our surroundings. To understand the brain systems responsible for helping us make these choices, we developed a risky decision-making task that rats can perform, called the “blackjack task”. In this task, rats choose between a risky lever that can give them a large sugar pellet reward, or another lever that always gives them a small sugar pellet reward. Before they make the choice, we play sounds for them which tell them whether the odds are good or bad to take that risk. In this experiment we block the neurotransmitter dopamine in a part of the rat’s brain called the nucleus accumbens and see that dopamine helps them make the best choices in this task.

# Preface

The research question this thesis sought to answer was conceived by the author, Shayden Schofield-Lewis, and his supervisor Dr. Stan Floresco. The data collection was led by the author. Tiffany Luo, an undergraduate research assistant, assisted with data collection. The author was taught the necessary techniques to complete this experiment by his colleagues, Jackson Schumacher and Mudi Zhao. Data analysis and manuscript writing was completed primarily by the author. Dr. Floresco assisted in data analysis, editing, and providing feedback for this thesis. All experiments were conducted in accordance with the Canadian Council for Animal Care and were approved by the Animal Care Committee of the University of British Columbia under protocol A22-0191.

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And to you, reading this page, thank you. By reading my thesis, you are in some way, small or large, helping me or the advancement of science. For that, I owe you my gratitude.

# Introduction

In order to maximize the utility of its environment, an organism must be able to make efficient decisions that integrate the costs and benefits of each potential outcome. These costs and benefits can take several forms. The reward may be temporally delayed, require a measure of physical exertion, have a varying magnitude, or have a probabilistic chance of being delivered. Cost/benefit decision-making often requires aspects of learning, as information regarding reward outcomes must be obtained, retained, and recalled in order to guide present and future choices. Thus, it reasonably follows that brain regions involved in learning and memory assist in guiding this decision-making process. Namely, converging evidence from both humans and animal research has implicated different regions of the frontal lobes, including the medial prefrontal (PFC) and orbitofrontal cortex (OFC), amygdala, and nucleus accumbens (NAc) in guiding decision-making behaviour.

## Anatomical properties of the nucleus accumbens

The NAc is a region of the ventral striatum that has been identified as distinct both anatomically and functionally distinct in comparison to other medial forebrain and striatal regions (Mogenson et al., 1980). The NAc receives direct glutamatergic input from the amygdala, hippocampus, and PFC, and dopaminergic input from the ventral tegmental area (VTA). The striatum contains at least five distinct cell types, two of which are projection types, with the remaining 3 being interneuron populations (Castro & Bruchas, 2019). These projection neurons are classified as medium spiny neurons due to their average soma size and their dendritic arbor being densely decorated with dendritic spines (Meredith et al., 2008). The NAc

itself has been categorized as having two distinct subregions, each characterized by unique connectivity, cell type populations, and behavioural functions: the medial “shell” and the more laterally located “core” (Zahm & Brog, 1992). These divisions are supported by differential expression of enzymes and cell population in early studies of the NAc (Záborszky et al., 1985). The NAc core subsumes the anterior commissure and is located ventrally to the lateral ventricle, shaped similar to an oval. The NAc core is sheathed by the NAc shell, whose shape extends from a position more medial than the NAc core to a more lateral terminus brushing up against the caudate putamen. Amygdalar and cortical inputs innervate both the core and shell subregions of the NAc yet are more pronounced in the core, while hippocampal innervations are more dominant in the shell (Britt et al., 2012). This topographical organization of afferent signals mesoaccumbal subregions is theorized to create neuronal ensembles which each subserve distinct functional roles in guiding behaviour. These ensembles interact with dopaminergic input, resulting in either attenuation or enhancement of NAc neural activity (Charara & Grace, 2003; Nicola & Malenka, 1997). It is precisely by this selective increase or decrease in firing that the NAc may exert its effects on biasing the behaviour of an organism.

## Behavioural functions of the nucleus accumbens

Since at least the 1970s, this nucleus has been suggested to act as a nexus linking together motivation and action within an organism (Mogenson et al., 1980). The NAc has long been implicated in reward & reinforcement, as demonstrated by seminal work by Olds & Milner in 1954 where stimulating electrodes implanted in the “septal area” which encompasses the NAc as well as other septal nuclei (Olds & Milner, 1954). The stimulated areas that were determined to be rewarding were initially termed “pleasure centers”, possibly contributing to the errant

belief that the area is responsible for hedonic pleasure and that its function is centralized rather than acting as part of a circuit with afferent and efferent connections (Olds, 1956). Building on the work by Olds & Milner, Roy Wise further implicates dopaminergic transmission as being key to the facilitation of self-stimulation as well as being exploited by drugs of abuse such as cocaine and amphetamine (Wise, 1980). This finding and its primary author are largely credited for the founding of the dopamine (DA) hypothesis of reinforcement, which argues that DA mediates the reinforcing effects of these natural reinforcers and drugs of abuse (Salamone & Correa, 2002). While DA circuitry had evolved to promote pursuit of natural reinforcers that would increase an organism's probability of survival and likelihood to reproduce, brain stimulation via drugs or self-stimulation by electrode implantation such as the experiment performed by Olds & Milner described above would "hijack" the brain's natural reinforcement system and produce reinforcing effects according to the DA reinforcement hypothesis. This claim was supported by following research using dialysis in freely moving rats finding elevated concentrations of synaptic dopamine especially in the nucleus accumbens following administration of drugs of abuse (Di Chiara & Imperato, 1988). The perspective on the role of the NAc with respect to its influence on behaviour evolved over the course of time. For many years, it was believed that dopaminergic transmission in the NAc mediated the reinforcing effects of primary reinforcers such as consuming food or water (Wise, 1982). In this manner, it was believed that drugs of abuse exploit this system to exert their hedonic and reinforcing effects.

DA transmission as part of the DA reward hypothesis is thought to be the "reward" neurotransmitter system, though the precise meaning of the term "reward" is often ill-defined (Salamone & Correa, 2012). In rodent behavioural studies, reward is often operationalized as

delivery of sugar pellet(s). These rewards can be delivered with varying magnitude, probability, or both. To clarify the role of accumbal DA in reward, work with careful manipulation of effort-related costs to obtain reward demonstrated that accumbal DA may play a role in the motivational aspect of exerting effort in order to obtain a reward (Salamone et al., 2003). Another rodent study found that transient dopamine activity in the NAc was related to reward-related action initiation (Syed et al., 2016). A behavioural assay commonly used in rodent studies of reward-related behaviour is the probabilistic discounting task. In this task rodents, typically rats, are presented with a choice between two levers. One lever is known as the 'certain' lever as it will always deliver a single sugar pellet with 100% certainty. The other lever is referred to as the 'large, risky' which delivers four pellets in a probabilistic manner. These probabilistic odds decrease between the blocks of trials in the session, consisting of 100, 50, 25, 12.5, and 6.25 percent chances of large reward delivery. Animals are trained on this task until they are able to adjust their choice behaviour across the blocks of probability in order to maximize the expected value of reward delivery. This manifests in prioritizing 'certain' choices when the risky odds are poor (at 12.5 and 6.25 percent), and prioritizing 'large, risky' choices when the odds are good (at 100 and 50 percent). Work previously from our laboratory has implicated dopamine in modulating performance on the probabilistic discounting task. In one experiment, stimulation of D1 (SKF81297) or D2 (bromocriptine) receptors with DA agonists administered systemically via intraperitoneal injection increased risky choice behaviour. Conversely, antagonism of D1 (SCH-23390) or D2 (eticlopride) receptors in subjects given amphetamine, a potent general DA agonist, resulted in attenuation of the increased preference for the 'large, risky' lever caused by amphetamine administration (St Onge & Floresco, 2009).

This study exclusively used drugs delivered systemically, and thus it logically followed to pursue an experiment where delivery of DA agonists or antagonists are delivered in specific dopaminergic brain regions involved in the decision-making process. One such follow-up involves inactivation of the NAc and its subregions via GABA agonists (muscimol). Inactivation of both NAc core and shell produced a reduced preference for the 'large, risky' lever and increased overall response latencies on the probabilistic discounting task. Further selective inactivation of the NAc core did not produce a significant change in choice behaviour (but did slow choice latencies), while inactivation of the NAc shell reduced win-stay tendencies, referring to the tendency to press the risky lever again following a rewarded risky lever press (Stopper & Floresco, 2011). This indicates that, when the NAc shell is inactivated, receiving a large probabilistic reward was less effective at biasing future risk-reward decision making. This finding is supported by the lack of an effect on lose-shift tendencies, showing that the win-stay effect is likely not due to risk aversion or a nonselective impairment. Notably, the reduction in risky choice and increased choices latencies observed when both subregions of the NAc were inactivated, suggests both regions work in concert to influence risky choice and motivation.

Subsequent studies examined how dopamine receptor-specific modulation in the NAc altered risk-reward decision-making behaviour. In this experiment, the area bordering the NAc core and shell was surgically targeted in order for infused drugs to exert their effects on both subregions simultaneously. Again utilizing the probabilistic discounting task, this study used both D1 and D2 agonists and antagonists. Here, D1 agonist treatment increased risky choice when the odds were good, and decreased risky choice when the odds were poor while neither blockade nor stimulation of NAc D2 receptors influenced risky choice. Comparatively, D1

receptor blockade reduced preference for larger, uncertain rewards and also increased response latencies and decreased locomotion (Stopper et al., 2013). Collectively, these data demonstrate the functional heterogeneity of the NAc core and shell, and suggest differential roles for D1 and D2 receptors within these subregions.

The probabilistic discounting task used in these aforementioned studies require subjects to develop an internal model of decision making, based on integrating information of choice-outcome contingencies and how these vary over a session to maximize profitable behaviour. However, naturalistic decision-making behaviour is often guided by cues gleaned from an organism's environment. These cues can signal the probability of which a reward may or may not be delivered. Human behavioural procedures such as the Cambridge Gamble Task give their participants information about the likelihood of probabilistic reward delivery (Grant et al., 2011). A real-life example of this is the casino game "Blackjack" where one face-up card signals the likelihood of the dealer having a good hand or a poor hand, and the players can choose to double-down or surrender depending on this cued information in order to maximize their potential profits. To research this aspect of risk-reward decision making, our lab has developed a novel behavioural assay coined as the "blackjack task". Similar to the probabilistic discounting task, the blackjack task involves a choice between a large, risky lever whose larger 4 sugar pellet reward is delivered probabilistically and a small, certain lever which always delivers a single sugar pellet with 100 percent certainty. Crucially, these choices are preceded by the presentation of one of two pseudorandomly presented auditory cues. These cues indicate the degree of risk involved in choosing the risky lever, which are deemed either good (50 percent) or poor (12.5 percent) odds. With training, rats develop a choice profile where they tend to

choose the risky option when the odds are good, and the certain option when the odds are poor. An initial experiment using the blackjack task inactivated the NAc core or shell using GABA agonists. Risky choice was found to be differentially affected by NAc core vs shell inactivation. Inactivation of the NAc shell induced an overall increase in risky choice without affecting latencies or locomotion. In comparison, inactivation of the NAc core led to near-random modes of responding on both good and poor-odds trials, and caused a general slowing of behaviour, demonstrated by increased choice latencies and lower locomotion counts. Conversely, the NAc shell inactivation had no effect on latency or locomotion (Floresco et al., 2018). A key control experiment used an auditory conditional discrimination task, which is in essence the blackjack task with the probabilistic component removed. In this task, subjects are exposed to distinct auditory cues that inform them that they will be rewarded if they press either the left lever when hearing one tone, or the right lever for the other tone. In this control task, NAc core inactivation also impaired performance, inducing near chance-levels of performance, and also increased choice latencies and omissions and decreased locomotion. NAc shell inactivation also impaired performance on this task, but the effects were more muted compared to the core, and only emerged later in the session. These results suggest that the NAc and its subregions play complementary yet distinct roles in refining action selection when discriminative stimuli that different actions may yield rewards, both when delivery of these rewards are certain or probabilistic.

Thus far, the NAc core and shell and mesolimbic DA transmission have been implicated in risk-reward decision making. However, the precise influence of specific DA receptor subtypes in the NAc on the cue-guided blackjack task remains unclear. To investigate this, the present study

assessed the role of dopamine D1 and D2 receptors in the NAc core and NAc shell by infusing intracranial doses of D1 or D2 antagonists in rats trained on the blackjack task. As inactivation of either subregion of the NAc also impaired conditional discrimination performance when reward delivery was deterministic, we also assessed how dopamine antagonism in these regions affected choice behaviour under these conditions.

# Methods

## Animals

One hundred four male and free-cycling female Long Evans rats (176-567g) arrived group-housed and were given one week to habituate to the novel environment after arrival. After the habituation period, the animals were split into pairs and would remain housed with their cage-mate for the remainder of the experiment. The colony room in which all animals were housed had its ambient temperature maintained at 21 degrees Celsius. The colony room had a 12-hour light/dark cycle (lights on 8am – 8pm). All training and experimental testing was performed during the light cycle phase of the colony room. Before beginning training, the animals were gradually food restricted to 15-17g of food per day (5053 – PicoLab Rodent Diet 20) which reduced their body weights to approximately 90% of their ad libitum feeding weights. All experiments were conducted in accordance with the Canadian Council for Animal Care and were approved by the Animal Care Committee of the University of British Columbia.

## Apparatus

Rats were trained and tested within operant chambers (30.5 × 24 × 21 cm; Med-Associates, St Albans, VT, USA) situated inside sound-attenuating enclosures. Internal fans were utilized to maintain a consistent temperature and to mask any external noise. Each chamber was outfitted with two retractable levers on each side of a food port, allowing for the delivery of 45 mg sweetened reward pellets (Bioserv Frenchtown, NJ, USA) through a pellet hopper. These chambers featured a 100mA house light for illumination. Auditory cues were presented using a speaker embedded in the wall opposite the levers, which was connected to a programmable sound generator (ANL-926, Med Associates). Four infrared photobeams were on the sides of the chambers to detect beam breaks, which served as an index of general locomotor activity.

## Stereotaxic surgery

Prior to behavioural training, rats underwent stereotaxic surgery. Initially, they were administered subanaesthetic intraperitoneal doses of ketamine (50 mg/kg) and xylazine (4 mg/kg) for sedation. Anesthesia was maintained using isoflurane throughout the surgical procedure which lasted approximately one hour from first incision.

Bilateral stainless steel cannulae (23-gauge, with beveled tips) were surgically implanted either in the NAc core region (coordinates: Anteroposterior [AP] = +1.8 mm; Mediolateral [ML] = +/− 1.8; Dorsoventral [DV] = −6.3 mm from dura) or the NAc shell region (coordinates: AP = +1.6 mm; ML = +/− 1.0; DV = −6.3 mm from dura). These cannulae were secured in place using four stainless steel skull screws and dental acrylic. Obdurators (30-gauge) were inserted into the cannulae and were routinely monitored to ensure their retention until and throughout the days of microinfusion testing. A minimum of one week was provided for the rats to recover before the commencement of food restriction and behavioural training.

## Behavioural training

### Initial lever press and pre-blackjack training

On the first day of training, the rats underwent a single session of magazine training. During this phase, 30 pellets were dispensed into the food port over a span of 30 minutes, with a variable interval of 60 seconds between deliveries. Subsequently, the rats were introduced to lever-press training using a fixed-ratio-1 schedule of reward. One lever was introduced into the chamber, baited with reward pellet dust, and remained extended until either 60 presses were performed or 30 minutes had elapsed, whichever came first. This training process was repeated on the following day with the other lever extended. The rats were subject to this training for a

minimum of two days and continued training, alternating between levers each day, until they met the criterion of completing 50 presses within a 30-minute timeframe, typically requiring 2 to 3 days.

Following the fulfillment of the fixed-ratio-1 criteria, the rats were further trained to interact with retractable levers. Each session comprised 90 trials. At 40-second intervals, the house light was illuminated, and 3 seconds later, one of the levers was extended into the chamber, with the specific lever being randomized in pairs. The rats were given 10 seconds to press the extended lever; otherwise, it would be retracted, and the trial was recorded as an omission. A successful lever press resulted in the delivery of one pellet with a 50 percent probability. The training on retractable lever pressing continued until the rats completed fewer than 10 omissions in a session, typically requiring 2 to 3 days.

After the lever-press training, the rats underwent additional training procedures to establish an association between one of the levers and a larger reward. Initially, they were trained on a reward magnitude discrimination task, consisting of four blocks that included both forced-choice and free-choice trials. At 40-second intervals, one or both levers were introduced into the chamber. Pressing one of the levers within 10 seconds of its insertion resulted in the delivery of four pellets, while the other lever yielded only one pellet. The assignment of the large and small reward levers was counterbalanced between rats and remained consistent throughout the experiment. In the initial session (comprising 48 trials, with 2 forced and 10 free-choice trials per block), both the large and small rewards were delivered with a 100 percent probability. In the subsequent two sessions (comprising 72 trials, with 8 forced and 10 free-choice trials per block), the large reward was delivered with a 50 percent probability.

## Blackjack task

After reward magnitude training, rats began training on the blackjack task. Within this task, one lever was designated as the small, certain option, which ensured the delivery of a single pellet with 100 percent certainty. The other lever was assigned as the large, risky option, delivering four pellets but subject to two varying probabilities, consistent with the lever associations established during reward magnitude training.

Trials were presented at intervals of 20 seconds, commencing with the illumination of the house light and the initiation of one of two distinctive auditory cues, a 3 kHz pure tone, or white noise. These auditory cues indicated the probability of obtaining the large, risky reward for the upcoming trial. One cue signaled a "good-odds" trial, where selecting the risky option would result in a reward with a 50 percent probability. The other cue indicated a "poor-odds" trial, where the risky choices were rewarded with only a 12.5 percent probability. The presentation of both cues occurred an equal number of times during the session, pseudorandomized in pairs. The large, risky option should be preferred during "good-odds" trials, whereas during "poor-odds" trials, the small, certain option holds greater expected value. The assignment of auditory cues to good vs. poor odds trials was counterbalanced across rats and remained consistent throughout the duration of the experiment.

Upon choosing either lever, both levers retracted. If the rat selected the large, risky option and received a reward, both the auditory cue and house light remained illuminated during the delivery of the four pellets, turning off 3 seconds after the choice. In cases where large, risky choices failed to yield a reward, the house light extinguished immediately, and the auditory cue terminated 2 seconds after the choice. The prolonged presentation of the auditory

cue aimed to facilitate the learning of their predictive value and the likelihood of different outcomes linked to these choices. In the event of an omission, both levers retracted, and the house light and auditory cue were extinguished.

Initially, the rats were trained on a forced-choice version of the task, involving 32 forced-choice trials followed by 20 free-choice trials. On the forced-choice trials, only one lever is extended at a time. When the large, risky lever was extended, the auditory cue indicated the respective probability of obtaining the large reward for that trial (50 or 12.5 percent). In forced-choice trials where the small, certain lever was introduced, an equal number of each auditory cue was presented within the session, ensuring that the rats learned that a response on this lever consistently yielded one pellet, regardless of the cue presented.

Following 14-19 days of training on the forced-choice version of the blackjack task, rats exhibited stable choice behaviour and progressed to the final iteration of the task, which encompassed 80 free-choice trials. Stable choice behaviour was confirmed over a 3-day period through a 2 x 3 repeated measures ANOVA, with days and odds as within-subject factors. This ANOVA showed a non-significant main effect of days and a non-significant days x odds interaction ($p > 0.1$), alongside a significant main effect of odds ($p < 0.05$). Rats were then trained on the 80 trial free-choice version of the task for a minimum of 5 days, or until they demonstrated stable choice behaviour, after which microinfusion tests commenced.

## Auditory conditional discrimination task

Distinct cohorts of rats underwent training in an auditory conditional discrimination task, a task that shares many similarities with the blackjack task (Auger et al., 2017). The initial training phases, encompassing fundamental lever-pressing and retractable lever training,

mirrored procedures akin to those employed in blackjack task training. However it differed during the retractable lever training phase, where a response would unfailingly result in a reward. In the subsequent training phase, rats learned to associate specific auditory cues (e.g., 3 kHz with the right lever, white noise with the left lever) with the delivery of a two-pellet reward. In essence, the presentation of a given cue signified that a "correct" response on one lever consistently led to a reward, whereas an "incorrect" response on the opposing lever yielded no reward. The association of each cue with the correct lever was counterbalanced across animals and remained consistent throughout the training regimen. Following a correct and rewarded choice, the auditory cue ceased 1 second after the response, and the house light extinguished 3 seconds post-response. In contrast, an incorrect response immediately extinguished the house light and auditory cue. In addition, this task involved 40 total trials within a session, with a 40 second intertrial interval. All other procedural aspects of this task paralleled those of the Blackjack task.

The training for the auditory conditional discrimination task unfolded in two phases. Resembling the Blackjack training regimen, the initial phase comprised 52-trial sessions (lasting 36 minutes). These sessions commenced with 32 forced-choice trials, succeeded by 20 free-choice trials. Each auditory cue was presented an equal number of times during the session, arranged in a pseudorandom order. During forced-choice trials, only the correct lever was introduced into the chamber. Rats received training in this version of the task for approximately 10 days, with the criterion for proficiency set at achieving over 70% correct responses for two consecutive days. Following this, they underwent an additional 4 days of training in the final iteration of the task, consisting of 40 free-choice trials. Subsequently, rats underwent surgical

procedures and were retrained to attain criterion performance before participating in their initial microinfusion test day.

## Microinfusion testing

Prior to the first microinfusion test day, rats underwent a mock infusion to acquaint them with the procedure. During these sessions, obdurators were temporarily removed, and 30-gauge wires attached to tubing were inserted into the guide cannulae for a duration of 2 minutes and 30 seconds. Subsequently, the rats were placed in their home cages for a 10-minute period prior to being transferred to the operant chamber for their behavioural session. Rats underwent their first microinfusion test day within 1-4 days following the mock injection, unless their behaviour significantly deviated from pre-mock baseline.

In all experimental groups, microinfusions were carried out with the drug vehicle and two doses of the drug, all counterbalanced. The infusions were delivered at a volume of 0.3 µl over a 90-second duration (at a rate of 0.27 µl per minute) using a Sage Instruments Model 341 syringe pump. To achieve dopamine D1 receptor blockade, a solution of the D1/D5 receptor antagonist SCH-23390 at concentrations of 0.1 µg or 1.0 µg per hemisphere was dissolved in saline and infused directly into either the NAc core or NAc shell. Dopamine D2 receptor blockade was achieved using a solution of the selective D2 receptor antagonist L-741,626 at concentrations of 0.5 µg or 5.0 µg per hemisphere was dissolved in a solution of saline and 5 percent Tween 80 (Polysorbate 80, Croda Americas, Inc). The infusions were administered through a 30-gauge injector that extended 0.8 mm beyond the guide cannula's end. Following the 90-second infusion period, the pumps were deactivated, and the injector remained in place for 60 seconds to facilitate diffusion. Subsequently, the rats were returned to their home cages

for a 10-minute interval before being transferred to the testing chamber for their respective test sessions.

All experiments followed a within-subject design, ensuring that each rat received both the vehicle and all doses of either SCH-23390 or L-741,626. After each test session, rats were retrained on a daily basis until they reattained their pre-test baseline performance, which typically required 1-10 days of retraining before the administration of the next, counterbalanced microinfusion.

## Histology

Upon the conclusion of the behavioural experiments, the animals were subjected to isoflurane anesthesia and subsequently euthanized with CO2. Their brains were then carefully excised and underwent a fixation process in 4% formalin for a minimum period of 24 hours before the commencement of sectioning. In the sectioning process, the brains were rapidly frozen and sectioned at 50 micrometers. These sections were mounted onto slides coated with gelatin and further processed through Nissl staining using Cresyl Violet dye. To ascertain the accuracy of cannula placements and infusion integrity, reference was made to the neuroanatomical atlas by Paxinos and Watson (Paxinos & Watson, 2006).

Rats with cannula placements that resided either outside the borders of the NAc core or shell, or on the boundary between core and shell subregions of the NAc were systematically removed from the analysis. This led to the exclusion of 12 rats with placements in the NAc core area and 12 rats with placements in the NAc shell area. The reported sample sizes for each experiment represent the final count of animals with placements deemed acceptable within the NAc core and shell, that completed all three tests and that were deemed “good players” (as

described below). The locations of all such appropriate infusion placements are visually depicted in Figure 3 for reference.

## Statistical analyses

The primary dependent measure was the percentage of choosing the large, risky option, calculated separately for good- and poor-odds trials, factoring out trial omissions. Additionally, comparisons were made regarding choice latencies and the frequency of trial omissions across various treatments.

The choice data derived from the blackjack task underwent analysis through a four-way between/within subjects mixed ANOVA. This included treatment (vehicle, low dose, high dose) and odds (good vs. poor) as two within-subject factors. Sex was one of the between-subject factors. In addition, because different subregions of the NAc have been shown to play either complementary or differential roles in different types of risk/reward decision making (Floresco et al., 2018; Stopper & Floresco, 2011), the analysis also included brain region (NAc core or shell) as a second between-subjects factor. The main effect of odds consistently yielded significance ($p<0.01$) in these analyses and was not subjected to further reporting. The majority of rats exhibited optimal choice strategies, demonstrating a pronounced bias toward the large, risky option on good-odds trials and selecting it less frequently on poor-odds trials. However, a subset of rats (n=10) did not develop an optimal strategy over training. These rats, classified as "poor players" following established criteria (van Holstein et al., 2020) chose the large, risky option on fewer than 40 percent of both good and poor odds trials. The analysis focused on the subset of "good players", that at least showed a bias towards the risky option on good-odds trials

Supplementary analyses examined how these manipulations affect feedback sensitivity, assessing the proportion of trials that rats chose the risky option after a rewarded risky choice on the preceding trial (win-stay), or shifted to the certain option after a risky loss (lose-shift). In attempting to parse these data out by trial type, there were very few win-stay responses when rats faced a good-odds trial, so we compared the proportion of win-stay responses on all trial types with a three-way ANOVA, with treatment as a within-subjects factor and brain region and sex as between-subjects factors. In comparison, all rats made at least a few lose-shift responses on both trial types. Thus, these data were subjected to a four-way ANOVA, incorporating treatment and trial type (good vs. poor odds) as within-subject factors, along with brain region and sex as between-subjects factors.

The analysis of latency and omission data derived from the blackjack task underwent analysis through a four-way between/within subjects mixed ANOVA. This included treatment (vehicle, low dose, high dose) and odds (good vs. poor) as two within-subject factors. Sex and brain region were the between-subject factors. With respect to locomotion data, some of these chambers had malfunctions in the photobeams, so these data were only obtained from a subset of rats. Analysis of these data used a three-way ANOVA similar to the above analyses.

A similar approach was used to analyze the data for rats tested on the auditory conditional discrimination. The primary measure was the percentage of correct choices, and this was analyzed with three-way ANOVAs with treatment as a within-subjects factor and region and sex as between subjects factor. Other performance measures were analyze using similar ANOVA models.

Following the detection of significant drug effects, multiple comparisons were conducted utilizing Dunnett's tests to assess dose effects. All analyses were performed using SYSTAT and R version 4.3.0. In R, the function ezANOVA() was used from the package “ez”, which by default uses type 2 sums of squares in its calculation of ANOVA.

# Results

## NAc D1 blockade

Data from 21 rats (9 male, 12 female) with acceptable placements and performance were included in this analysis (Fig 3). Data from 4 rats with acceptable placements were excluded due to their classification as “poor players” under control conditions. A four-way ANOVA on choice data from rats deemed “good players” revealed no significant effect of treatment ($F(2,34) = 1.80$, $p = 0.179$, Fig 4A, B), treatment by odds interaction ($F(2,34) = 0.67$, $p = 0.520$), treatment by sex interaction ($F(2,34) = 0.42$, $p = 0.662$), or treatment by region interaction ($F(2,34) = 0.43$, $p = 0.655$). D1 treatment also did not produce significant effects in reward sensitivity measured by win-stay ($F(2,34) = 1.44$, $p = 0.250$, Fig 5A) and lose-shift ($F(2,34) = 0.26$, $p = 0.770$, Fig 5B). Despite the lack of effect on choice, these treatments were behaviourally active, evident by their effects on other performance measures. D1 antagonism increased omissions ($F(2,34) = 5.90$, $p = 0.006$, Fig 8A, B) and choice latencies ($F(2,34) = 4.68$, $p = 0.016$, Fig 6A, B), while at the same time decreasing locomotion ($F(2,20) = 13.91$, $p < 0.001$, Fig 7A, B). These data indicate that D1 blockade in either subregion of the NAc did not affect choice behaviour, but did produce a more general slowing of behaviour.

## NAc D2 blockade

Data from 19 rats (9 male, 10 female) with acceptable placements and performance were included in this analysis (Fig 3). Data from 6 rats with acceptable placements were excluded due to their classification as “poor players” under control conditions. A four-way ANOVA on risky choice revealed a treatment by region effect ($F(2,30) = 3.79$, $p = 0.034$). This interaction reflected that D2 blockade in the NAc core caused an overall reduction in risky choice across both trial types, whereas similar treatments in the NAc shell tended to increase

risky choice. Curiously, these effects were maximally prominent following treatment with the low (0.5 ug, Dunnett's, $p<0.05$) but not high (5.0 ug) dose of L-741,626 (Fig 9A, B).

Additional analyses examined how D2 blockade in NAc core and shell altered feedback win-stay and lose-shift behaviours. No main effects of treatment or interactions with any of the factors were observed for win-stay behaviour (all Fs<1.0, n.s., Fig 10A). In contrast, analysis of the lose-shift data revealed a treatment by region interaction ($F(2,30) = 5.73$, $p = 0.008$, Fig 10C), with no other interactions (all Fs, 2.1, n.s.). Partitioning this interaction by subregion with one-way ANOVAs and Dunnett's test revealed that the reduction in risky choice induced by D2 antagonism in the NAc core was associated with an increase in lose-shift behaviour when rats faced either good or poor-odds trials ($F(2,16) = 3.78$, $p = 0.045$, Fig 10C) following treatment with the low dose, whereas the effects of the high dose did not reach statistical significance. On the other hand, infusions of the low dose of L-741,626 into the NAc shell tended to increase lose-shift behavior on both trial types, this difference did not achieve statistical significance.

With respect to other performance measures, D2 treatment was found not to have a significant effect of treatment on choice latencies (all Fs < 1.7, n.s., Fig 11A, B). However, these treatments induced a curious decrease in omissions ($F(2,30) = 3.98$, $p = 0.029$) with this effect being most prominent in females ($F(2,30) = 4.81$, $p = 0.015$, Fig 12A). A three-way ANOVA on locomotion did not reveal any significant effects of treatment ($F(2,20) = 0.20$, $p = 0.82$, Fig 13A, B) or any significant interactions. Taken collectively, these data suggest D2 receptors in the NAc play a role in modulating reward sensitivity in cue-guided risk-reward decision making.

## Auditory conditional discrimination

Data from 13 rats with acceptable placements in the NAc shell, and 13 separate rats with acceptable placements in the NAc core (14 males, and 12 females for a total of 26 rats) were included in the subsequent analysis of D1 and D2 receptor blockade on the auditory conditional discrimination task (Fig 3). With respect to D1 receptor blockade, analysis of the percentage correct data did not yield a significant main effect of treatment ($F(1,22) = 2.90$, $p = 0.102$ Fig 14A, C) or any significant interactions with the other factors (all Fs < 1.8, n.s.). On the other hand, these treatments did increase response latencies ($F(1,22) = 5.11$, $p = 0.034$, Fig 15A, C) in a manner that did not vary by sex or NAc subregion (all Fs< 1.7, n.s.). These treatments also reduced locomotion irrespective of brain region (main effect of treatment, ($F(1,22) = 2.0.51$, $p < 0.001$, Fig 17). Omissions were not affected by D1 antagonism ($F(1,26) = 1.27$, $p = 0.270$, Fig 16A).

In a separate group of rats, blockade of D2 receptors in the accumbens also did not affect performance or response latencies (main effect of treatment and interactions with the treatment factor, all Fs <1.0, ns, Fig 14B, D). Omissions were not affected by D2 blockade ($F(1,26) = 1.22$, $p = 0.279$, Fig 17B). However, analysis of the locomotion data revealed a Treatment x Sex x Brain region interaction (($F(1,21) = 8.22$, $p = 0.009$). This interaction reflected the observation that D2 blockade in the core reduced locomotion in both males and females (main effect of treatment, ($F(1,11) = 11.30$, $p = 0.006$; treatment x sex interaction, ($F(1,11) = 0.17$, n.s., Fig 18A). In comparison, D2 blockade in the NAc shell reduced locomotion in males, but actually increased in females (treatment x sex interaction, ($F(1,10) = 15.47$, $p = 0.003$, Fig 18B). These results show that D1 blockade produced similar latency impairments to those seen

in the blackjack task without affecting task performance on a simpler conditional discrimination. Similarly, unlike the alteration in choice induced by D2 blockade on the blackjack task, similar manipulations failed to disrupt performance on the simpler auditory conditional discrimination task.

# Discussion

The present study had the goal of clarifying the role of dopamine D1 and D2 receptor subtypes in the NAc core and shell subregions on a cue-guided risk-reward decision-making task. It was found that D2 but not D1 antagonism in the NAc core maximally increased loss sensitivity, with this effect more prominent at a low dose. This suggests that D2 receptors in the NAc core are involved in biasing subsequent action selection after experiencing a risky loss. D1 receptors did not produce an effect on choice, however D1 antagonism resulted in an overall slowing in behaviour, as represented by increased latency, omissions, and decreased locomotion counts. These data provide evidence for the specific roles of dopamine receptor subtypes D1 and D2 in modulating cue-guided risk-reward decision making.

## NAc D1 blockade

Contrary to expectations, D1 receptor blockade in the NAc core or NAc shell did not significantly affect choice behaviour in the blackjack task. Previous work has demonstrated in rodent models that D1 blockage tends to reduce risky choice tendencies in behaviour (Jenni et al., 2017; Milienne-Petiot et al., 2017; Stopper et al., 2013). However, these studies did not utilize external auditory cues which signal alternating probabilities to guide risky decision making in their behavioural assays. For example, a mouse version of the Iowa Gambling Task (IGT) was developed and used to test the effects of D1 antagonism on risk-taking behaviour. In the original version of the task, human participants had to choose between four decks of cards—two advantageous decks with smaller magnitude rewards and losses, and two disadvantageous decks with large magnitude rewards and larger losses. In these initial experiments with human patients with prefrontal damage, a region rich with dopaminergic

transmission, it was found that their choice behaviour was deficient (Bechara et al., 1994). In the mouse model of the IGT, mice similarly have to choose between nosepoking in one of four illuminated holes, two with low net gain and two with high net gain. Here, it was found that D1 blockade reduced advantageous choice behaviours in safe-preferring mice and reduced disadvantageous choice in risk-preferring mice (Milienne-Petiot et al., 2017). Further evidence is provided by studies using the probabilistic discounting task. In one study, NAc D1 receptor blockade reduced risky choice preference across all blocks, no matter whether the large, risky choice was advantageous, equal, or disadvantageous (Stopper et al., 2013). Crucially, these behavioural assays require each subject, whether rodent or human, to develop, maintain, and update an internal model of decision making in response to rewarded or unrewarded choices. D1 and D2-like receptors in the NAc have been implicated in aiding memory consolidation in a inhibitory avoidance task (Managò et al., 2009). Since the blackjack task does not require constant maintenance of an internal model but rather the retrieval of an externally signalled model of probability of reward, it is plausible that the blackjack task is less taxing on working memory and consolidation processes because the probability of reward is always indicated by the external cue. Indeed, the influence that DA exerts on NAc neurons is most impactful when those neurons are in an excitable state driven by other afferent inputs, but is not impactful when the neurons are in a state of low activity (Floresco, 2015). Recent work by our lab has also found that, contrary to expectations, D1 blockade in the prelimbic or infralimbic medial prefrontal cortex did not produce statistically significant effects on choice behaviour in the blackjack task (Schumacher, 2020). Given that dopamine, acting on D1 receptors, is one of the

many converging inputs to the NAc, it may be that the specific burdens of the blackjack task are not as dependent on these receptors compared to other measures of decision making.

Despite a lack of significant effects on risky choice behaviour, the D1 blockade was behaviourally active. D1 antagonism increased choice latencies, omissions, and decreased locomotion. This slowing of behaviour as a result of D1 antagonism in the NAc is consistent with previous work using the probabilistic discounting task (Stopper et al., 2013). Further, in the conditional discrimination control experiment of the present study, D1 antagonism also produced significant increases in choice latencies and reduced locomotion counts. Hence, although D1 antagonism did not affect choice behaviour in the blackjack or conditional discrimination control task, these receptors do modulate the rapidity of choice initiation and overall movement.

## NAc D2 blockade

D2 antagonism in the NAc produced differential effects between the two subregions. In the NAc core risky choice was ablated by D2 antagonism, while D2 antagonism in the NAc shell heightened risky choice. Intriguingly, these effects were maximal at the low dose and not the high dose. Parsing this effect further, it was revealed that the reduction in risky choice behaviour caused by D2 antagonism in the NAc core was associated with an increase in lose-shift behaviour across both trial types. These effects were not associated with significant effects on locomotion or choice latencies, however there was a significant decrease in omissions, most prominent in females, due to D2 antagonism. In the conditional discrimination task, D2 blockade did not affect choice behaviour or latencies, but did produce a sex and region dependent effect on locomotion. In the NAc core, D2 antagonism reduced locomotion across

both sexes, while in the NAc shell locomotion was decreased for males but increased for females.

The effect on risky choice in the blackjack task was not accompanied by an effect on conditional discrimination performance, suggesting that D2 receptor activity plays a more critical role in situations involving the integration of varying reward magnitudes and probabilities versus a simpler discrimination of auditory cues. However, the integration of external cues to guide risky decision making may be crucially modulated by D2 receptor activity. In a D2 receptor blockade experiment on the probabilistic discounting task, no significant impairments on performance were found (Stopper et al., 2013). This suggests that the effects produced by the present experiment are at least in part dependent on the particularities of the blackjack task's design. Looking to the region-specific effects, it has been previously demonstrated that NAc core inactivation produced a reduction in good-odds risky choice and increase in poor-odds risky choice while NAc shell inactivation produced a selective increase in poor-odds risky choice (Floresco et al., 2018). Here, D2 blockade in the NAc core reduced risky choice but D2 blockade in the NAc shell increased risky choice, both regions affecting both trial types similarly. This finding is similar to previous work using a D2 blockade on the blackjack task, where prelimbic medial prefrontal cortex D2 antagonism also reduced risky choice on good-odds trials (Schumacher, 2020). The increase in lose-shift behaviour when D2 is antagonized in the NAc core represents a lower tendency of the subject to use environmental cues to bias the direction of risky choice behaviour. Given that the blackjack task provides external cues to indicate the presently optimal choice, it is not non-optimal to use the result of the previous probabilistic outcome to guide the subsequent choice since each trial's odds are not dependent

on the result of the immediately prior trial. Integrating this finding with the current perspective of the literature surrounding the NAc core, it can be argued that the ability of the NAc core to bias behaviour towards motivationally relevant stimuli, in this case the lever associated with the larger reward when signalled by a good-odds cue, is suppressed by a D2 receptor blockade. Without a potent "go" signal given by the NAc core, the subject may be more likely to utilize ultimately irrelevant information to guide subsequent risky choice.

Curiously, the findings of the present experiment on choice were most effective at the low dose of the D2 antagonist. This observation is not without precedent, however. In a study using systemic D2 antagonists on the Rodent Gambling Task, it was observed that the two lower doses (0.01 and 0.03mg/kg) but not the highest dose (0.06mg/kg) improved performance on the task (Zeeb et al., 2009). Taken collectively, the findings of this study are consistent with the notion that DA neurotransmission in the NAc acts as a modulatory dial on the afferent inputs to the NAc in order to guide appropriate behavioural responses to motivationally relevant stimuli in complex situations involving reward (Charara & Grace, 2003; Floresco et al., 2001b, 2001a).

## Dopamine modulation of decision making

Thus far it is clear that dopamine has an important role in the modulation of decision making, and that its role can be pronounced, subtle, or undetectable depending on the intricacies and characteristics of the task or situation at hand. Taking these knowledge fragments into the holistic mosaic of dopamine's role in decision making, we can see that our present results suggest a modulatory role of D2 but not D1 receptors in modulating cue-guided risk-reward decisions. While D1 receptors have been shown previously to have a role in tasks measuring risk-reward decision making, in the externally guided blackjack task these receptors

seem to play a less crucial role in guiding risky choice patterns. Depletions of DA in the NAc have been shown to disrupt the processes that allow for organisms to overcome behavioural constraints such as effort in obtaining a reward (Correa et al., 2002). Thus, it may be tempting to view the disruption to choice in this experiment as a reduction in the ability to exert effort to obtain reward. However, it has been previously shown that the ability of DA depletions in the NAc to influence effort-related behavioural deficits is dependent on the level of effort required. In experiments with a low effort to reward ratio, such as a fixed-ratio 1 schedule, there were no effects of NAc DA depletion (Aberman & Salamone, 1999). In the blackjack task, subjects similarly only are required to commit themselves to a single lever press in order to attempt to receive a food reward. Moreover, the conditional discrimination task provides the same amount of behavioural costs, yet produced no deficits in performance due to D1 or D2 receptor blockades. Collectively, these results continue to support the role of the NAc in biasing the behavioural action selection of an organism particularly when the reward is uncertain or ambiguous.

## Implications for human research and medicine

The ability of an organism to harmonize internal motivations with external cues and effectively coalesce these factors into an action is an adaptive characteristic of organic life on Earth. Dysregulation of this ability is a hallmark of many neurological or behavioural disorders, such as Parkinson's disease or gambling addiction. In humans, it has been observed that grey matter volume loss in the NAc precedes the development of apathy, a reduction in goal-directed behaviour, in patients with Parkinson's disease (Morris et al., 2023). Errant dopamine transmission is associated with "pathologic gambling" (PG) in a study with patients with

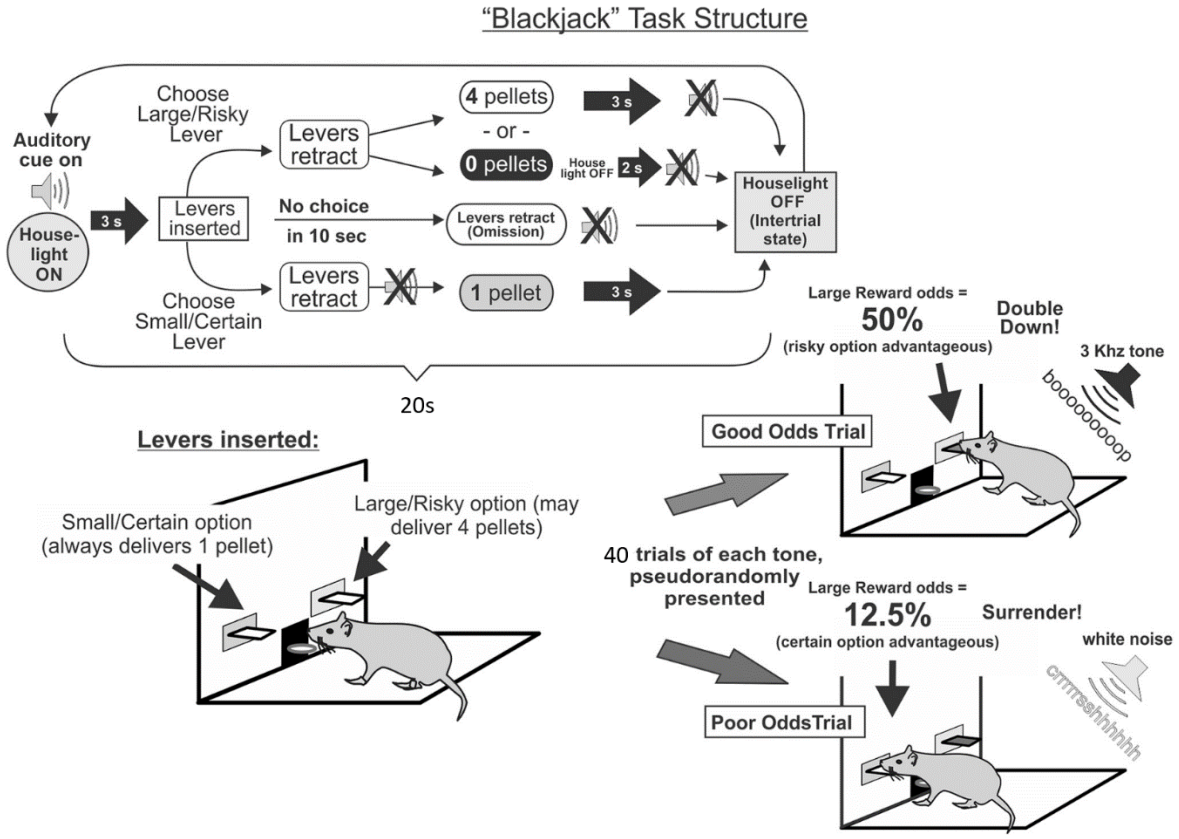
Parkinson's disease. The prevalence of PG in the general population is 1%, 3.4% in a Parkinson's disease clinic, and 7.2% in patients who had in their lifetime been on dopamine agonists (Voon et al., 2006). In a PET study with patients with Parkinson's disease using a radiotracer with affinity for D2/D3 receptors, it was found that the degree of the radiotracer binding was correlated with trait impulsivity and was significantly higher in those with a history of PG. This suggests that dysfunctional dopamine transmission is related to dysfunctional decision making, particularly in domains of decisions where reward is uncertain or ambiguous. The results shown by this thesis provide the information that NAc D2 receptors are involved in integrating external cues to guide appropriate decision-making behaviour under conditions of risk. This information may be used to help guide the development of treatments used for disorders with these deficits.

## Summary and conclusions

The findings reported here represent evidence for the role of D2 but not D1 receptors in the NAc modulating a region-specific effect on risky choice when information concerning the odds of the risk are externally signalled. Within this domain of decision making, D2 receptors in the NAc shell, reduce risky choice, while D2 receptors in the NAc increase preference of choosing the risky option. The results of this experiment are in line with the literature regarding the role of NAc dopamine's influence on decision making, further elucidating the manner in which specific dopamine receptors affect this process. Deeper understanding of these neural dynamics provides a clearer picture of what guides organisms to make riskier or safer decisions in the context of their environment. A table summarizing the effects of dopamine and NAc manipulations on the probabilistic discounting and blackjack tasks is presented in Figure 19.

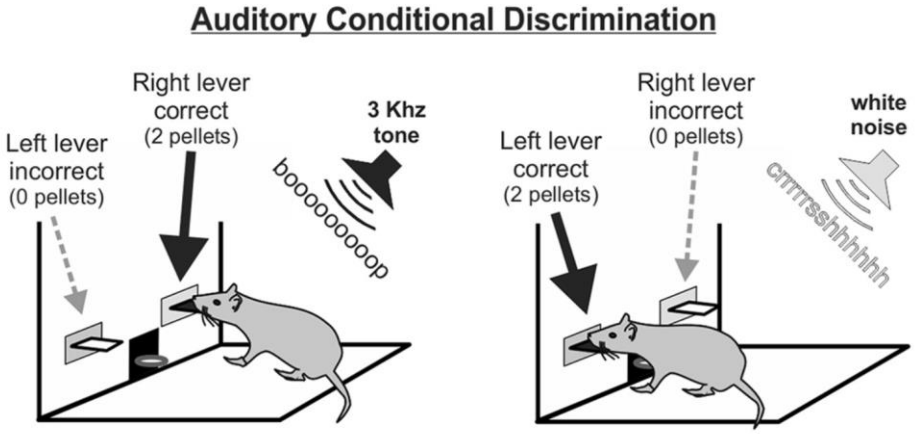
# Figures

## Fig. 1: Blackjack Task Structure



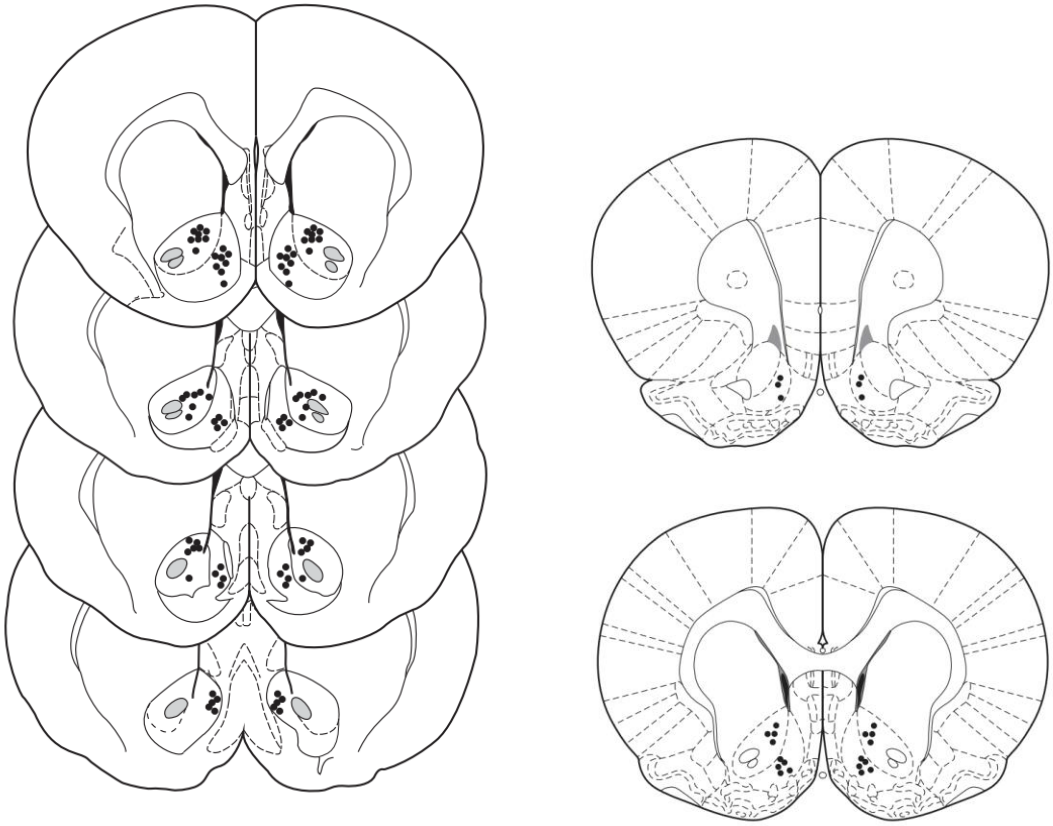

Visual depiction of the blackjack task. Adapted from (Floresco et al., 2018).

## Fig. 2: Auditory Conditional Discrimination Task Structure



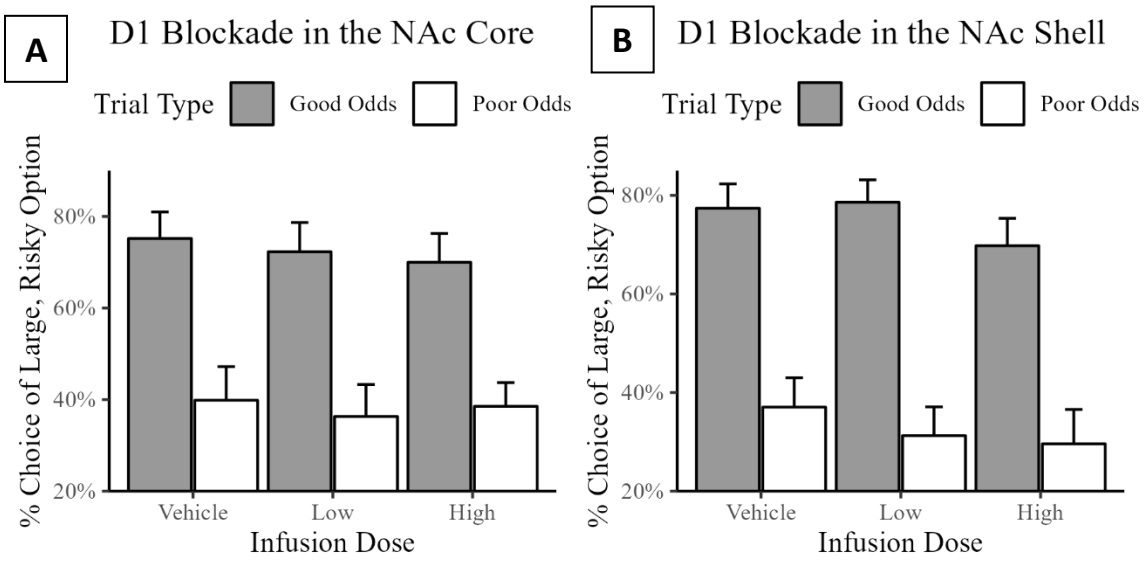

Visual depiction of the auditory conditional discrimination task. Adapted from (Floresco et al., 2018).

# Fig. 3: Histology



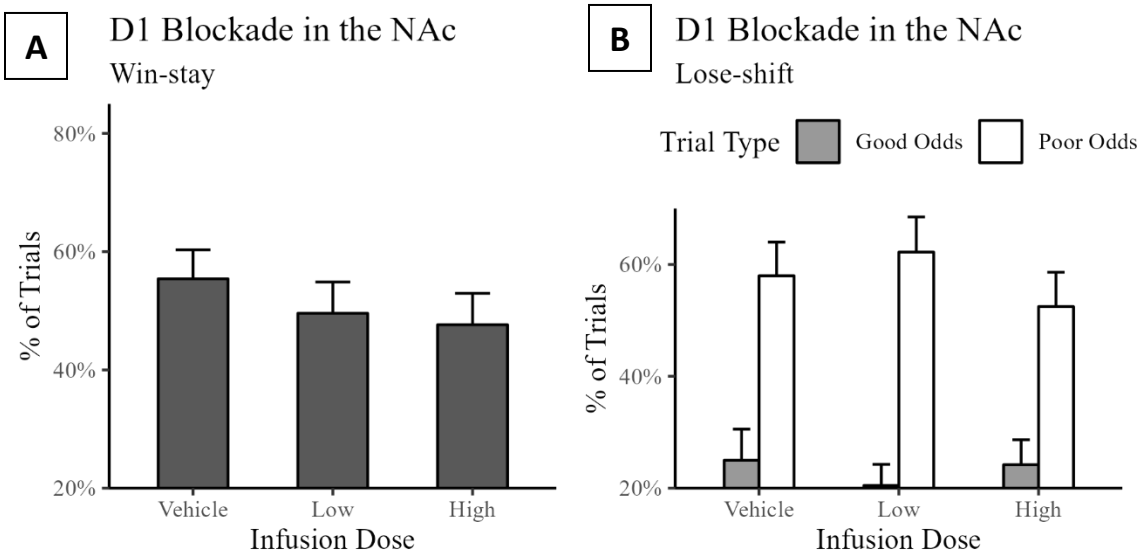
Coronal sections of the rat brain where circular black dots represent placements of all acceptable infusions into the NAc core or NAc shell (Paxinos & Watson, 2006).

## Fig. 4: Blackjack D1 Blockade- Choice



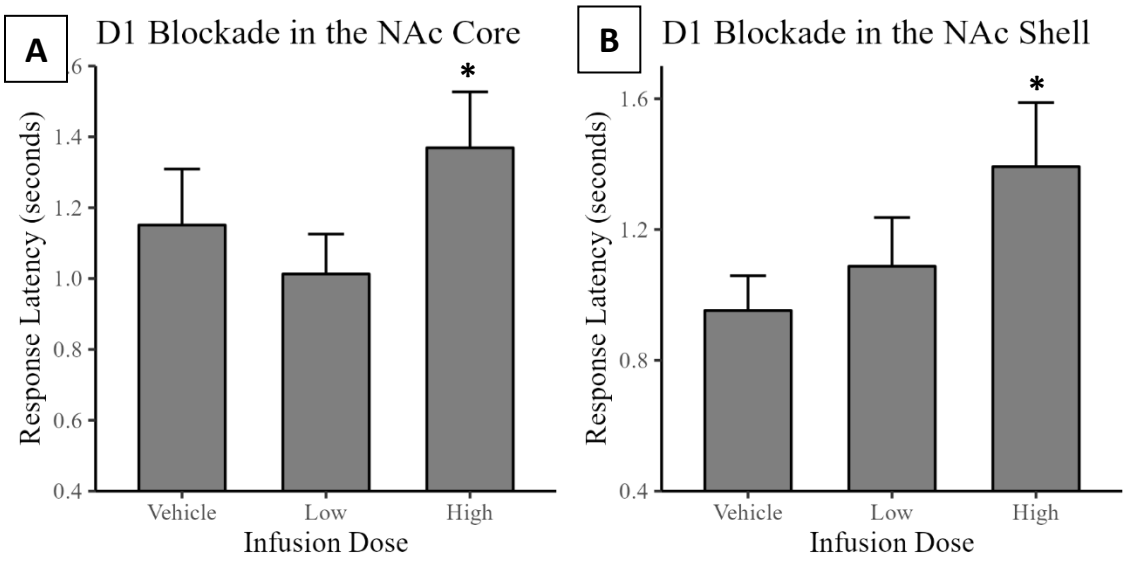

Averaged choice data for rats receiving the D1 antagonist into the NAc core (A) or shell (B).

## Fig. 5: Blackjack D1 Blockade- Reward Sensitivity



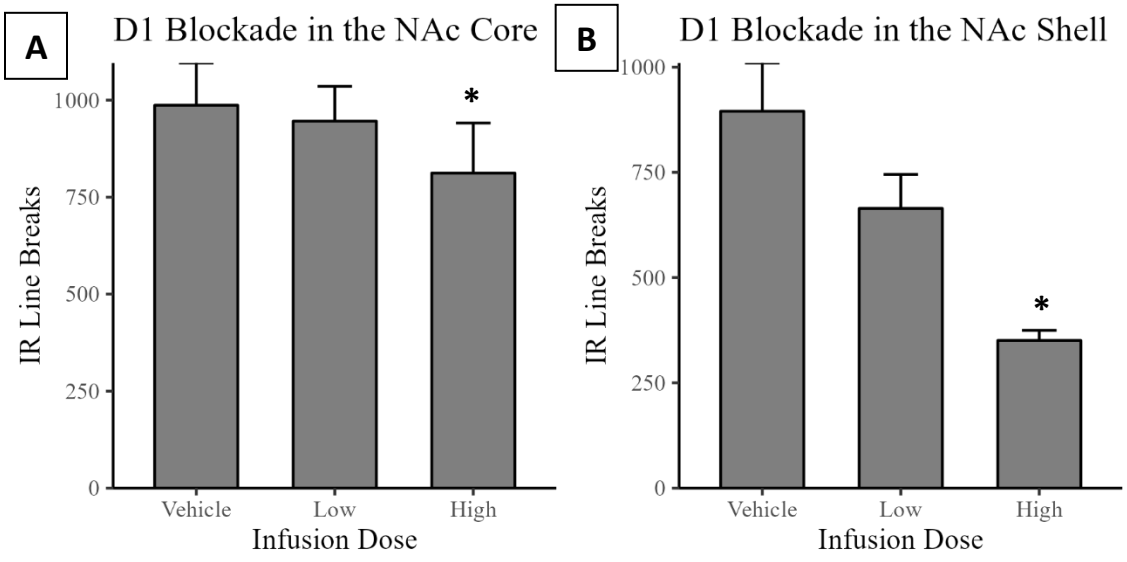

Averaged win-stay (A) or lose-shift (B) data for rats receiving the D1 antagonist into the NAc core and shell.

## Fig. 6: Blackjack D1 Blockade- Latency



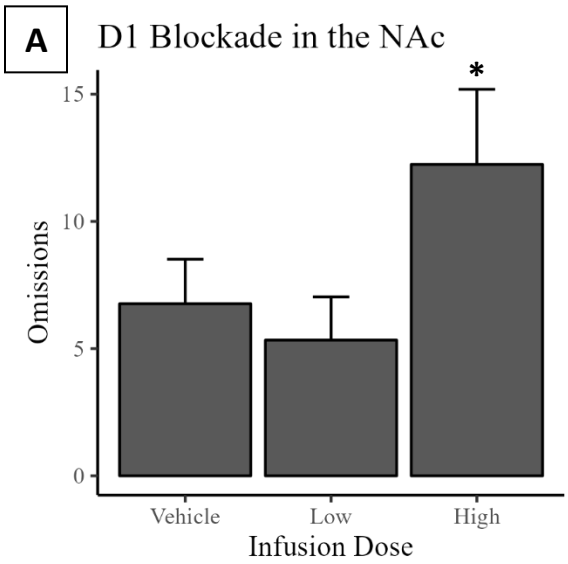

Averaged latency data for rats receiving the D1 antagonist into the NAc core (A) or shell (B).

## Fig. 7: Blackjack D1 Blockade – Locomotion



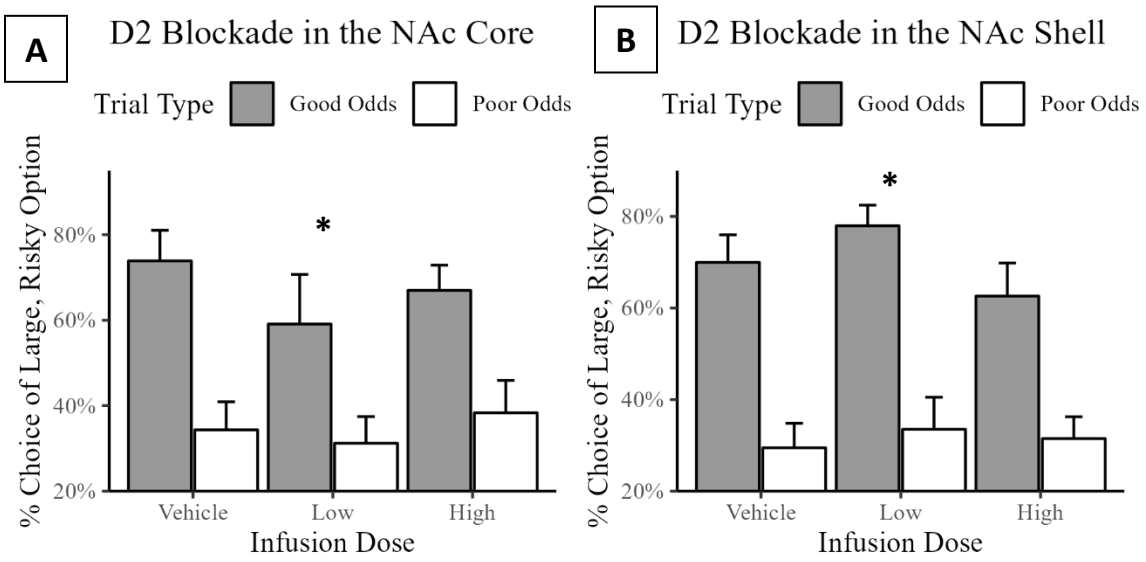

Averaged omission data for rats receiving the D1 antagonist into the NAc core (A) or shell (B).

# Fig. 8: Blackjack D1 Blockade – Omissions



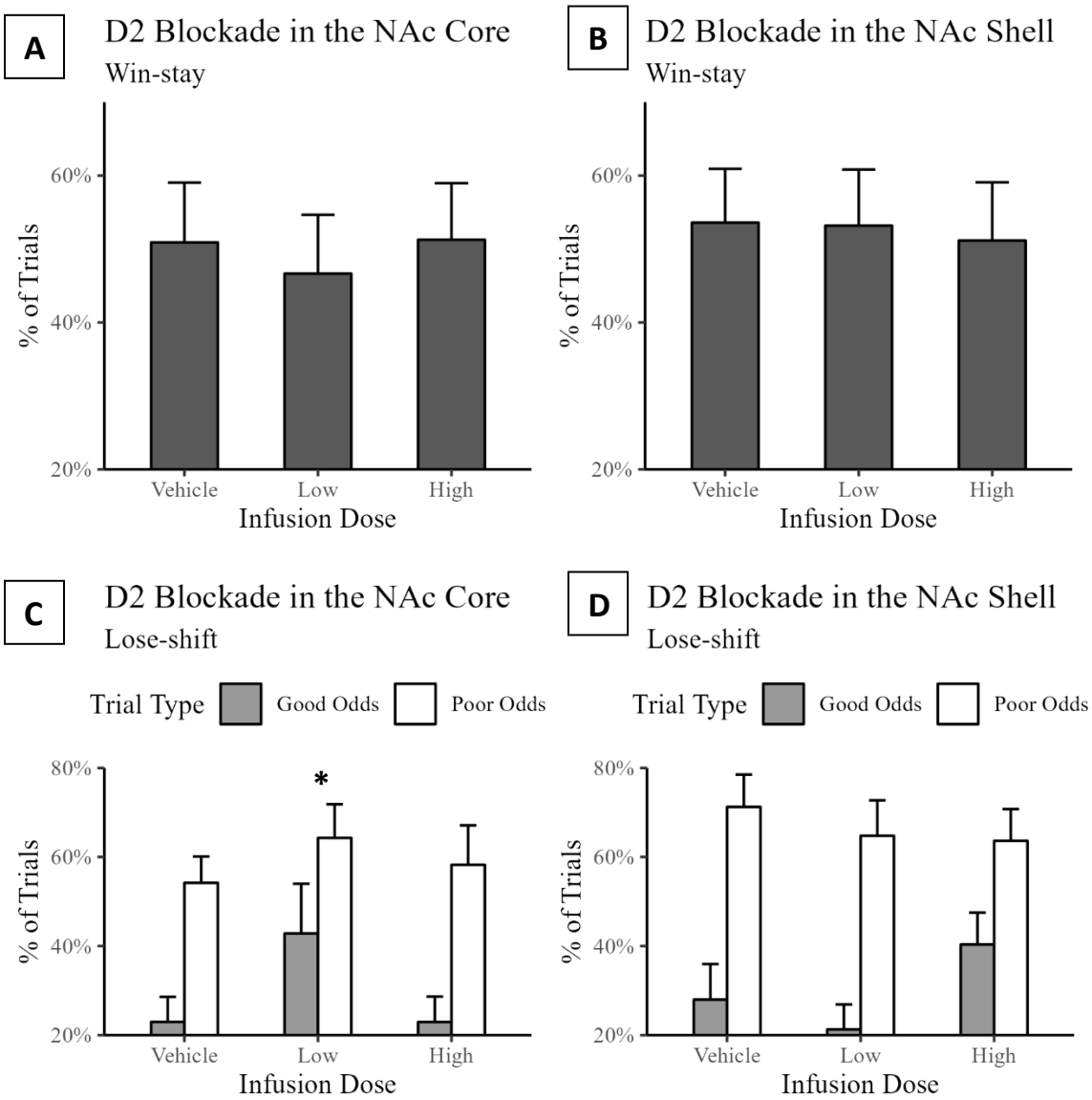

Averaged locomotion data for rats receiving the D1 antagonist into the NAc (A).

# Fig. 9: Blackjack D2 Blockade – Choice



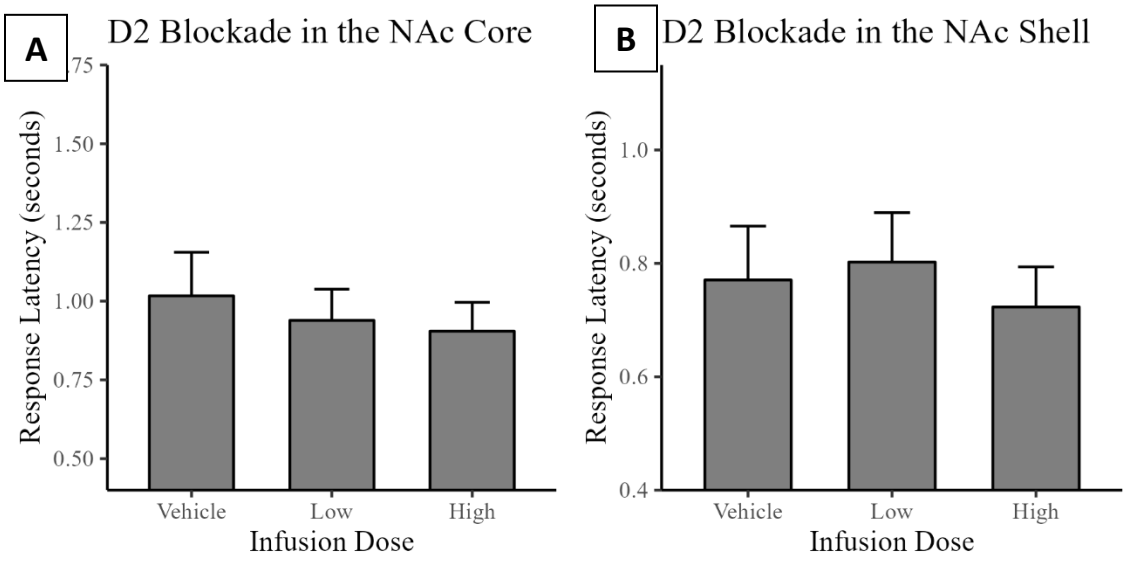

Averaged choice data for rats receiving the D2 antagonist into the NAc core (A) or shell (B).

# Fig. 10: Blackjack D2 Blockade – Reward Sensitivity



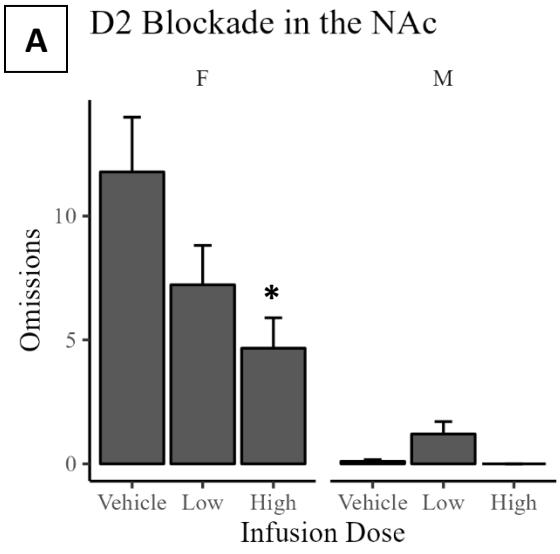

Averaged win-stay data in the NAc core (A) or shell (B) for rats who received the D2 antagonist. Lose-shift data for the NAc core (C) or NAc shell (D) for rats who received the D2 antagonist.

## Fig. 11: Blackjack D2 Blockade- Latency



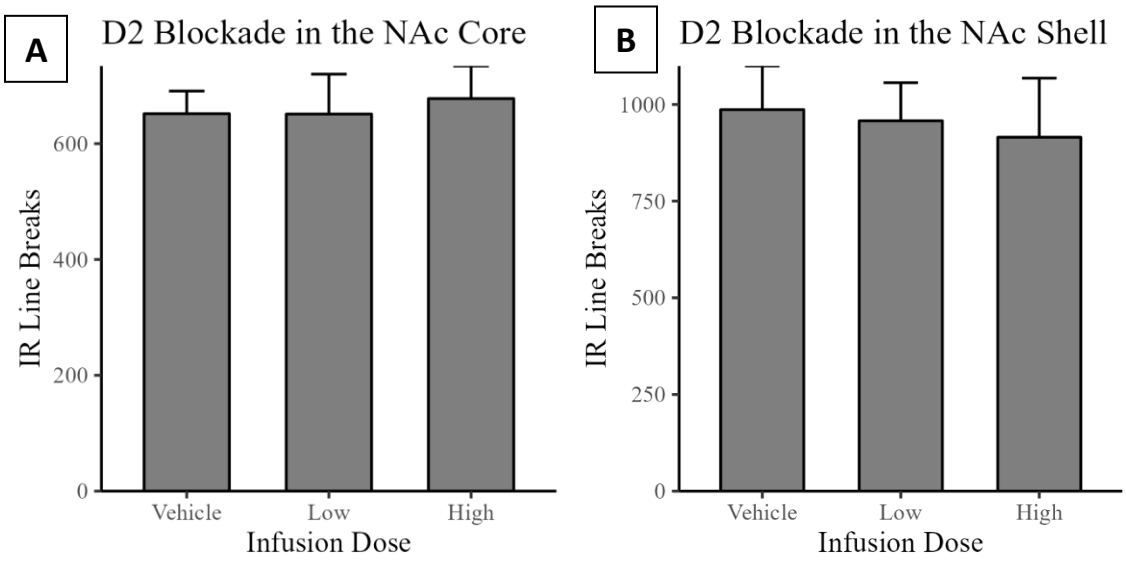

Averaged latency data for rats receiving the D2 antagonist into the NAc core (A) or shell (B).

## Fig. 12: Blackjack D2 Blockade- Omissions



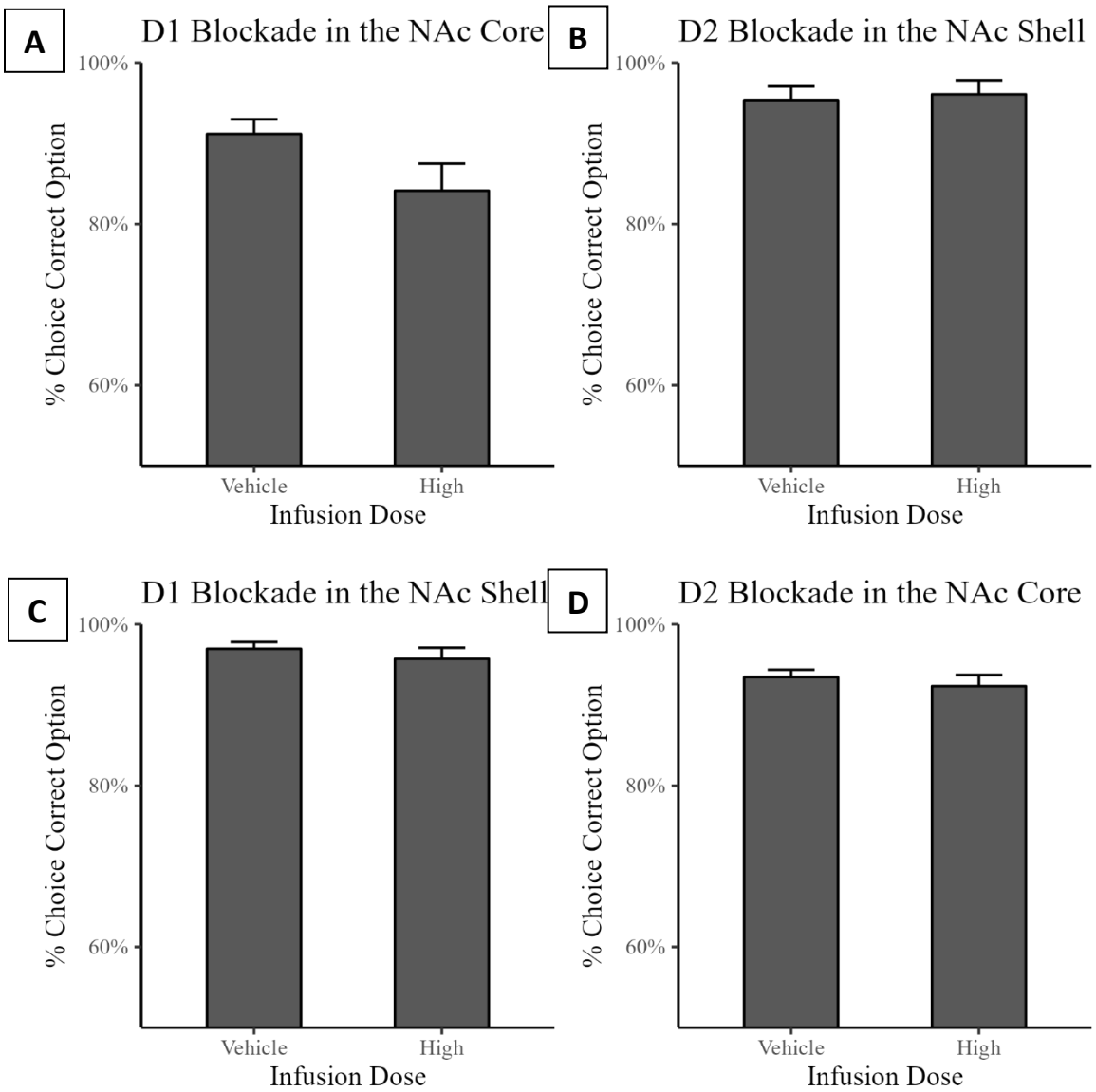

Averaged omission data for rats receiving the D2 antagonist into the NAc (A), separated by sex.

# Fig. 13: Blackjack D2 Blockade – Locomotion




Averaged locomotion data for rats receiving the D2 antagonist into the NAc core (A) or shell (B).

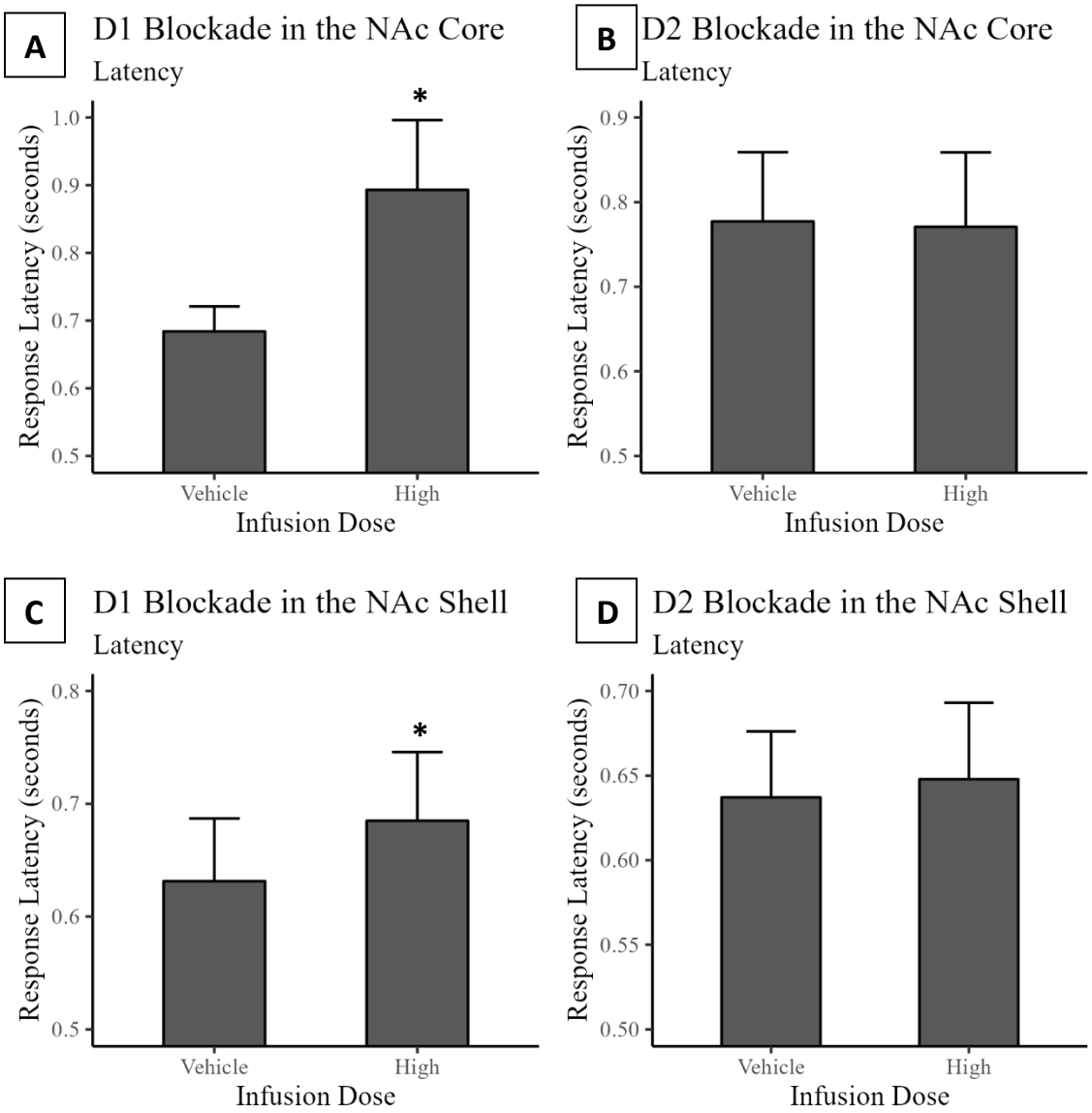
# Fig. 14: Conditional Discrimination DA Blockade – Choice




Averaged choice data for rats receiving the D1 antagonist into the NAc core (A) or shell (C).

Averaged choice data for rats receiving the D2 antagonist into the NAc core (B) or shell (D).

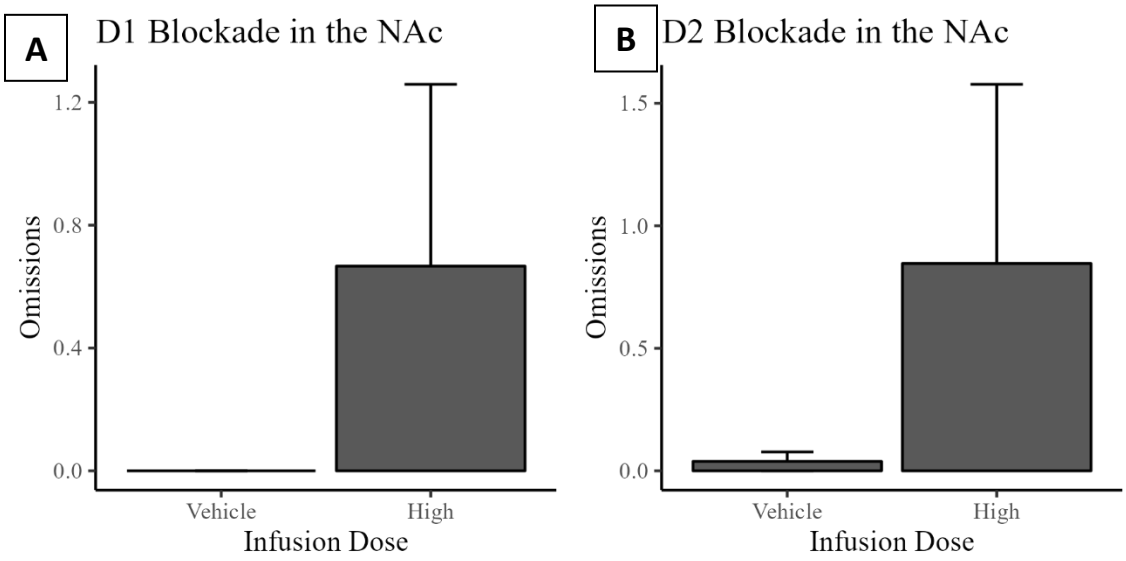
# Fig. 15: Conditional Discrimination DA Blockade- Latency




Averaged latency data for rats receiving the D1 antagonist into the NAc core (A) or shell (C).

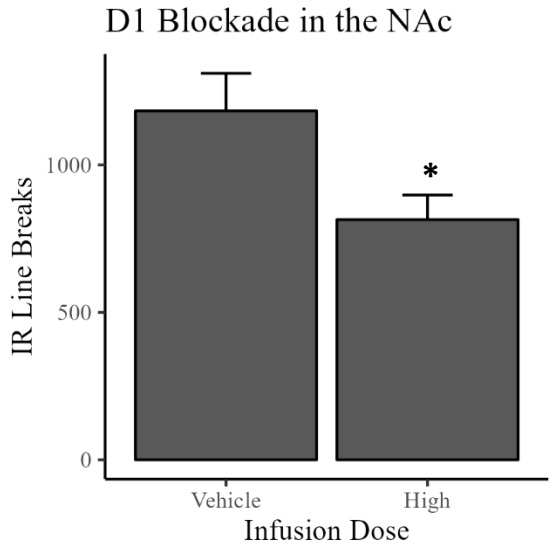
Averaged latency data for rats receiving the D2 antagonist into the NAc core (B) or shell (D).

## Fig. 16: Conditional Discrimination DA Blockade- Omissions



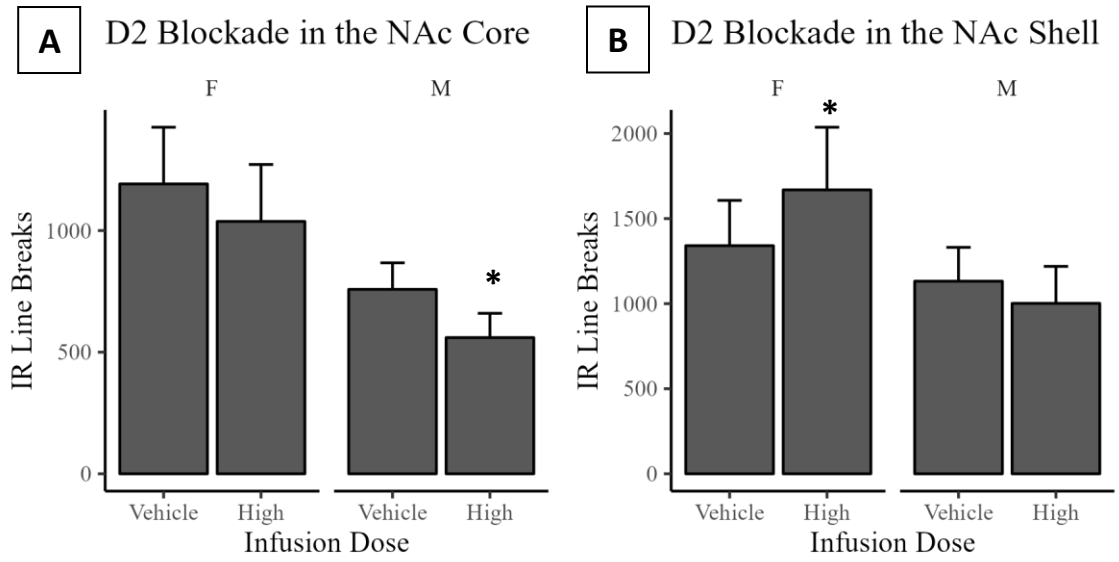

Averaged omission data for rats receiving the D1 (A) or D2 (B) antagonist into the NAc.

## Fig. 17: Conditional Discrimination D1 Blockade- Locomotion




Averaged locomotion data for rats receiving the D1 antagonist into the NAc.

# Fig. 18: Conditional Discrimination D2 Blockade- Locomotion




Averaged locomotion data for rats receiving the D2 antagonist into the NAc core (A) or shell (B).

## Fig. 19 – Table Summary of Findings Between Probabilistic Discounting and Blackjack Tasks

| | Probabilistic Discounting | Blackjack Task |
|---|---|---|
| Systemic D1 antagonism | ↓ reduced risky choice | ↕ disrupted choice patterns |
| | ↑ increased latencies | — no effect on latencies |
| Systemic D2 antagonism | ↓ reduced risky choice | ↕ disrupted choice patterns* |
| | ↑ increased latencies | — no effect on latencies |
| NAc core inactivation | — no effect on choice | ↕ random choice patterns |
| | ↑ increased latencies | ↑ increased latencies |
| NAc shell inactivation | ↓ reduced risky choice | ↑ increased risky choice |
| | — no effect on latencies | — no effect on latencies |
| NAc D1 antagonism | ↓ reduced risky choice | — effect on choice |
| | ↑ increased latencies | ↑ increased latencies |
| NAc D2 antagonism | — no effect on choice | Core: ↓ reduced risky choice<br>Shell: ↑ increased risky choice |
| | — no effect on latencies | — no effect on latencies |

A table summarizing work performed in our lab contrasting the effects of dopamine receptor and nucleus accumbens manipulations between the probabilistic discounting task and the blackjack task. Results for systemic dopamine antagonists on the blackjack are currently unpublished. The results of this thesis help dissociate the broader impairments from NAc core and shell inactivation into the specific contributions of dopamine receptor subtypes.

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