Neostriatal Dopamine Modulates Motivation:

Incentive Salience Generation in the Neostriatum

by

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Abstract

Motivational processes are constantly at work to focus us on relevant stimuli for survival. The mesolimbic dopamine system has been strongly linked to motivation and reward-directed behavior, especially in regions such as the nucleus accumbens and ventral tegmental area. However, the nigrostriatal (substantia nigra to neostriatum) pathway has also been implicated in reward and motivation. Incentive salience is the motivational value placed on cues paired with reward. This study aimed to test whether increased dopamine in the neostriatum could enhance incentive salience of a learned cue. Differing levels of a D1/D2 dopamine agonist cocktail were combined with an autoshaping paradigm as a measure of incentive salience using both dorsomedial and dorsolateral neostriatum cannula placements. Results indicate that dopamine stimulation may increase incentive salience in the direction of predictive cues in the dorsolateral neostriatum but increase incentive salience in a more general fashion in the dorsomedial neostriatum.

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Motivational processes are essential for our daily lives, but they often go unnoticed. If something is desired or needed, there is a good chance that a motivated attempt will be made toward the goal, whatever that goal may be. Many neural mechanisms have been implicated in the production and execution of motivation, both consciously and unconsciously. Classically, the mesolimbic dopamine system, including the nucleus accumbens and ventral tegmental area, has been viewed as the neural center for reward (Berridge, 2004). However, recent evidence has suggested that other brain structures may play an equally important role in reward processing.

Throughout the last half century, it has become increasingly apparent that dopamine is the neurotransmitter most important for reward (Berridge, 2007; Dayan & Balleine, 2002; Wise 2009). However, only recently has evidence pointed to a specific role for dopamine within the scope of reward. Namely, dopamine is responsible for the 'wanting' portion of reward, rather than 'liking' and, to a lesser extent, learning (Berridge, 2007; Berridge & Robinson, 1998). 'Wanting' refers specifically to the incentive salience process of motivation. Incentive salience is an association between natural rewards and learned stimuli that are paired in a Pavlovian fashion. The reward (an unconditioned stimulus) is paired with some sort of cue (a conditioned stimulus), and eventually the same motivational behaviors normally directed at the reward are then aimed at the cue as well. In this sense, the cue becomes 'wanted' as much as the natural reward, and is attractive enough to warrant approach and consummatory behaviors normally directed at the reward (Berridge, 2007). Incentive salience is an anticipatory process, with the reward-paired cues causing motivational responses. These responses are locked to the moment of cue presentation, which is the moment that 'wanting' can be observed.

A major site of dopamine innervation is the neostriatum, often referred to as the dorsal striatum or caudate putamen. It is a major crossroads of connectivity between the mesolimbic dopamine system and the neocortex (Thorn, Atallah, Howe, & Graybiel, 2010; Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004). Along with cortical projections, inputs from the substantia nigra, ventral tegmental area, and nucleus accumbens all converge in the neostriatum (Haber & Knutson, 2010; Yin, Ostlund, & Balleine, 2008). The neostriatum is traditionally viewed as a site of stimulus-response (S-R) learning, which involves the learning of strict responses to stimuli that can be classified as habits or rigid responses. However, there is growing evidence that dopamine in the neostriatum plays a role in motivation as well.

Dopamine in the neostriatum has been proven necessary for motivated behaviors by depletion studies. A strain of dopamine-deficient mice, which show a complete lack of motivation for natural rewards and must be given L-DOPA daily for survival, show a rescue in eating behavior, locomotion, and reward-learning with the addition of a dopamine-promoting virus into the center of the neostriatum. Motivated behaviors are not rescued when the virus restores dopamine only to the nucleus accumbens, which may indicate that the neostriatum is essential for motivational processing and output while the nucleus accumbens modulates more particular aspects of motivation (Palmiter, 2008). This demonstrates the importance of the neostriatum in the manifestation of motivated behaviors and suggests that it is sufficient for the integration and output of motivated behaviors carried out by the dopaminergic subcircuit.

Other studies have also indicated that neostriatal dopamine signaling is central to motivational processes. Evidence exists in reward learning, where substantia nigra neurons fire with almost identical patterns to ventral tegmentum neurons in the presence of stimuli paired with rewards (Hollerman & Schultz, 1998). Extracellular dopamine levels increase in the

neostriatum during presentation of a drug-paired cue, suggesting that the expectation of reward is sufficient to cause neostriatal dopamine release and has a larger role than simple motor response production (Ito, Dalley, Robbins, & Everitt, 2002; Robbins & Everitt, 1992). There is evidence that dopamine in the neostriatum, while involved in the execution of motor activity, also has a role in drug-seeking behavior. The blockade of dopamine receptors in the dorsal striatum inhibits cocaine-seeking behavior, with rats showing a reduced response to cocaine-paired drug cues (Vanderschuren, Di Ciano, & Everitt, 2006). In humans, the neostriatum has been shown to act in a similar fashion. Neostriatal dopamine release is evoked by the presentation of drug cues to cocaine addicts and can accurately predict feelings of drug craving (Volkow et al., 2006). This suggests that dopamine in the neostriatum plays an important role in drug craving, and subsequently the 'wanting' portion of reward as described by incentive salience.

One way to model incentive salience attribution in the laboratory is the autoshaping phenomenon, which involves the attribution of motivated responses to a conditioned stimulus paired with an unconditioned stimulus. With autoshaping, the UCS reward is delivered regardless of the effort put forth to receive it. This procedure results in the attribution of a conditioned response to the reward cue, such as a lever presented with a cue light before a food reward is given. Some animals will show approach and interactions with the cue object, while others are more inclined to interact with the area of reward presentation, such as a food cup. The animals that interact with the cue are called sign-trackers, and those that interact with the reward cup are called goal-trackers. For both of these groups behavior is locked to the cue presentation, which means that behaviors focused on the prepotent stimulus will only be enhanced during the presence of the reward-predictive cue. Even though the two targets differ, both may express incentive salience.

A classic example of the autoshaping phenomenon is observed by Brown and Jenkins (1968). Pigeons were presented with a lighted food tray that appeared for a four-second interval either before or after another lighted cue was presented. The group that experienced the cue light before the food tray was presented (forward pairing) began to approach and peck the cue light (Brown & Jenkins, 1968). Other examples of this phenomenon exist, such as a raccoon that was trained to put coins in a piggy bank and then rewarded as reinforcement. Eventually, the coins themselves became a sort of reward for the raccoon, as he would not let go of them, even if reward was no longer given (Breland & Breland, 1961). These reward-paired cues elicit a dopamine response, as has been illustrated in a great number of studies (Berridge, 2007; Berridge & Robinson, 1998; Ito et al., 2002; Robbins & Everitt, 1992).

When investigating the role of dopamine in the neostriatum, it is important to note the different responses that dopamine signaling is capable of producing. Five types of dopamine receptors (D1-D5) are known, and these are divided into two families of receptors. The D1 family contains the D1 and D5 receptors and the D2 family contains the D2, D3, and D4 receptors. The striatum contains mostly D1 and D2 receptors, and D5 receptors to a lesser extent (Humphries & Prescott, 2010). One known function of dopamine is the regulation of glutamate signaling in medium spiny neurons, which are the most prevalent neuronal population in the striatum (Surmeier, 2007). This glutamate signaling in D1 receptor-expressing neurons has been shown to be necessary for the formation of associations between cue and reward in incentive learning processes (Novak et al., 2010). Although dopamine signaling and its subsequent cascades are much more complicated than simple excitation and inhibition, in general D1 receptors increase glutamate signaling and responsiveness postsynaptically (neuronal excitability), while D2 receptors seem to decrease downstream glutamate signaling (neuronal

inhibition) in the striatopallidal system through NMDA and AMPA receptor regulation (Palmiter, 2008; Surmeier, 2007). Since dopamine responses are somewhat complicated, it may be most useful to look at the effects of general dopamine excitation in the neostriatum before looking at the roles of individual receptor subtypes.

The division between medial and lateral neostriatum has been identified by several studies. This division is based on both neural connectivity and function. In habit learning, the medial and ventral neostriatum show different patterns of activation during the learning of paired associations, and these differences also manifest themselves in the motivational output that they produce (Thorn et al., 2010). The largest distinction in connectivity and function seems to be along the ventromedial to dorsolateral striatal gradient. This would suggest that the dorsomedial and dorsolateral neostriatum also show a division of function, as the gradient is not the simple dorsal-ventral arrangement that was previously thought (Voorn et al., 2004). Lesions of medial and lateral portions of the neostriatum produce different behavioral effects, which illustrate that a division of function accompanies this topographical division (Yin et al., 2008). Additionally, connections between the cortex and neostriatum respond differently in terms of synaptic plasticity over a medial to lateral gradient. This could indicate that anatomical differences underlie the differences in functional output generated by both the medial and lateral neostriatum (Smith, Musleh, Akopian, Buckwalter, & Walsh, 2001). Overall, evidence is beginning to point to more specific areas of the neostriatum in regards to connectivity and function, so it is necessary to break the neostriatum down into smaller pieces to determine whether this division can be better explained.

While it is obvious that dopamine is important in reward and plays a role in the function of the neostriatum, there are still areas in which dopamine is poorly understood. The following

set of experiments will address the question of incentive salience generation in the neostriatum, particularly by dopaminergic activation. To address this question, an autoshaping paradigm will be employed to test sign-tracking and goal-tracking behaviors in the presence and absence of a general dopamine receptor agonist (as well as a mu-opioid receptor agonist, used to verify an effect already observed in this laboratory [DiFeliceantonio & Berridge, 2010]). In addition, fos immunofluorescence will quantitatively measure specific neuronal activation under vehicle and dopamine agonist conditions. Most importantly, all experiments will be examining both dorsolateral and dorsomedial neostriatal cannulae placements to observe whether a medial/lateral separation of function is present in the neostriatum in respect to incentive salience processing.

Method

Subjects

Female Sprague-Dawley rats, born at the University of Michigan, were housed in pairs or in groups of three at 21 degrees Celsius and on a 12 hour reverse light/dark cycle. Rats were over three months old and between 240 and 400 grams in weight at the time of surgery. Rat chow and water were provided *ad libitum* during housing. All animals were handled regularly to accustom them to human interaction and lower their environmental stress during testing. Animal studies meet all standards and were approved by the University Committee on Use and Care of Animals (UCUCA) of the University of Michigan, along with national regulations set by the National Institute of Health.

Surgery

The same surgical procedures were used prior to all experiments. Rats were anesthetized with ketamine (80 mg/kg), xylazine (7 mg/kg), and atropine sulfate (0.04 mg/kg), along with carprofen (5mg/kg) as analgesic and chloramphenicol sodium succinate (60 mg/kg) to prevent

infection. After administration of anesthesia, 14 millimeter steel brain cannulae were implanted bilaterally. The implanted cannulae were attached to the skull with four steel screws and dental cement, and closed off with a stylet. Placements for the cannulae are described in Table 1.

Rats were monitored for one week after surgery before any behavioral testing took place. A dose of carprofen (5mg/kg) was administered on the first day after surgery as an analgesic. If necessary, a low dose of chloramphenicol was used to prevent or improve infection. No rats died before, during, or after the surgical process.

Drug Microinjections

All experiments (autoshaping, food intake, and fos immunofluorescence) required drug microinjections to administer either the tested drug or a vehicle control. Four drug conditions exist for these experiments. Both a high dose (.6mg/mL) and low dose (.3mg/mL) of a dopamine agonist cocktail, consisting of quinpirole (D2 agonist, Sigma Aldrich) and SKF-82958 (D1/D5 agonist, Sigma Aldrich), were used to test the effects of general dopamine stimulation (mixture of these agonist cocktails is described in the Appendix A). A 20:80 DMSO:saline mixture was used to allow the SKF and quinpirole to fully dissolve into solution. The vehicle control used was also a 20:80 DMSO:saline mixture. The final drug condition is a DAMGO dose, a mu-opioid receptor agonist, dissolved in artificial cerebrospinal fluid (this dose used to verify a result already observed in this laboratory). All rats used in the autoshaping and food intake experiments were tested under both dopamine conditions and vehicle, and most were also tested under a DAMGO condition. Thus, four days of testing were usually required per animal for each type of experiment, with at least one day break between test days. The fos study requires a naïve drug microinjection by nature, and thus each animal in the fos groups were only administered one drug condition.

Drug microinjections were administered by a microinfusion pump that allows a slow, steady delivery of drug into the desired brain region. Microinjections occurred directly before experimentation so the drug effects could be most potent. A dose of .5µL of drug was given over a two-minute span, and another minute was allowed before microinjection tubes were removed to ensure all drug was delivered. The dose of .5µL means that an effective dose of .3µg/.5µL was given for the S/Q High dose and .15µg/.5µL for the S/Q Low dose. After the microinjection was complete, a clean stylet was inserted into the cannulae and the rats immediately began experimentation.

Autoshaping

A total of 21 rats underwent autoshaping testing ($n_{dorsomedial} = 11$, $n_{dorsolateral} = 10$). The autoshaping chambers used for the first set of experiments consisted of a 4.5 cm x 2 cm cue lever that would appear with a cue light and 2.9 kHz continuous noise (CS+), an identical neutral lever that was present at all times (CS-), and a recessed food cup (CS_{cup}), also present at all times and used to administer a sucrose pellet after the presentation and withdrawal of the cue lever. Each cue lasted eight seconds, and twenty-five total cue presentations occurred in each experimental session. The inter-trial interval (time between CS+ presentations) varied between cues, but on average was 90 seconds. The CS+ appeared with the cue signals, which makes it more associated with the prediction of the reward delivery. The CS_{cup} is also the delivery location of the UCS sucrose reward, but is not directly predictive of reward because it is never retracted.

A program was created with MedPC Software to run the series of cue presentations on a random interval, and all video was recorded for scoring using Sony RealShot Manager. During training and testing, food was restricted to 10g chow/rat/day for the first few days and eventually raised to 15g chow/rat/day. This food restriction may have increased hunger, and thus

motivation for the sucrose reward, but also was useful in putting the animals in the same homeostatic state throughout all days of testing.

After surgery and prior to experimentation, rats were acclimated to the autoshaping chambers for six days. The first day consisted of a magazine training program, which simply gives a cue light and sound along with the delivery of a sugar pellet into the food cup. 20 total pellets were delivered, which taught the rats when and where to expect a sugar pellet in the chambers. The next five days consisted of autoshaping training, which consisted of the normal experimental procedure for all 25 cues, but in the absence of drug. These five days were a period of Pavlovian Instrumental training, where the rats soon identified the cue presentation with the sucrose reward.

Based on the amount of time spent interacting with the food cup or the autoshaping lever, rats were characterized as either sign-trackers or goal-trackers. Sign-trackers interact more with the autoshaping lever during cue presentation under absence of drug, while goal-trackers interact more with the food cup under absence of drug. These two classifications occurred within the already present conditions of cannula placement. This left four distinct test groups, which are dorsomedial lever-oriented, dorsomedial cup-oriented, dorsolateral lever-oriented, and dorsolateral cup-oriented.

Autoshaping Video Scoring

Video analysis was done in slow motion and took place blind of drug condition. When scoring the data, only the first, fifth, tenth, fifteenth, twentieth, and twenty-fifth CS+ presentations were scored and assumed to be a representative sample of the overall interactions that took place during the trial. Of these scored trials, the first was omitted from the data because it usually consisted of behaviors that were uncharacteristic of the rat during the rest of the test

run, possibly due to the added stress immediately after the microinjection. Thus, five cues were scored per rat in each drug condition. In addition to the eight seconds scored during each cue, the eight seconds prior to each cue presentation were also scored to determine whether any subsequent drug effects are general or specific to the time of cue presentation.

Scored behaviors include nibble-sniffs, slow bites, approaches to cue, and looks toward the opposite cue. Latency to the cue was also measured (in seconds) from the time of cue presentation to the time of first contact with the lever or first movement into the food cup. These behaviors were scored for both the lever and food cup, which leaves a total of 10 scored items per test run. Nibble-sniffs include physical contact with either the lever or food cup by many small, quick bites or focused sniffing. Slow bites involve more contact with the lever or food cup and are longer in duration, also concentrated to one area (whereas nibble-sniffs can be seen while the animal's head is moving from one part of the lever or cup to another). In this sense, the slow bites are more concentrated to one area and involve less movement of the animal. Approaches are measured when the animal either moves toward a cue for the first time during presentation or when she switches cues during presentation. In contrast, a look consists of visual contact being made with the opposite cue when already having approached the first cue, but without approach behavior being made to the second cue. For example, if a rat were committing nibble-sniffs to the autoshaping lever then stopped, turned her head toward the food cup, but then continued showing consummatory behaviors toward the lever, a food cup look would be recorded rather than a food cup approach.

Food Intake

Two surgery groups were subjected to a food intake trial after autoshaping testing. 13 rats ($n_{dorsomedial} = 6$, $n_{dorsolateral} = 7$) were used in this experiment. Rats were taken off of food

restriction prior to experimentation, which occurred with a one-day break between drug conditions. Drug microinjections with the same four conditions preceded one-hour intervals in food intake chambers. These chambers are clear to allow video scoring, with each rat getting her own chamber. Water was provided *ad libitum* in the chambers along with 20 grams of M&Ms and 20 grams of regular rat chow. Three neutral wood blocks in the shape of the rat chow were also present as a control for focused eating rather than a simple locomotor effect. The final amounts of M&Ms and rat chow were measured and recorded.

Video scoring was again done in slow motion and blind to drug condition. Scored eating behaviors were chow eating events and time spent eating, M&M eating events and time spent eating, and wood chew events. Drinking bouts, food-sniffs directed at either M&Ms or chow (focused sniffing, but no eating), and M&M carries and chow carries (food picked up and brought to another part of the cage) were also recorded. Cage crosses (front to back, or vice versa), treading, grooming, sleeping (bouts and amount of time), and rearing are general behaviors that were recorded to determine whether large locomotor effects of the drugs could be playing a role in the data.

Brain Extraction for Autoshaping and Food Intake

Brains were extracted after all sets of experimentation to check cannula placement. Rats were given a lethal injection of pentobarbital (between .65mL and .80mL) and decapitated. After decapitation, brains were manually extracted and incubated overnight in a 10% paraformaldehyde solution. After 24 hours, the brains were switched to a solution of 30% sucrose in .1M NaPB. After the brains sank, which was between two and three days, the brains were rapidly frozen and cut into 60µm slices. These slices were then mounted on slides and

allowed two days to dry. A Nissl stain colored the brain tissue, and cover glass was applied to keep the brains from decaying. The Nissl stain procedure is explained in-depth in Appendix B.

After staining and mounting, cannula placements were verified. Data for two animals were excluded because of incorrect cannula placement. Rat 6965 had a cannula placement into the ventricle, which forced it to be excluded from data. Rat 7119 had a placement that was too far dorsal and did not cross the corpus callosum into the neostriatum, also forcing it to be excluded from data. All other rats had acceptable cannula placements.

Fos Immunohistochemistry

A fos study was used to determine neuronal activation during drug microinjection. The same surgical procedure, including placements for dorsomedial and dorsolateral neostriatum, were employed. 18 rats were used in the experiment, 9 dorsomedial, 8 dorsolateral, and 1 normal. The normal rat, used as a control, was given only screws and dental cement without implanted cannulae. Of the dorsomedial placements, 3 were given vehicle, 3 S/Q Low dose, and 3 S/Q High dose. The dorsolateral placements consisted of 3 vehicle, 2 S/Q Low dose, and 3 S/Q High dose. The rats were handled five times before the date of microinjection to minimize fos activation due to stress rather than due to microinjection. The naïve rats were given microinjections of their drug condition and sacrificed forty minutes later, which was estimated to be the peak time of activation for the S/Q drug conditions.

Brains were extracted with the same procedure as previously stated, but decapitation was preceded by a transcardial perfusion which prepared the brains for immunohistochemistry. In addition, the slicing procedure varied slightly. Brains were sliced to 40µm, with every other slice being placed in one of two scintillation vials, which left two viable sets of tissue per brain in the event that one vial was destroyed. The backup vial was stored in cryoprotectant at -20^{o}F.

After incubation in primary and secondary antibody over a two day period, brains were mounted on slides and covered once dry. The fluorescently labeled fos was observed under a microscope and transferred onto digital images to be scored. The scored area was taken directly behind the center microinjection point, where less tissue damage occurred but still close enough to the injection point to allow drug to diffuse to that area. For a more detailed description of the fos procedure, see Appendix C.

Fos scoring took place with a grid placed upon the digital image. Boxes down the x- and y-axes, along with two other axes on a 45 degree angle from x and y, were used as a scoring template. Each fluorescent dot was counted as a fos positive cell, and only the fos marks in the boxes counted in the fos total. These total fos counts were included in the data.

Data Analysis and Graphing

Data for all of the above experiments were originally entered into Microsoft Excel, where they were organized into relevant categories. Data were then transferred to SPSS for statistical analysis. Graphs of the data were created on Microsoft Excel and edited in Adobe Illustrator.

Results

Autoshaping

For all autoshaping data, a repeated-measures ANOVA with a Bonferonni correction on subsequent t-tests was applied. An α level of .05 was used in determining whether data was statistically significant. Since there were a low number of rats in each experimental group, data trends are also discussed as percentage of vehicle for drug conditions. Results are reported in terms of S/Q Low and S/Q High stimulation for both cannula placements and in terms of autoshaping tendancy (lever-oriented and food-cup oriented).

Dorsomedial Lever-Oriented Animals. The S/Q Low dose appears to increase nibblesniffs and slow bites with both the food cup and cue lever during presentation, while the S/Q High dose showed only an increase in nibblesniffs and slow bites with the prepotent stimulus, the cue lever. These results are shown below.

S/Q Low Dose for Dorsomedial Lever-Oriented Animals. Data for dorsomedial lever-oriented animals under the S/Q Low dose showed an increase in prepotent interactions (109% of vehicle; $M_{\text{nibblesniffs}} = 5.88$, $SD = 1.39$, $M_{\text{slow interactions}} = 1.24$, $SD = 1.65$), described as lever nibblesniffs plus lever slow bites, along with a slight increase in non-prepotent interactions (104% of vehicle; $M_{\text{slow interactions}} = 0.04$, $SD = 0.09$). This accompanied a small increase in latency to the prepotent cue (112% of vehicle; $M_{\text{latency}} = 1.44$, $SD = 0.79$) and no change in latency to the non-prepotent cue. An increase in the probability of approaching both lever and food cup (from zero to four percent, $F(2, 4) = 368.57$, $p < .001$) was also observed, which follows the observed increase in interactions with both food cup and cue lever. Overall, the S/Q Low dose shows an increase in nibblesniffs and slow bites focused on both the prepotent and non-prepotent stimulus.

S/Q High Dose for Dorsomedial Food Cup-Oriented Animals. The S/Q High dose showed a decrease in prepotent interactions (95% of vehicle; $M_{\text{nibblesniffs}} = 4.96$, $SD = 1.34$, $M_{\text{slow interactions}} = 1.24$, $SD = 1.49$) but an increase in non-prepotent interactions (116% of vehicle; $M_{\text{nibblesniffs}} = 0.12$, $SD = 0.18$). Rats were slower to the prepotent cue (130% of vehicle; $M_{\text{latency}} = 1.68$, $SD = 1.06$) and quicker to the non-prepotent cue (94% of vehicle; $M_{\text{latency}} = 7.51$, $SD = 0.68$), along with being more likely to approach both cues during presentation (from zero to 12% probability). Overall, the S/Q High dose shows an increase in only nibblesniffs and slow bites for the non-prepotent stimulus.

Dorsomedial Food-Cup Oriented Animals. The S/Q Low dose shows an increase in nibblesniffs and slow bites with both the prepotent and non-prepotent stimuli during cue presentation, while the S/Q High dose shows and increase in nibblesniffs and slow bites with only the prepotent stimulus.

S/Q Low Dose for Dorsomedial Food Cup-Oriented Animals. In dorsomedial food-cup oriented animals under the S/Q Low dose, a large increase in prepotent interactions (food cup) was observed (140% of vehicle, $F(2, 4) = 3.00$, $p = .008$; $M_{\text{nibblesniffs}} = 3.28$, $SD = 1.45$, $M_{\text{slow interactions}} = 1.44$, $SD = 1.13$), which accompanied a decrease in latency to the food cup (61% of vehicle; $M_{\text{latency}} = 1.77$, $SD = 0.84$). An increase in cue lever interactions was also seen (142% of vehicle; $M_{\text{nibblesniffs}} = 0.68$, $SD = 0.89$, $M_{\text{slow interactions}} = 0.12$, $SD = 0.27$) along with a decrease in latency to the lever (88% of vehicle; $M_{\text{latency}} = 6.26$, $SD = 1.95$). The probability of approaching both cues also increased from vehicle levels (from 52% to 64% probability, $F(2, 4) = 41.66$, $p = .009$). Like the lever-oriented animals with a dorsomedial cannula placement, this dose showed an increase in nibblesniffs and slow bites with both the prepotent and non-prepotent stimuli during cue presentation.

S/Q High Dose for Dorsomedial Food-Cup Oriented Animals. For the S/Q High dose, prepotent interactions increased (149% of vehicle; $M_{\text{nibblesniffs}} = 2.92$, $SD = 1.22$, $M_{\text{slow interactions}} = 2.04$, $SD = 1.81$) while latency to the food cup decreased (74% of vehicle; $M_{\text{latency}} = 2.14$, $SD = 1.77$). Non-prepotent interactions decreased (92% of vehicle; $M_{\text{nibblesniffs}} = 0.52$, $SD = 1.05$, $M_{\text{slow interactions}} = 0.08$, $SD = 0.18$) as cue lever latency also decreased (90% of vehicle; $M_{\text{latency}} = 6.43$, $SD = 2.71$). Another large distinction from the S/Q Low dose was in the probability of approaching both cues during presentation, which decreased greatly (from 52% to 36% probability). Unlike the lever-oriented animals with a dorsomedial cannula placement, the S/Q

High dose showed an increase in nibblesniffs and slow bites with only the prepotent stimulus for this cannula placement.

Dorsolateral Lever-Oriented Animals. For lever-oriented animals with a dorsolateral cannula placement, the S/Q Low dose increases nibblesniffs and slow bites in the direction of the cue lever, while also showing a decrease in these interactions with the food cup. The S/Q High dose showed the opposite effect.

S/Q Low Dose for Dorsolateral Lever-Oriented Animals. In dorsolateral lever-oriented animals, the S/Q Low dose increased the number of interactions with the cue lever (118% of vehicle; $M_{\text{nibblesniffs}} = 5.84$, $SD = 1.37$, $M_{\text{slow interactions}} = 1.4$, $SD = 1.21$, Figure 1) while also decreasing latency to the lever (82% of vehicle; $M_{\text{latency}} = 1.18$, $SD = 0.49$). Interactions with the food cup decreased (74% of vehicle; $M_{\text{nibblesniffs}} = 0.36$, $SD = 0.50$, $M_{\text{slow interactions}} = 0.2$, $SD = 0.28$ Figure 1), while latency increased (109% of vehicle; $M_{\text{latency}} = 6.55$, $SD = 1.74$). The probability of approaching both cues during cue presentation remained at vehicle level. Overall, the cue lever gains interactive behavior from the rat while the food cup loses these interactions.

S/Q High Dose for Dorsolateral Lever-Oriented Animals. For the S/Q High dose, interactions with the food cup increased (114% of vehicle; $M_{\text{nibblesniffs}} = 0.2$, $SD = 0.45$, $M_{\text{slow interactions}} = 0.2$, $SD = 0.45$, Figure 2), while interactions with the cue lever decreased (31% of vehicle, $M_{\text{nibblesniffs}} = 5.72$, $SD = 1.60$, $M_{\text{slow interactions}} = 1.32$, $SD = 1.49$, Figure 2). Latency did not change from vehicle for the cue lever, while it increased for the food cup (116% of vehicle; $M_{\text{latency}} = 6.98$, $SD = 2.29$). The S/Q High caused increased interactions with the food cup and fewer with the cue lever.

Dorsolateral Food-Cup Oriented Animals. For food-cup oriented animals with a dorsolateral cannula placement, the S/Q Low dose showed an increase in nibblesniffs and slow

bites with the cue lever and a decrease in these interactions with the food cup. The S/Q High dose showed a general increase in these interactions with both cues.

S/Q Low Dose for Dorsolateral Food-Cup Oriented Animals. In dorsolateral food-cup oriented animals, the S/Q Low dose promoted a decrease in interactions with the food cup (82% of vehicle; $M_{\text{nibblesniffs}} = 1.25$, $SD = 0.53$, $M_{\text{slow interactions}} = 0.45$, $SD = 0.5$, Figure 2) while causing increases in cue lever interactions (130% of vehicle; $M_{\text{nibblesniffs}} = 0.05$, $SD = 0.1$, Figure 2). Latency to the food cup increased greatly (186% of vehicle; $M_{\text{latency}} = 3.09$, $SD = 1.01$) while it decreased slightly for the food cup (95% of vehicle; $M_{\text{latency}} = 6.97$, $SD = 1.26$). The effect seen here is a decrease in sniffing and biting behavior of the food cup and an increase in this behavior toward the cue lever, an effect that is very similar to the S/Q Low dose in lever-oriented animals with a dorsolateral cannula placement.

S/Q High Dose for Dorsolateral Food-Cup Oriented Animals. For the S/Q High dose, an increase in interactions with the food cup was seen (111% of vehicle; $M_{\text{nibblesniffs}} = 1.6$, $SD = 1.10$, $M_{\text{slow interactions}} = 0.8$, $SD = 0.71$) along with a decrease in interactions with the cue lever (90% of vehicle; $M_{\text{nibblesniffs}} = 0.1$, $SD = 0.2$). Latency to both cues increased for the high dose (lever = 109% of vehicle; $M_{\text{latency}} = 8$, $SD = 0$; food cup = 118% of vehicle; $M_{\text{latency}} = 1.96$, $SD = 1.52$). Overall, the S/Q High dose showed a general increase in interactions with both cue lever and food cup.

All Dorsomedial Animals. Since results for prepotent and non-prepotent interactions were similar for the dorsomedial placement, they were grouped together. The data can be better explained in terms of the overall effect in this placement, rather than distinguishing between sign-trackers and goal-trackers. For the dorsomedial neostriatum cannula placement, interactions with the prepotent stimulus (cue lever for sign-trackers, food cup for goal-trackers) increased for

the S/Q Low dose (142% of vehicle, $F(2,9) = 2.751$, $p = .002$, Figure 3), as did the number of interactions with the non-prepotent stimulus (131% of vehicle; $M_{\text{non-prepotent interaction}} = 1.48$, $SD = 1.01$, Figure 3). A slightly decreased latency to both the prepotent cue (97% of vehicle; $M_{\text{latency}} = 1.77$, $SD = 0.84$) and non-prepotent cue (93% of vehicle; $M_{\text{latency}} = 6.26$, $SD = 1.95$) was also observed. The probability of approaching both cues was also increased (52% of trials to 64% of trials, $F(2,9) = 159.648$, $p < .001$).

All Dorsolateral Animals. Since the effect shown by both dorsolateral lever-oriented animals and dorsolateral food-cup oriented animals was very similar in terms of cue lever and food cup interactions, data was combined to observe the overall area effect. The effect produced by the S/Q Low dose is better described in terms of lever interactions and cue interactions, rather than prepotent/non-prepotent interactions. In this area, lever interactions increased (118% of vehicle; $M_{\text{lever interactions}} = 4.33$, $SD = 3.57$, Figure 4) while latency to the cue lever did not change from vehicle level ($M_{\text{latency}} = 3.76$, $SD = 3.34$,). In contrast, a decrease in food cup interactions (86% of vehicle; $M_{\text{food cup interactions}} = 1.13$, $SD = 1.32$) is accompanied by a large increase in latency (497% of vehicle; $M_{\text{latency}} = 5.01$, $SD = 2.95$).

Autoshaping: Combining Placements to Determine Effects. Since the dopamine agonist cocktail used in this study had been previously unused, it was necessary to use both the high dose and low dose to see which one produced a more reliable effect. When referencing the above results, the S/Q Low dose appears to produce the more reliable result. Therefore, the focus of the discussion will be on those results, along with possible explanations for the discrepancies between doses. As an overall effect, combining all animals with a dorsomedial cannula placement shows an increase in nibblesniffs and slow bites with both the prepotent and non-prepotent stimulus for the S/Q Low dose. That contrasts with the dorsolateral placement,

where animals showed an increase in nibblesniffs and slow bites with the only autoshaping lever, along with a decrease in these interactions with the food cup.

Fos immunohistochemistry

Fos counts performed on images of the fluorescently labeled tissue yielded results that differed slightly from the autoshaping data. Counts were performed on a grid of ten 0.05mm x 0.05mm boxes along both the x- and y-axis and at 45^{o} to each axis. For animals with a dorsomedial placement, the S/Q Low dose remained at vehicle levels of activation (100% of vehicle, Figure 5) for almost all distances from the center of the grid, up to the final scored point at 0.50mm from center. However, a much higher level of activation was recorded for the S/Q High dose in the dorsomedial animals (between 175% and 225% of vehicle activation at all points, Figure 5). Animals with a dorsolateral placement displayed similar levels of activation up to 0.20mm from center (about 125% to 150% of vehicle, Figure 6) for both S/Q doses, but showed differing activation at more distant points. While the S/Q Low dose continued to show activation at 125% of vehicle and less at 0.50mm, the S/Q High dose increased activation for distant points, up to around 200% of vehicle at 0.50mm (Figure 6). This data shows an unexpected effect when the autoshaping data is referenced, as the S/Q Low dose showed more behavioral effects during autoshaping but the S/Q High dose showed more fos activation.

Food Intake

S/Q Low Dose for the Dorsomedial Placement. For animals with a dorsomedial cannula placement, the S/Q Low dose elicited increases in both eating and locomotor behaviors. The amount of M&Ms eaten (in grams) did not show a change from vehicle ($M_{\text{M\&M eaten}} = 7.38$, $SD = 2.31$) and the number of M&M eating bouts decreased only slightly ($M_{\text{M\&M bouts}} = 14$, $SD = 1.41$), as did the total amount of time spent eating ($M_{\text{M\&M time}} = 561.5$, $SD = 34.65$). However,

the number of focused M&M sniffs increased greatly (233% of vehicle; $M_{\text{M\&M sniff}}$ = 22.5, *SD* = 10.61, Figure 7). These effects accompanied large increases in cage crosses (158% of vehicle; $M_{\text{cage cross}}$ = 75, *SD* = 19.80) and rearing events (140% of vehicle; M_{rear} = 146, *SD* = 67.88). For the S/Q Low dose, focused sniffs toward the M&Ms increased, as did the locomotor response to the drug.

S/Q High Dose for the Dorsomedial Placement. For the S/Q High dose, very similar effects were seen. The amount of M&Ms eaten did not change from vehicle ($M_{\text{M\&M eaten}}$ = 7.25, *SD* = 1.33) and M&M eating bouts decreased slightly (83% of vehicle; $M_{\text{M\&M bouts}}$ = 12.5, *SD* = 0.71). Total time spent eating did not change from vehicle ($M_{\text{M\&M time}}$ = 535, *SD* = 149.91), but focused sniffs at the M&Ms doubled (199% of vehicle; $M_{\text{M\&M sniff}}$ = 29, *SD* = 0). Again, cage crosses (182% of vehicle; $M_{\text{cage cross}}$ = 86.5, *SD* = 10.61) and rearing bouts (141% of vehicle; M_{rear} = 146.5, *SD* = 58.69) increased. For the S/Q High dose, focused sniffs toward M&Ms doubled from vehicle levels, and the locomotor response to the drug also increased.

S/Q Low Dose for the Dorsolateral Placement. Animals with a dorsolateral cannula placement showed a different response to the dopamine agonist drugs. For the S/Q Low dose, there was no change from vehicle in the amount of M&Ms eaten ($M_{\text{M\&M eaten}}$ = 8.29, *SD* = 2.31), the number of M&M eating bouts ($M_{\text{M\&M bouts}}$ = 12, *SD* = 1.73), or the amount of time spent eating M&Ms ($M_{\text{M\&M time}}$ = 749, *SD* = 143.45). M&M sniffs decreased slightly (92% of vehicle; $M_{\text{M\&M sniff}}$ = 15.67, *SD* = 4.51, Figure 7), as did rearing (88% of vehicle; M_{rear} = 59.33, *SD* = 0.58). An increase in cage crosses was seen (117% of vehicle; $M_{\text{cage cross}}$ = 37, *SD* = 5.29). For the S/Q Low dose, no large change from vehicle was seen for M&M related interactions and a mixed effect was seen for locomotor behaviors.

S/Q High Dose for the Dorsolateral Placement. For the S/Q High dose, the total amount of M&Ms eaten decreased (79% of vehicle; $M_{\text{M\&M eaten}} = 6.46$, $SD = 3.22$), as did the number of M&M sniffs (88% of vehicle; $M_{\text{M\&M sniff}} = 15$, $SD = 1.42$). M&M eating events increased slightly (111% of vehicle; $M_{\text{M\&M bouts}} = 13$, $SD = 4.24$), as did the total time spent eating M&Ms (106% of vehicle; $M_{\text{M\&M time}} = 815.5$, $SD = 204.35$). Locomotor activities did not differ much from vehicle, but did increase very slightly. Cage crosses (106% of vehicle; $M_{\text{cage cross}} = 33.5$, $SD = 6.36$) and rears (108% of vehicle; $M_{\text{rear}} = 73$, $SD = 9.90$) showed these slight increases. For the S/Q High dose, results did not differ strongly from vehicle for both M&M related interactions or locomotor behaviors.

Discussion

The data presented here demonstrate a motivational role for dopamine in the neostriatum. The most interesting pieces of evidence are in the autoshaping results for the S/Q Low dose of both dorsomedial and dorsolateral animals. In all animals with a dorsolateral cannula placement, interactions with the cue lever increased while interactions with the food cup decreased. In addition, animals were quicker to the cue lever and slower to the food cup under dopamine stimulation. This demonstrates a shift in focus to the predictive cue, the lever, during presentation. For the dorsomedial cannula placement, all animals (sign-trackers and goal-trackers) showed an increase in interactions with both the cue lever and the food cup. This is a more general increase, not just limited to the predictive stimulus. A decrease in latency to both cues was also seen for this placement. Together, these results may indicate that dopamine in the dorsomedial neostriatum may prime a more general motivated state for obtaining rewards, while dopamine in the dorsolateral neostriatum seems to increase incentive salience in the direction of the predictive cue.

The food intake experiment also gives evidence for incentive salience generation by dopamine signaling in the neostriatum. The increased number of focused sniffs on the M&Ms under the S/Q Low condition in the dorsomedial placement indicates a heightened anticipatory response of the M&M reward itself, which is exactly what would be predicted by incentive salience. The sight of the M&M is a food cue and generates a cue-paired response, which only takes place prior to consumption. The anticipatory nature of the sniff does not transfer to actual consumption of the M&M, as is seen in the results (no change in amount of M&Ms eaten for the dorsomedial placement), because it is associated only with the 'wanting' portion of the reward. M&M sniffs are comparable to nibblesniffs and slow bites on the cue lever in the autoshaping portion of the study because they both signal the upcoming availability of reward. However, the consummatory phase is not initiated because these behaviors are specific to the cue, not the reward.

With previous research suggesting a more goal-oriented role for the dorsomedial neostriatum and an S-R role for the dorsolateral neostriatum, it was interesting to note that the data obtained in this study did not fully support these ideas. Yin et al. speak of the dorsolateral neostriatum as being an area of strict S-R habit production (Yin et al., 2008). If this were the case, then S-R habits should have been strengthened with increased dopamine levels. Sign-tracking animals did show an increased number of interactions with the cue lever and fewer with the food cup, which would be consistent with an S-R hypothesis; however, goal-tracking animals did not show a similar strengthening of their prepotent stimulus – they also showed an increase in lever interactions and a decrease in food cup interactions. These data together show that rather than increase habitual action, incentive salience is instead increased, demonstrated by a flexible change in behavior. The rats seem to experience a shift in focus away from the food cup

and in the direction of the predictive cue under dopamine stimulation of the dorsomedial neostriatum.

While a distinct shift in focus was not seen under increased dopamine in the dorsomedial neostriatum, an increase in interactions with both the prepotent and non-prepotent cues was seen along with decreased latencies to both cues. This could indicate that an increased motivational state was primed by dopamine, which made both stimuli more attractive. General motivated behaviors are restored to dopamine-deficient mice when a dopamine promoting virus is injected into the dorsomedial neostriatum, but less effective with an injection into the dorsolateral neostriatum (Palmiter, 2008). The non-specific activation observed in this study may also underline the more general role of rescuing motivation for many different processes, which in the Palmiter study include feeding, locomotion, and reward-based learning (Palmiter, 2008).

A study by Volkow et al. involving video presentation of drug cues to cocaine-addicted humans showed that the cues themselves are sufficient to elicit dopamine release in the neostriatum, especially along the nigrostriatal pathway. The study speaks mostly of drug "craving" as a correlate of "wanting" and suggests that it is the main reason for actions used to obtain the drug reward (Volkow et al., 2006). Much like the Volkow study, this study sees the moment of cue presentation as the time when dopamine release has its largest noticeable effect in the neostriatum. The prepotent stimulus is comparable to a drug cue, and the increased craving resembles incentive salience (Volkow et al., 2006). Even though drug cues for human addicts are very different from reward-paired cue presentations in this study, they both relate to the moment of reward anticipation and thus may include a common neural pathway. Recently, another form of cue-based activation has been associated with the neostriatum. Striatal dopamine signaling increases in response to food cues in humans and to a greater extent in obese

humans (Stice, Yokum, Burger, Epstein, & Small, 2011; Volkow, Wang, & Baler, 2011). Over-activation of the neostriatum can also be observed in children that have a high risk for developing obesity (Stice, et al., 2011). This increase in eating behavior caused by striatal activity correlates to the increase in motivated behaviors shown toward the predictive cues in this study. Dopamine modulation in the neostriatum has obvious ties to addictive behavior, both for drugs and food, and may be an important predictor of susceptibility to addiction later in life.

One seemingly contradictory result of this study is the enhanced level of neostriatal fos activation seen with the S/Q High dose when compared with the S/Q Low dose. With the autoshaping data showing an increased number of interactions with the prepotent stimulus in the dorsomedial placement for the S/Q Low dose, along with a more consistent pattern of activation, it was strange that the S/Q High dose elicited a stronger level of fos activation. This could be explained by the type of activation the dopamine agonist cocktails provided. It is possible that the activation of D1 and D2 receptors has a different overall response at low or high levels of activation. SKF-82958 is a D1/D5 receptor agonist, which has been shown to cause neuronal activation of downstream neurons, while quinpirole is a D2 receptor agonist, which causes neuronal depression (Palmiter, 2008; Surmeier, 2007). It has been demonstrated that D1 activation produces a large activation of the fos protein, while D2 stimulation does not (Paul, Graybiel, David, & Robertson, 1992). The heightened levels of the D1 agonist in the high dose may have been sufficient to raise fos levels independently of the D2 stimulation. It is also possible that the increased fos activation seen was due to an overall increase in dopamine receptor activation and their subsequent signaling cascades, but the competition between the D1 and D2 response systems lowered the motivational response as observed in the results.

The discrepancy in motivational output based on overall dopamine stimulation may be explained by the activation of both direct and indirect dopamine pathways. The direct pathway, activated by D1 receptor stimulation, seems to have an excitatory function throughout a cortico-basal ganglia loop. In contrast, the indirect pathway, marked by D2 activation, has an inhibitory function through the same kind of loop (Graybiel, 2000). When activated simultaneously, these pathways may balance each other in some aspect but also have unexpected effects that influence the overall motivational output generated in the neostriatum. In this experiment, D1 receptor activation by SKF-82958 may be more potent in the S/Q Low dose while quinpirole activation of D2 receptors may function more efficiently at a higher concentration, which could cause internal competition to determine the absolute activation or inhibition that dopamine signaling will elicit. A good way to test this hypothesis would be to test each drug individually. This would also help clarify the effects seen in the autoshaping portion of the experiment and give more specificity to the role of each dopamine receptor subtype in the neostriatum.

As seen in the results of this study and as reported by numerous other studies, a division of function occurs along the medial/lateral gradient of the neostriatum (Faure, Haberland, Condé, & El Massioui, 2005; Thorn et al., 2010; Yin et al., 2008). However, this is the first study that has specifically targeted dopamine modulation along this gradient in terms of the incentive salience process. Because of this, the evidence obtained from this study was intriguing enough to warrant a more in-depth look with future studies. Another gradient that would be useful to study is the rostral/caudal gradient of the neostriatum, which may show a similar distinction of activation between the two areas. One study has investigated the role of the posterior medial neostriatum in regards to the learning of goal-directed actions, which could be initiated there (Yin, Knowlton, & Balleine, 2005). This division could be measured with an autoshaping study

as well, which would help determine whether the posterior medial neostriatum differs from its more anterior portion in respect to motivational processing.

Studies that measure a potential motivational role for dopamine in the neostriatum have interpreted the processes seen as goal-directed behaviors or reward-based habit learning rather than assigning a concrete motivational role, such as incentive salience, to the neostriatum (Ito et al., 2002; Palmiter, 2008; Volkow et al., 2006). However, this study presents evidence that the motivational process of incentive salience may be directly influenced by dopamine receptor activation in the neostriatum. This result opens up an exciting area of research, as the motivational role of dopamine in the neostriatum is largely unstudied. It would be beneficial to replicate this study with more specificity; first, at a larger scale, and then with split conditions for D1/D5 receptor activation and D2 receptor activation. Another possible separation of function may occur along the rostral/caudal gradient of the neostriatum, so further division of the neostriatum may prove useful in determining specific areas of motivational functioning.

While this study helps identify the neostriatum as a brain region that should be more closely studied in a motivational sense, it only begins to identify the motivational functions that could be seen there. Debates about the role of dopamine are still occurring, and further implicating the neostriatum in motivational responding contributes to the debate. The nigrostriatal pathway is now considered by several studies to be a possible contributor to the same incentive processes that the mesolimbic dopamine system has been attributed (Berridge & Robinson, 1998; Faure et al., 2005; Wise, 2009). Understanding the functions of dopamine, especially in the motivational sense of incentive salience, is important because of its correlation with addictive behavior. Drug cues can elicit a dopamine response in the neostriatum, which in turn may indicate possible chance of relapse or further drug-seeking behavior (Ito et al., 2002;

Vanderschuren et al., 2006; Volkow et al., 2006). Along the same line, binge eating is another possible result of dopamine activation caused by cue presentation (Stice et al., 2011). The fact that both of these pathways both show increased dopamine in the neostriatum indicates that a common mechanism may contribute to drug addiction and food addiction. Targeting cue-specific dopamine release in the neostriatum could possibly lower the likelihood of a drug- or food-seeking response to normally predictive stimuli. As the neural circuits that determine motivational responses to predictive stimuli become better understood, it will become possible to target specific aspects of the pathway and lower the conditioned response. Perhaps the framework of this experiment, along with the known anatomical relationships of the neostriatum, will help provide insight about the answers to these important clinical questions.

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Table 1

Cannula Placements for All Surgeries

	Cannula Placement (Millimeters from Bregma)		
Placement	Anterior/Posterior	Medial/Lateral	Dorsal/Ventral
Dorsomedial	+1.0	+/- 1.8 - 1.9	-3.0
Dorsolateral	+1.0	+/- 3.5 - 3.6	-3.0

Note. Placements are based on Paxinos & Watson, 1998.

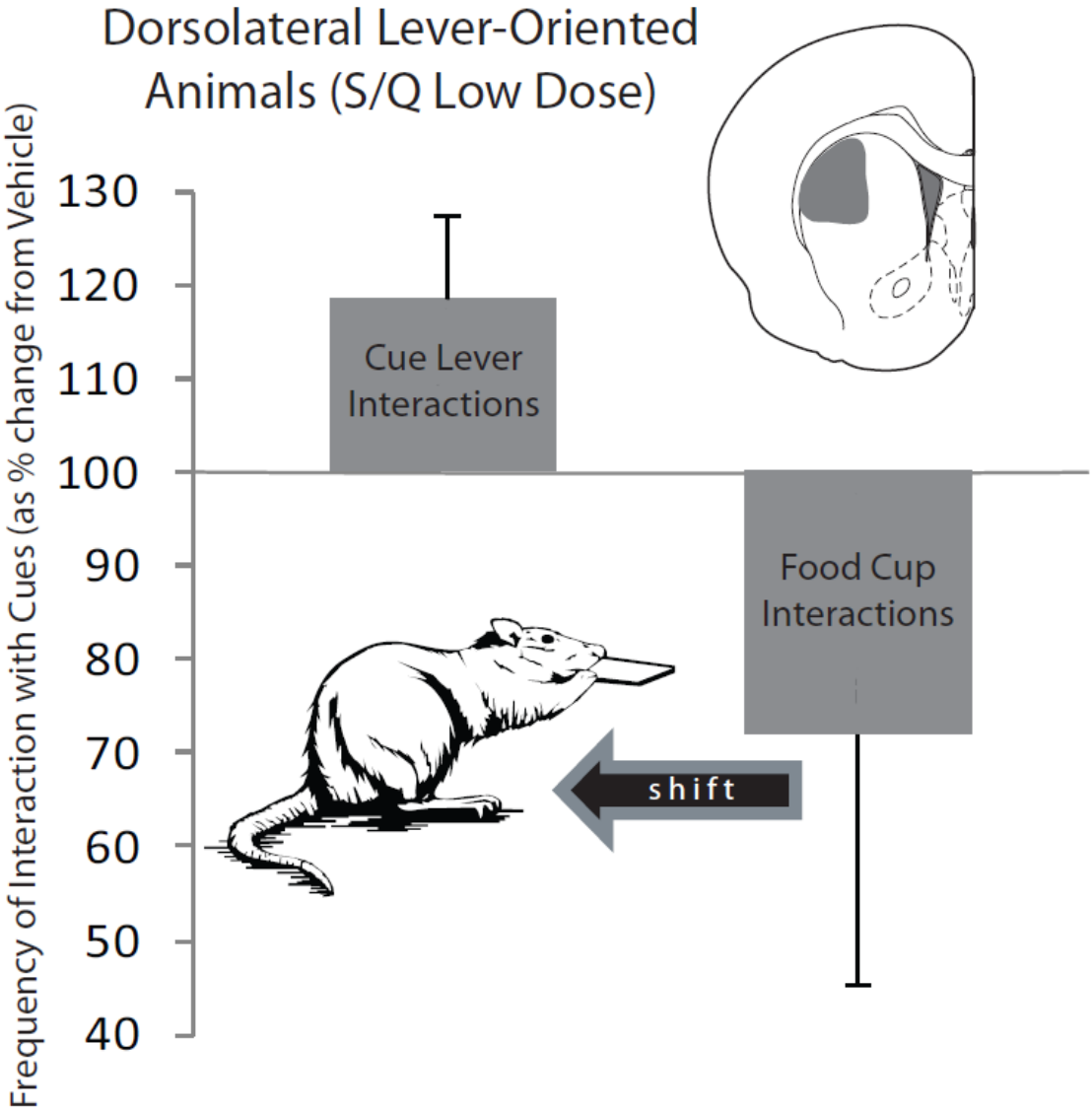

Figure 1. Illustrates the increase in cue lever interactions and decrease in food cup interactions as seen in lever-oriented animals with a dorsolateral cannula placement. Under the S/Q Low condition, interactions with the cue lever increased (118% of vehicle; $M_{\text{nibblesniffs}}$ = 5.84, *SD* = 1.37, $M_{\text{slow interactions}}$ = 1.4, *SD* = 1.21) while interactions with the food cup decreased (74% of vehicle; $M_{\text{nibblesniffs}}$ = 0.36, *SD* = 0.50, $M_{\text{slow interactions}}$ = 0.2, *SD* = 0.28). This result shows an increase in focus for the prepotent stimulus (the cue lever) and may indicate an increased 'wanting' of the predictive stimulus.

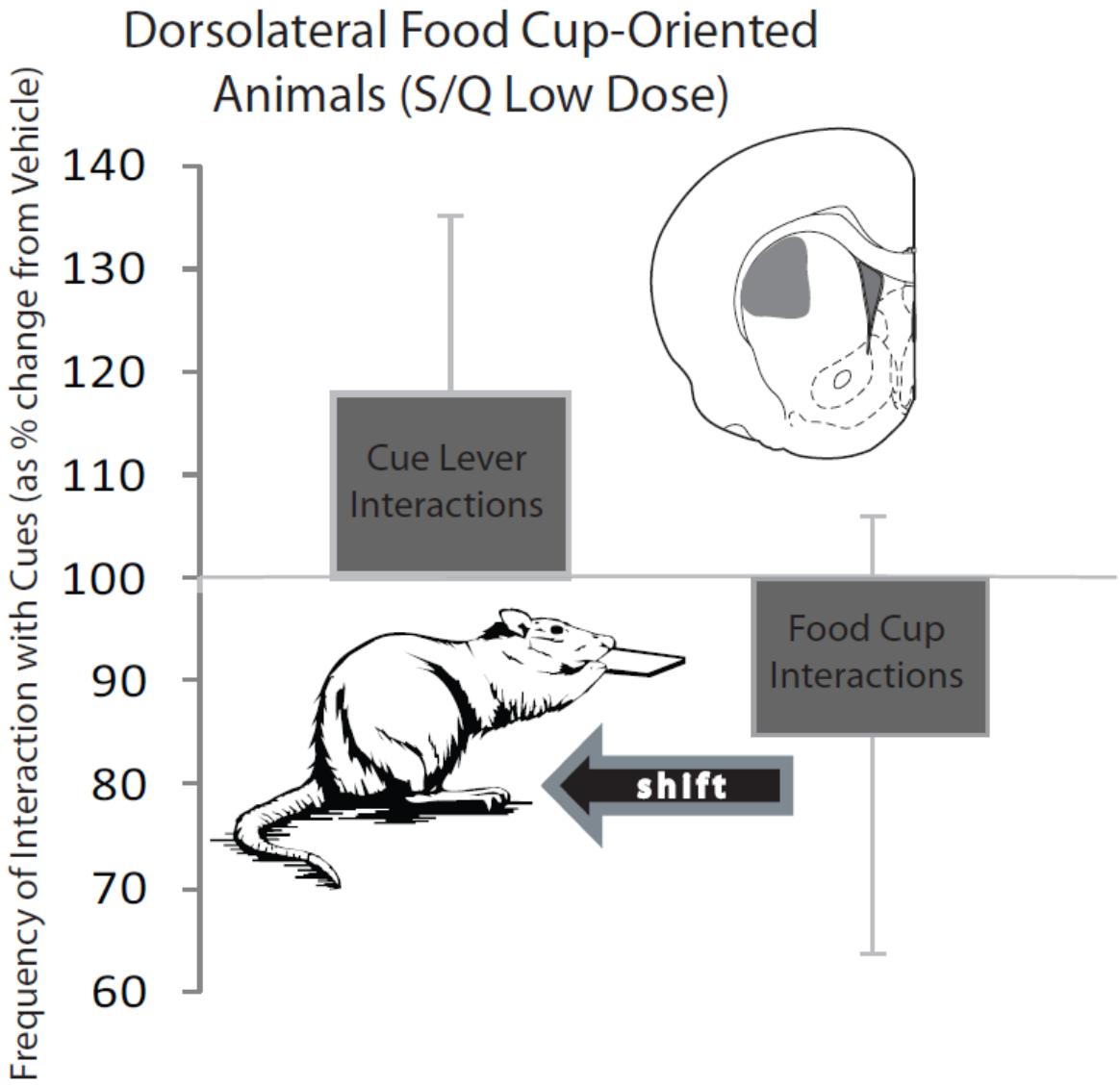

Figure 2. Illustrates the Increase in cue lever interactions and decrease in food cup interactions for food cup-oriented animals with a dorsolateral cannula placement. Cue lever interactions increased under the S/Q Low dose (130% of vehicle; $M_{\text{nibblesniffs}} = 0.05$, $SD = 0.1$) while food cup interactions decreased (82% of vehicle; $M_{\text{nibblesniffs}} = 1.25$, $SD = 0.53$, $M_{\text{slow interactions}} = 0.45$, $SD = 0.5$, Figure 2). This illustrates a shift in focus from the food cup to the cue lever, possibly indicating that the predictive stimulus becomes more 'wanted' with dopamine transmission.

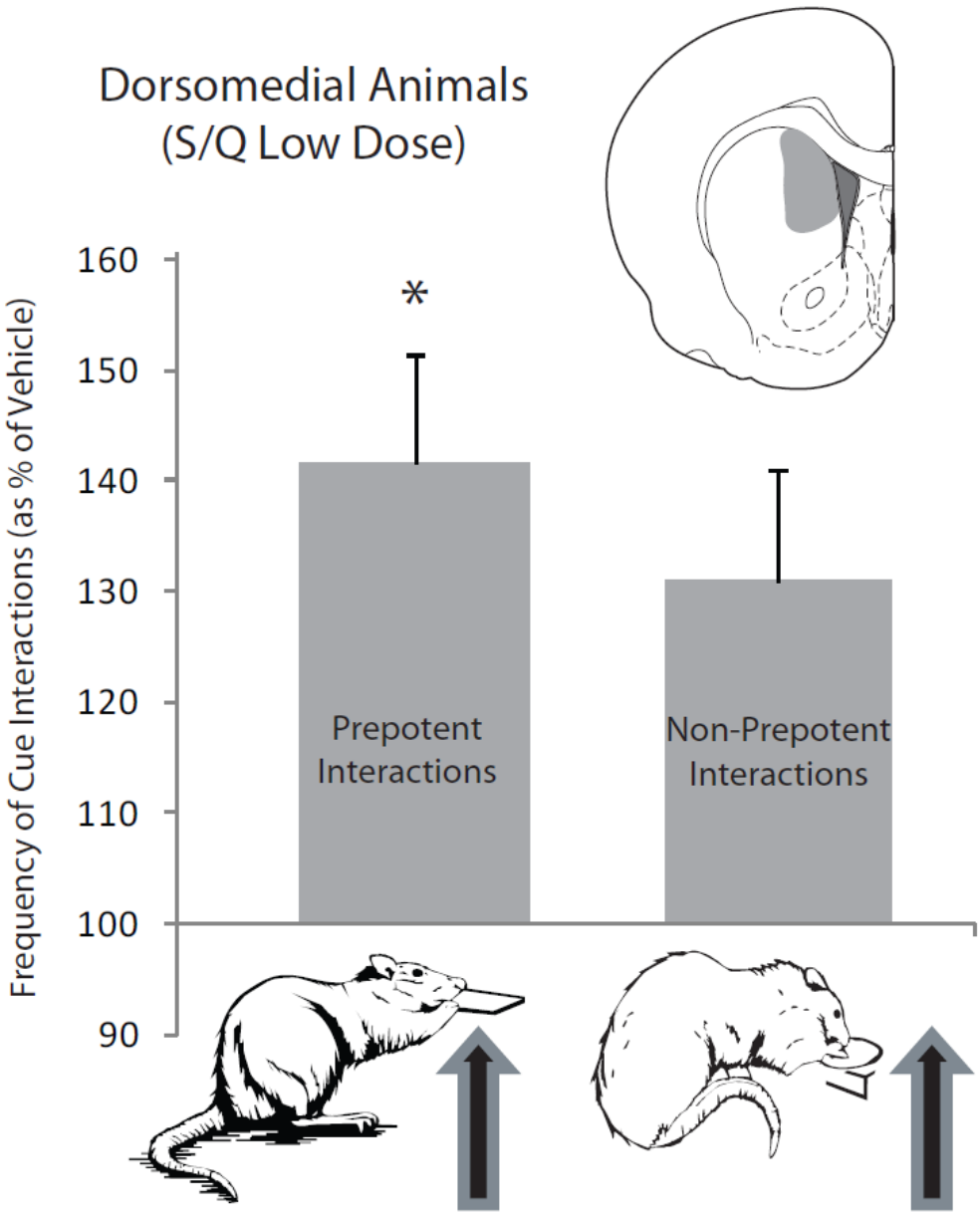

Figure 3. Illustrates the increases seen for interactions with both the prepotent and non-prepotent cues for the S/Q Low dose in animals with a dorsomedial cannula placement. Interactions with the prepotent cue (142% of vehicle, $F(2,9) = 2.751$, $p = .002$) show that an increased incentive value may have been evoked upon cue presentation. However, this increase is not specific to the prepotent stimulus, as interactions with the non-prepotent stimulus also show an increase (131% of vehicle; $M_{\text{non-prepotent interaction}} = 1.48$, $SD = 1.01$). Dopamine in the dorsomedial neostriatum may increase general motivational state rather than focus on the prepotent stimulus or shift to the predictive stimulus. * denotes $p < .05$.

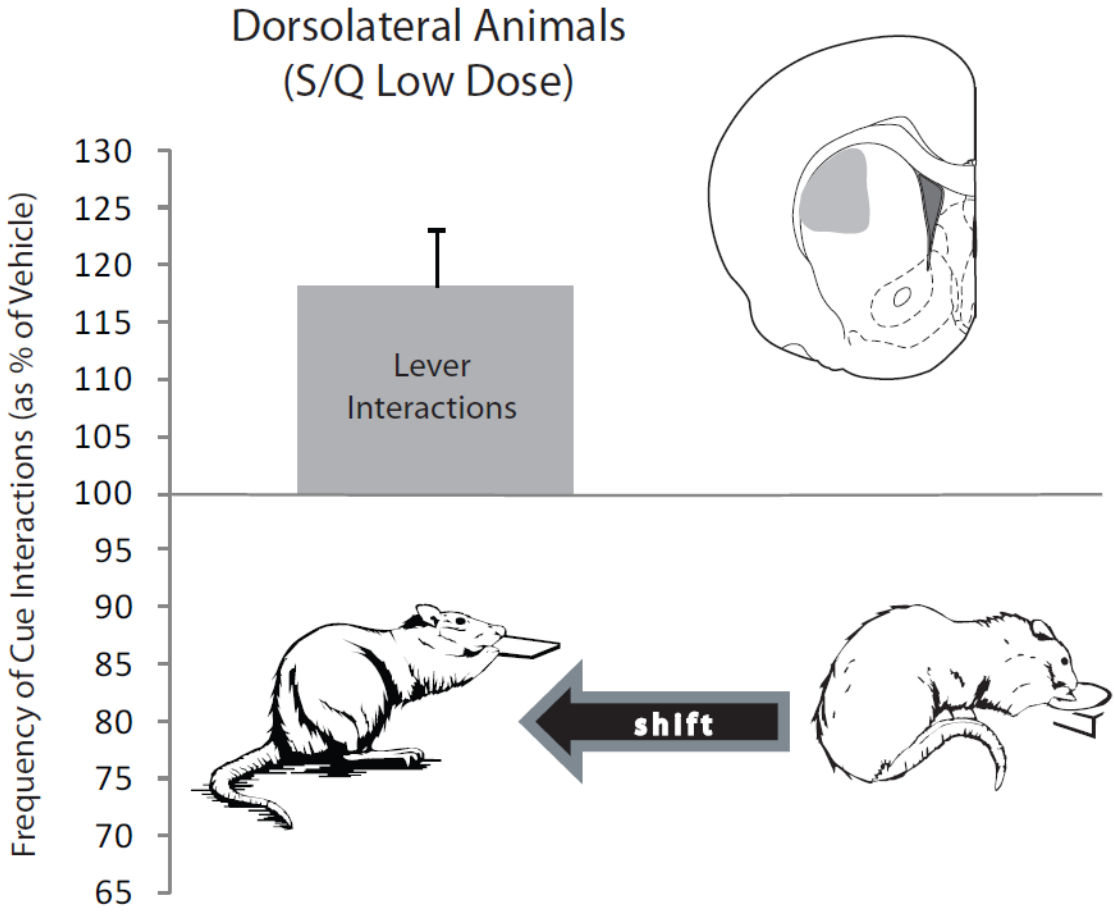

Figure 4. Illustrates the overall shift in focus from food cup to cue lever for all animals with a dorsolateral cannula placement under the S/Q Low condition. Focus was transferred from the prepotent stimulus to the non-prepotent stimulus for food cup oriented animals, whereas lever-oriented animals showed an increased number of interactions with their prepotent stimulus only (118% of vehicle; $M_{\text{lever interactions}} = 4.33$, $SD = 3.57$). This shift in focus shows an increase in 'wanting' of the predictive stimulus, the cue lever.

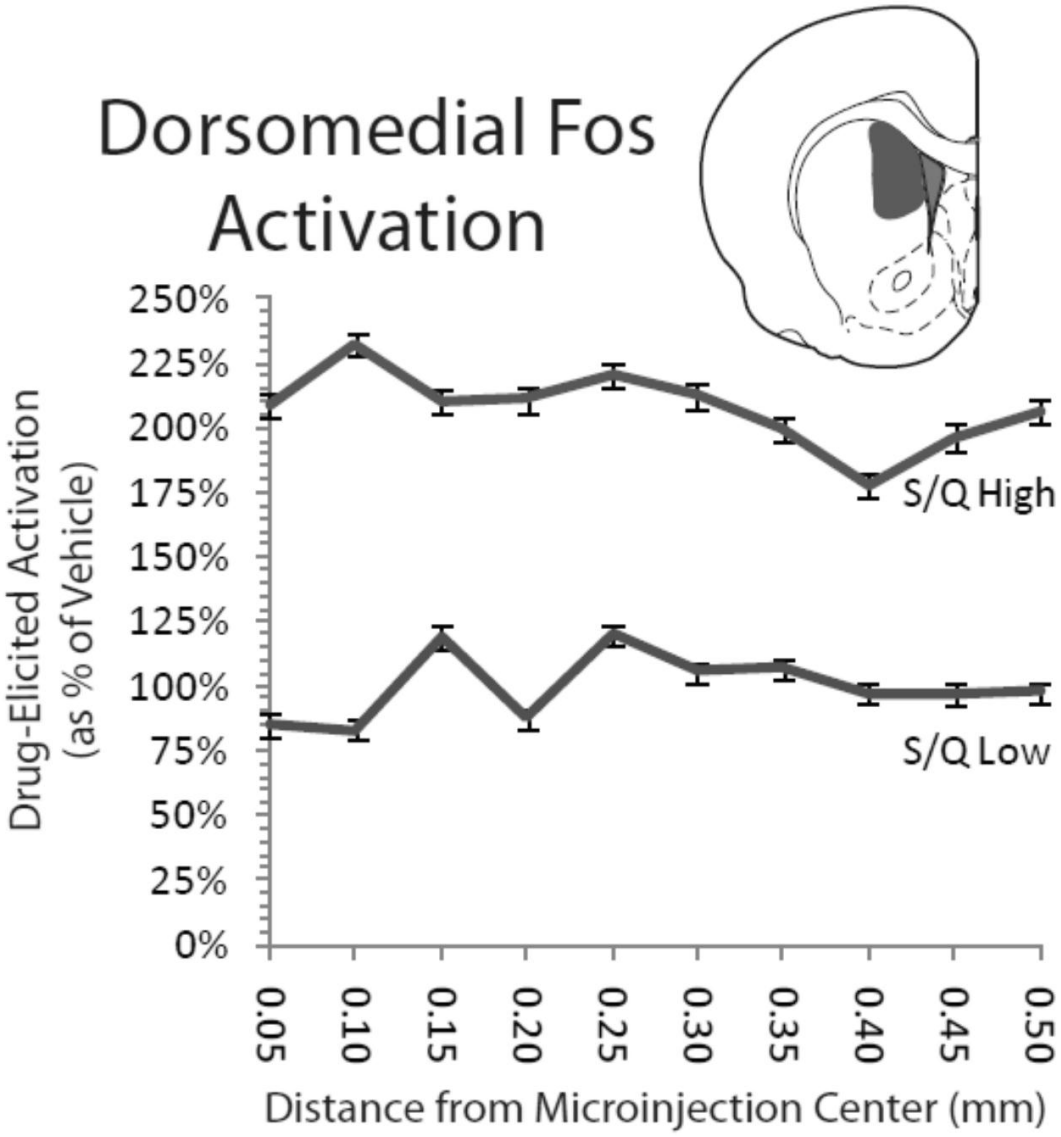

Figure 5. Illustrates the trend of fos activation caused by the S/Q High and S/Q Low doses in animals with a dorsomedial cannula placement. At all points from the microinjection center, fos activation levels remained close to vehicle levels for the S/Q Low dose. That contrasts with the S/Q High dose, which caused all points from the microinjection center to show close to 200% levels of vehicle activation.

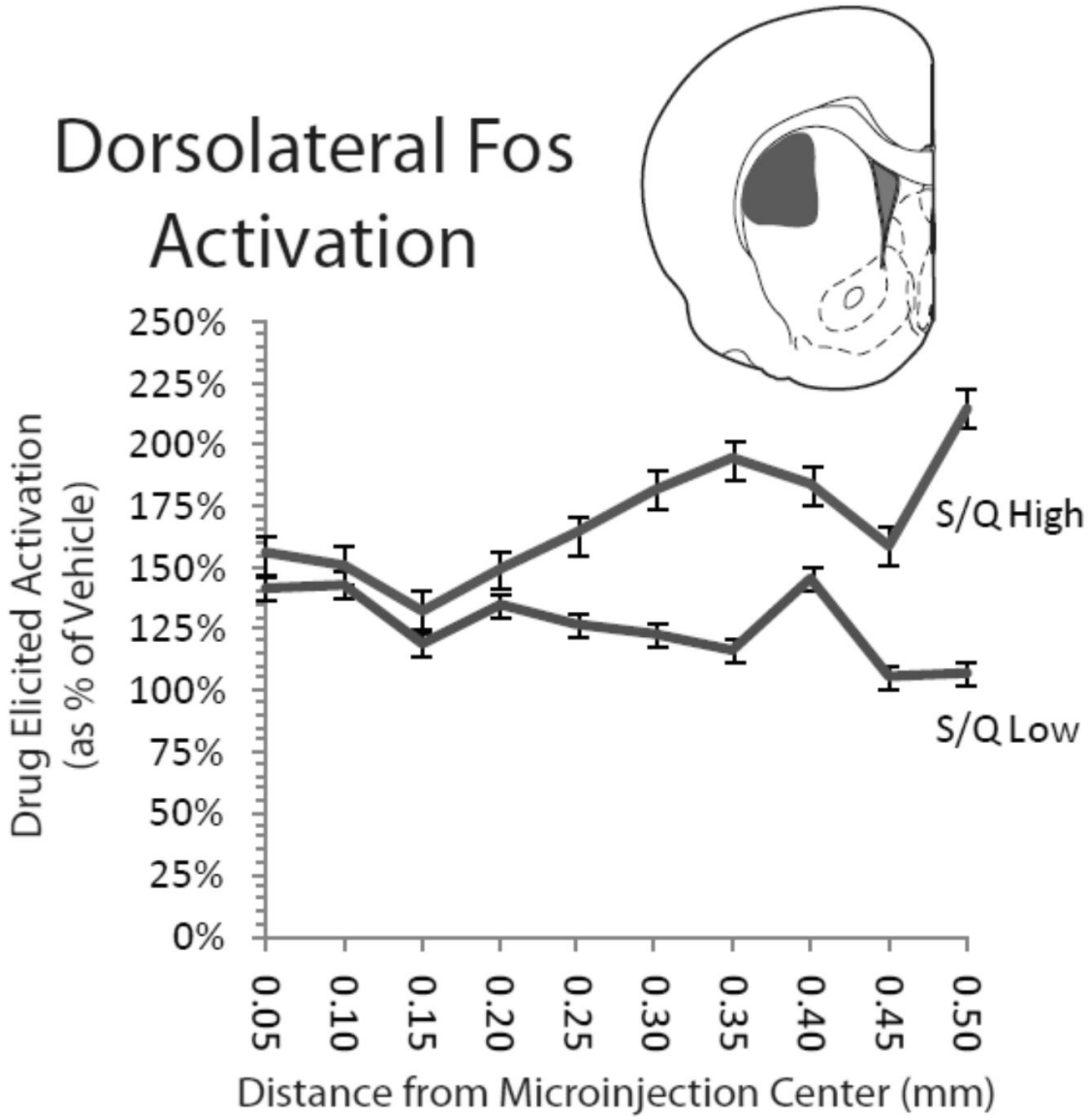

Figure 6. Illustrates the trend of fos activation caused by the S/Q High and S/Q Low dose for animals with a dorsolateral cannula placement. Up to about .20mm from the microinjection center, both the S/Q High and S/Q Low doses showed similar fos activation (125-150% of vehicle levels). However, at points further from the microinjection center, the S/Q High dose reaches levels of between 175 and 225% of vehicle levels, while the S/Q Low dose remains near 125% and as low as normal vehicle levels of activation.

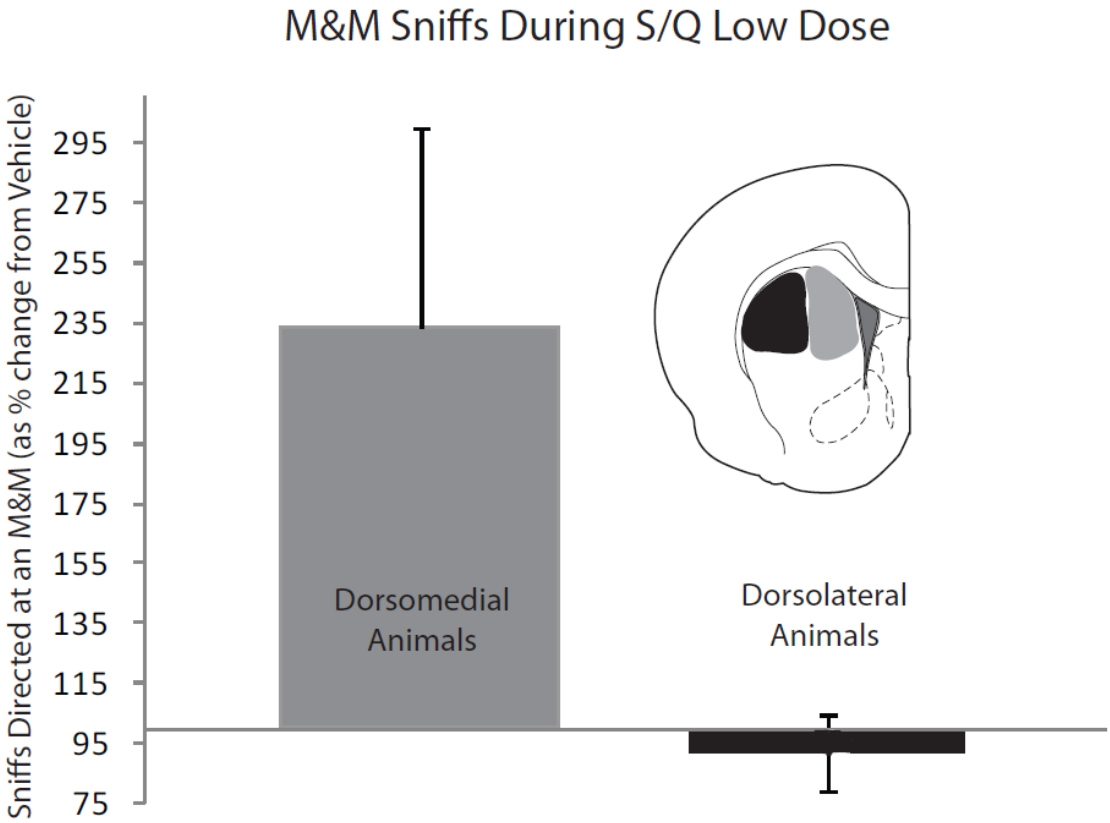

Figure 7. Illustrates the increased number of focused sniffs on the M&Ms during the food intake experiment. For the S/Q Low dose in animals with a dorsomedial cannula placement, M&M sniffs increased greatly (233% of vehicle; $M_{\text{M\&M sniff}} = 22.5$, $SD = 10.61$). However, no change from vehicle levels of activation were observed for animals with a dorsolateral cannula placement under the S/Q Low dose (92% of vehicle; $M_{\text{M\&M sniff}} = 15.67$, $SD = 4.51$). This is another example of anticipatory behavior being shown toward a cue. However, the lack of change from vehicle for animals with a dorsolateral placement may indicate that the method of cue presentation determines the motivational response in that brain region.

Appendix A

Mixture of Drugs

To get a drug that activated both D1/D5 and D2/D3 dopamine receptors, a mixture of quinpirole and SKF-82958 was created. The following procedure details the mixture of the drugs into a high and low dose.

1. 5mg SKF-82958 dissolved in 5mL of 1:4 DMSO:saline solution, resulting in a 1:1 mixture of drug to vehicle.
2. 10mg quinpirole dissolved in 5mL of 1:4 DMSO:saline solution, resulting in a 2:1 mixture of drug to vehicle.
3. In a single vial, .6mL of the SKF-82958 solution was mixed with .3mL of the quinpirole solution, along with .1mL vehicle (1:4 DMSO:saline) to bring the solution to 1mL. This solution contains .3mg SKF-82958 and .3mg quinpirole, giving a total concentration of .6mg of drug per 1mL solution.
4. This same concentration was diluted to half strength by adding another 1mL vehicle, which halved the concentration to .15mg SKF-82958 and .15mg quinpirole, giving a total concentration of .3mg of drug per 1mL solution.

The product of step 3 produced the S/Q High dose, and the product of step 4 produced the S/Q Low dose.

Appendix B

Nissl Stain Procedure

The Nissl stain used to mark the sliced tissue was performed on all extracted brains of the 21 non-perfused animals. Sliced tissue was first mounted on slides and allowed at least two days to dry. The completed procedure resulted in a blue stain that allowed the brains to be easily viewed under simple magnification, which was used to map out the sites of microinjection in each animal. The procedure is as follows:

1. 5 minute incubation in Cresyl Violet.
2. Dip twice in distilled water to remove excess cresyl violet.
3. 30 second incubation in 50% ethanol solution.
4. 30 second incubation in 75% ethanol solution.
5. 30 second incubation in 90% ethanol solution.
6. 1 minute incubation in 100% ethanol solution.
7. 1-2 minute incubation in Xylenes.

After removal from the xylenes, cover glass was applied and the tissue was given several days to dry.

Appendix C

Fos Immunohistochemistry Procedure

After the testing procedure, perfusions, and tissue sectioning, the following procedure was used for fos immunofluorescence. For each brain, the following chemicals and antibodies were used:

400µL Normal Donkey Serum (Jackson ImmunoResearch, 017-000-121)
20µL 10% Sodium Azide (Sigma-Aldrich, 438456)
4µL 1:10 dilution (w/NAPB rinse) c-Fos goat polyclonal IgG (Santa Crus Biotechnology, SC-52-G)
8µL Alexa Fluor 488 donkey-anti-goat (Invitrogen, A11055)
4 drops Image-iT FX signal enhancer (Invitrogen, I36933)

In addition, NaPB and Triton rinses were also used. The first day of the procedure consisted of three 10 minute rinses of the tissue, the first two being NaPB and the third being Triton rinse. The tissue was then placed into scintillation vials with 5% primary antibody pre-block for 30 minutes. Tissue was incubated overnight at 4^{o}C in new scintillation vials containing the primary antibody solution. All of the above steps were accompanied by a shaker at 30 RPM.

The second day began with three 10 minute Triton rinses. A 5% secondary antibody pre-block was prepared and the tissue was placed in it for 30 minutes. The Tissue was then added to the secondary antibody solution (Alexa Fluor 488 donkey-anti-goat) along with two drops of signal enhancer, and the tissue was then incubated for 2 hours. Three more 10 minute Triton rinses followed immediately, and the tissue was mounted after the final rinse. All of the above steps were also accompanied by a shaker at 30 RPM.