GABAergic Elicitation of Fear and Feeding in Nucleus Accumbens Shell Does Not Depend on Local Dopamine

by

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Abstract

Striking similarities exist in the generation of appetitive and defensive motivated behaviors by both glutamate disruption and GABAergic inhibition in nucleus accumbens shell. Rostral accumbens shell inhibition produces intense eating, whereas more caudal inhibitions generate increasingly fearful behaviors. Yet, these two forms of amino acid inhibition modulate very different sources of input: glutamate blockade largely affects corticolimbic glutamate inputs, whereas GABAergic inhibition mimics intrinsic, or subcortical, GABA inputs. GABA signals also exhibit an additional ability to mediate hedonic pleasure and disgust, a process known to be dopamine-independent. Considering that glutamate-induced motivated behaviors have been shown to be dependent on local endogenous dopamine, this experiment examined whether appetitive and defensive behaviors generated by the $GABA_A$ agonist muscimol in accumbens shell exhibit similar dopamine dependence. Presence of a local dopamine blockade did not significantly affect GABAergic elicitation of feeding and fear, suggesting that GABA-generated motivation does not depend on local endogenous dopamine.

Keywords: nucleus accumbens shell, GABA, mesolimbic dopamine, motivation

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The neurocircuitry of reward and the processes underlying incentive motivation represent significant areas of study in recent years, with growing evidence for their implication in the generation of both positive and negative valence motivation, manifested via appetitive and fearful behaviors (Faure, Reynolds, Richard, & Berridge, 2008; Faure, Richard, & Berridge, 2010; Reynolds & Berridge, 2001, 2002, 2003; Richard & Berridge, 2011). It is important to examine such mesolimbic reward and decision-influencing motivational systems because disruptions within this circuitry are believed to contribute to mood disorders and psychiatric diseases, such as anxiety and schizophrenia, as well as to substance abuse and addiction (Diekhof, Falkai, & Gruber, 2008). By better understanding the mechanisms of such mesolimbic circuitry, critical therapeutic targets can be identified, and effective treatments for such ailments can be designed.

The nucleus accumbens has long been considered an important mesolimbic structure implicated in the mediation of reward and reinforcement. Upon its original discovery the nucleus accumbens was believed to be a uniform structure; however, decades of research have contributed consistent support for its characterization as having two subregions that are functionally and anatomically distinct: the nucleus accumbens core and the nucleus accumbens shell (Stratford & Kelley, 1997). There has been great emphasis on signaling in the nucleus accumbens shell in particular as it relates to the elicitation and modulation of motivational processes, with the prominent neurotransmitters examined including GABA, glutamate, and dopamine.

Studies of GABA-mediated behaviors have established the strong potentiation of feeding behaviors and food intake resulting from administration of the $GABA_A$ agonist muscimol into nucleus accumbens shell (Faure et al., 2010; Reynolds & Berridge, 2001, 2002; Wirtshafter & Stratford, 2010). Specifically, this upregulation of eating results from the GABA-induced inhibition of chronically activated spiny projection neurons, which should disinhibit and excite targets in lateral hypothalamus and ventral pallidum (Stratford & Kelley, 1997). Evidence continues to accumulate supporting a motivation-enhancing role for this food-reinforced behavior rather than the simple activation of a motor pattern generator as had been speculated in prior studies (Hanlon, Baldo, Sadeghian, & Kelley, 2004). One such experiment demonstrated that muscimol injections in accumbens shell increased the "breaking point," a measure of reward incentive value, in food-reinforced progressive ratio schedules (Wirtshafter & Stratford, 2010). Wirtshafter and Stratford (2010) found large increases in breaking point following the administration of muscimol, implying an enhancement of some motivational property of the food reinforcement rather than motor activation.

While studies of motivational circuitry in the nucleus accumbens shell are traditionally known for directing focus toward appetitive behaviors and food intake, recent investigations have also taken into account the differential generation of positive and negative motivational behaviors. Enhancement of GABA inhibition has dynamic effects on motivational processes that vary according to precise anatomical localization, indicating the presence of a rostrocaudal gradient in medial shell of the nucleus accumbens (Faure et al., 2010; Reynolds & Berridge, 2001, 2002). It has consistently been demonstrated that infusion of the $GABA_A$ agonist muscimol into accumbens shell of rats markedly intensifies appetitive behaviors (Reynolds & Berridge, 2001, 2002). At rostral sites in accumbens shell, GABA enhancement certainly

generates patterns of increased food intake, robust eating, and elevated appetitive behavior expression in rats when compared with vehicle. However, such GABAergic inhibitory inputs also display an ability to elicit aversive behaviors, depending on specific location of infusion (Reynolds & Berridge, 2001, 2002). At caudal sites of administration, muscimol has been shown to produce intensely fearful and defensive behaviors in rats, most strongly exhibited by incidences of distress vocalizations, escape attempts, bite attempts, and defensive treading, an instinctive anti-predator behavior used by rodents to kick sand or dirt at threats or to build protective mounds in front of them (Coss & Owings, 1978; Faure et al., 2010; Reynolds & Berridge, 2001, 2002; Treit, Pinel, & Fibiger, 1981). Manipulations at intermediate sites evoke mixtures of appetitive and defensive behaviors in rats, depending on relative position along the gradient.

Glutamate disruptions in accumbens shell (induced via infusion of the AMPA glutamate antagonist DNQX) generate an equivalent valence of motivated behaviors according to the same rostrocaudal gradient (Reynolds & Berridge, 2003). The valence of positive/incentive and negative/aversive behaviors produced by either of these manipulations according to location along the gradient indicates comparable abilities of glutamate and GABA circuits in controlling the elicitation of motivated behaviors and the switch between desire and dread. It is important to note, though, that while DNQX and muscimol both generate appetitive and defensive motivated behaviors organized along a similar rostrocaudal gradient in accumbens shell, they were not found to have the same effects on hedonic pleasure and disgust (Faure et al., 2010). Only the enhancement of subcortical GABA inhibition was demonstrated to additionally regulate hedonic "liking" and "disliking" reactions in rats (Faure et al., 2010; Reynolds & Berridge, 2002), indicating an influential process specific to GABAergic signaling. These findings suggest the

existence of differential processes by which glutamatergic and GABAergic circuits affect and elicit behaviors.

Dopamine, a neurotransmitter known for its crucial role in reward processes, has also been investigated regarding its mediation of incentive motivation. The role of dopamine has been broadly examined in relation to both food and drug reinforced behaviors (Bari & Pierce, 2005; Wirtshafter & Stratford, 2010). For instance, administration of a D_1 and D_2 receptor antagonist mixture into accumbens shell was found to decrease the reinforcing effectiveness of cocaine in rats as measured by self-administration under a progressive ratio schedule, yet it did not appear to have an effect on food reinforcement (Bari & Pierce, 2005). Such findings illuminate the complexity associated with influence of dopamine signals, especially concerning dopamine's mediation of motivated behaviors.

Some dopamine-induced behaviors have also been briefly compared to reinforcing processes of GABA-generated motivation. One investigation revealed that administration of D-amphetamine led to significant increases in both food and water reinforced behaviors but not in food intake, whereas muscimol potentiation would increase food intake and only occurred for food reinforcement but not water (Covelo, Wirtshafter, & Stratford, 2012). Furthermore, GABA inputs have the ability to modulate pleasure and disgust in addition to motivational processes (Reynolds & Berridge, 2002), while dopamine manipulations have no effect on such hedonic mechanisms (Berridge, 2007). These observances support the existence of different functional mechanisms for GABA-induced motivation and dopamine-mediated behaviors, although it is unclear whether those processes are related or distinct. Possible explanations have included overlapping mechanisms, distinct activation of separate populations of cells sensitive to one

signal or the other, and different patterns of activation in the same population of neurons (Covelo et al., 2012); however, only further experimentation will lend insight into these theories.

The physiological influence of dopamine on glutamatergic signaling has been widely examined thus far. Studies of synaptic plasticity at glutamatergic synapses have revealed evidence that the postsynaptic activation of dopamine receptors is involved in the expression of long-term potentiation and the induction of long-term depression (Calabresi, 1997; Lovinger, 2010). Dopamine has also been investigated regarding its influence on the elicitation of fear and feeding behaviors, specifically those generated by glutamate disruptions in medial shell (Faure et al., 2008; Richard & Berridge, 2011). Local dopamine receptors were assessed for their roles in mediating glutamate-induced motivated behaviors, and dopamine antagonism was found to significantly affect generation of desire and dread. Glutamate disruptions via DNQX alone in accumbens shell elevate feeding and fearful behaviors in rats, dependent on position along the rostrocaudal gradient (Reynolds & Berridge, 2003). However, the introduction of a local combined blockade of dopamine receptors, prepared by incorporating a combined D_1 and D_2 antagonist mixture in the same microinjection with DNQX, was demonstrated to prevent DNQX from amplifying these motivated behaviors, specifically appetitive behaviors at rostral sites and defensive behaviors at caudal sites (Faure et al., 2008). These microinjections containing the mixture of DNQX plus D_1 and D_2 antagonists elicited no change in expression of feeding or defensive behaviors when compared with vehicle performance, indicating an ability of endogenous dopamine to modulate glutamatergic circuits in the nucleus accumbens involved in the generation of motivated behaviors.

Potential physiological mechanisms for the role of dopamine in the mediation of signaling at GABAergic synapses, however, have not yet "been explored in any detail"

(Lovinger, 2010, p. 956). Studies of physiological interactions between dopamine and GABAergic signaling have produced disjointed evidence, and investigations have mainly focused on the presynaptic effects of dopamine on GABA release from GABAergic interneurons. For example, one examination of the actions of endogenous dopamine in the striatum revealed an excitatory effect on fast-spiking GABAergic interneurons via direct depolarization through D_1 receptors and also by the reduction of synaptic inhibition through D_2 receptors (Bracci, Centonze, Bernardi, & Calabresi, 2002). A different study of the effects of D_1 dopamine receptor activation on fast-spiking GABAergic interneurons in the neocortex suggests a modulatory role by means of reducing inhibitory postsynaptic currents (IPSCs) between fast-spiking cells (Towers & Hestrin, 2008). Similarly, the stimulation of D_2 dopamine receptors with both amphetamine and cocaine led to the attenuation of GABA-mediated IPSCs (Centonze et al., 2002). Although these findings indicate some presynaptic mechanisms by which dopamine may be able to affect GABergic interneurons, whether dopamine has any influence on GABAergic inhibition of medium spiny neurons and the related generation of motivated behaviors remains largely unexplored.

In contrast, one investigation proposed that certain GABAergic processes involving medium spiny neurons do exhibit dopamine independence. Dopamine transporter knockdown mice were generated to examine the physiological effects of elevated levels of extracellular dopamine on dorsal striatum medium-sized spiny neurons (Wu, Cepeda, Zhuang, & Levine, 2007). Wu and colleagues (2007) observed that although glutamate-mediated synaptic currents were altered in the knockdown mice, GABA-receptor mediated synaptic currents remained unaffected. Without more evidence, few conclusions and inferences about dopamine's

physiological modulation of GABAergic signaling can be extrapolated from the given data. This represents an important area of research that should be examined in greater detail.

Although a few potential physiological interactions have been investigated, hardly any study has been done on the influence of dopamine manipulation on the generation of GABA-induced motivational processes. The purpose of this experiment was to establish whether endogenous dopamine plays an active role in mediating GABA-elicited feeding and fearful behaviors by inducing a local dopamine blockade via a combination of D_1 and D_2 receptor antagonists. Considering the similarities between the processes by which glutamatergic and GABAergic circuits have been demonstrated to mediate motivation (Faure et al., 2010) as well as the interactions found between dopamine and glutamate in the generation of appetitive and fearful behaviors (Faure et al., 2008; Richard & Berridge, 2011), it is compelling to examine the influence of endogenous dopamine on GABA-induced enhancement of intense motivated behaviors. Although glutamate-elicited motivation shows dependence on local dopamine, glutamate and GABA circuits display stark contrasts in their effects on hedonic processes (Faure et al., 2010) as well as in their distinct physiological sources of input (see Figure 1) and different receptor-mediated responses to elevated extracellular dopamine (Wu et al., 2007). Such findings suggest the possibility that GABAergic generation of motivation may exhibit even more distinctions from comparable glutamatergic processes.

Method

Subjects

Male Sprague Dawley rats (*N*=18) born at the University of Michigan were housed in pairs in a controlled temperature and pressure colony room maintained at ~21 °C. The room operated on a 12 hour light/dark reverse cycle with lights turning on at 9:00pm. Food pellets

(Purina Rat Chow) and water (tap water) were available *ad libitum*, and all animals weighed between 280 and 450 grams at the time of surgery. The University of Michigan's University Committee on the Use and Care of Animals (UCUCA) provided approval for the use of these rats and the experimental procedures conducted.

Surgery

Each animal was handled for a total of 15 minutes spread over the two days prior to surgery in order to habituate them to handling procedures. Rats were anesthetized with a mixture of ketamine HCl (80 mg/kg) and xylazine (5 mg/kg) and were pretreated with atropine (0.04 mg/kg) by way of injection into the intraperitoneal cavity. Following this, rats were placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the incisor bar set at 5.0 mm above interaural zero to avoid the lateral ventricles. All animals underwent bilateral implantation of 14.0 mm stainless steel microinjection guide cannulae (23 gauge) aimed at 2.0 mm above the intended target area due to the fact that the tips of the microinjector cannulae extend an additional 2.0 mm beyond the ventral tips of the guide cannulae.

Bregma was utilized as the reference point for all stereotaxic coordinates. Placements were targeted at one of two anteroposterior (AP) values: +3.1 mm or +2.4 mm anterior to bregma, signifying either a rostral or caudal placement in nucleus accumbens shell. The mediolateral (ML) and dorsoventral (DV) coordinates for all animals, however, stayed constant. Mediolateral coordinates were ±1.0 mm lateral to midline, and dorsoventral placements extended -5.7 mm below the skull surface. Coordinates were chosen in accordance with previous studies on nucleus accumbens shell (Richard & Berridge, 2011). Placements for two rats were later found to target nucleus accumbens core and were considered misses during statistical analysis. Once the guide cannulae were surgically implanted, they were anchored to the skull with four

bone screws and acrylic cement. Each rat had a stainless steel stylet placed into the guide cannulae to help prevent occlusions from occurring. Immediately following surgery, each rat received subcutaneous injections of cefazolin (75 mg/kg) for antibiotic protection, carprofen (5 mg/kg) for analgesic purposes, and saline solution for hydration. Carprofen was administered again the following day approximately 24 hours post-surgery. At least seven days were allowed for recovery before behavioral testing began, and rats were handled briefly each day during the recovery period.

Drugs and Microinjections

A local blockade of endogenous dopamine was induced via a mixture of D_1 and D_2 receptor antagonists (3 μg SCH 23390 and 5 μg raclopride, respectively, per side) dissolved in 0.5 μl artificial cerebrospinal fluid (aCSF). This combination of D_1/D_2 antagonists was chosen based on results of prior studies examining the mediation of DNQX-induced motivated behaviors by dopamine (Faure et al., 2008; Richard & Berridge, 2011). Muscimol (Sigma, St. Louis, MO) was chosen as the $GABA_A$ receptor activator and delivered at a concentration of 75 ng dissolved in 0.5 μl aCSF. This dose (75 ng per side) was selected based on previous findings demonstrating it to produce maximum appetitive behavior when administered to rostral accumbens shell and significant defensive treading behavior for caudal accumbens shell (Reynolds & Berridge, 2001, 2002). This dose was also shown to produce motivated behaviors at a level of expression comparable to that of DNQX in previous studies examining mediation of motivation signals (Faure et al., 2010). Vehicle conditions were induced with 0.5μl aCSF microinjections. The final microinjection condition contained a mixture of muscimol (75 ng) and the local D_1 (3 μg SCH23390) and D_2 (5 μg raclopride) dopamine antagonists and was administered to each side (in a total injection volume of 0.5 μl per side).

Stainless steel microinjection cannulae (28 gauge) were attached to a syringe pump via PE-20 tubing and were inserted into the guide cannulae. Rats were bilaterally infused with a volume of 0.5 µl at a rate of 0.3 µl/min. Following the infusion, injectors were kept in place for an additional 60 seconds to allow the drug to completely diffuse away from the cannulae before their removal and replacement with the stylets. The rat was then immediately placed into an individual testing chamber.

Behavioral Testing

Prior to behavioral testing, there were four days of habituation of the rats to the testing chambers, conditions, and environment (60 minutes each day). On the fourth day of habituation, typically the day before the first testing day, a 0.15 M sterile saline vehicle microinjection was administered to the rat preceding placement in the cage to simulate testing conditions as accurately as possible and ensure that any behavioral effects observed during testing would not be attributable to a novel environment or procedure.

Before administering microinjections, the testing room was set up with transparent testing cages. To provide for appetitive behavior expression, each cage contained a pre-weighed amount (~20 g) of food pellets and a bottle of water available *ad libitum*. The bottom of the cage was covered with a layer of granular bedding approximately 3 cm deep to make defensive treading behaviors more pronounced and easier to observe. All testing was conducted in a previously determined standard and relatively neutral lab environment, a testing room free from excessive light or auditory intensity as well as undisturbed by people during the testing period.

Each rat was tested with all four drug conditions in a counterbalanced order, with testing sessions spaced 48 hours apart to ensure that any preceding drug condition had completely been eliminated before testing of a new condition. Immediately following the microinjections on each

test day, each rat was placed into its individual transparent testing cage for 60 minutes and videotaped for subsequent offline behavioral video analysis. At the completion of each testing session, rats were removed from the testing chambers by the experimenter using a slow-approach technique where the experimenter would slowly reach toward the rat with a gloved hand and note any distressed or aggressive reactions in response to gentle touch and pick-up.

Behavioral Analysis

Upon removal of the animal, distressed behaviors including audible vocalizations, escape efforts, and bite attempts were recorded. Food in the testing cage was then re-weighed, and the amount consumed during testing was recorded. In later offline videotape analysis, each 60 minute testing session was scored by an experimenter blind to testing condition, and the total duration (in seconds) as well as the number of bouts of the following behaviors were recorded: eating behaviors (characterized by the rat's mouth coming in contact with food and subsequent chewing and swallowing motions), drinking behaviors (continuous licking of water spout), and defensive treading behaviors (rapidly thrusting forepaws in an alternating manner that pushes or sprays bedding away from the animal).

Other appetitive behaviors noted include the number of food sniffs (continuous sniffing action near food) and the number of food carries (picking up food in mouth and moving it) that occurred during the testing period. Additionally, the numbers of grooming bouts (alternating forepaw strokes over face followed by licking of the body), cage crosses (crossing the midline of the cage), rearing motions (forepaws raised an inch above ground for at least 0.5 seconds), burrow attempts (burying head under bedding with a forward motion), and burrow-treads (combination of burrowing and treading) were recorded as well as the total duration of time spent sleeping (in seconds) during testing. These behaviors and criteria were selected based on

prior studies involving elicitation of appetitive and defensive behaviors in rats (Reynolds & Berridge, 2001, 2003).

Histology

After completion of behavioral testing, rats were deeply anesthetized with a sodium pentobarbital overdose and decapitated. Brains were removed and placed in a 10% paraformaldehyde solution for 1-2 days. They were subsequently transferred to a 25% sucrose in 0.1 M NaPB solution and then allowed to remain there for three days. In order to determine microinjection site location, brains were coronally sliced at a thickness of 60 μm using a freezing microtome and were then mounted on slides, allowed to air dry, and stained using cresyl violet. The slides were analyzed, and cannulae placement sites were mapped onto a rat brain atlas. Nucleus accumbens shell placements were classified as rostral sites if they were located +1.4 to +2.7 mm anterior to bregma and caudal sites if they were located +0.48 to +1.4 mm anterior to bregma.

Statistical Analysis

A two-way mixed within- and between-subjects ANOVA (drug [muscimol vs. vehicle] by placement [rostral vs. caudal]) was used to evaluate effects of muscimol on behaviors and confirm appetitive and defensive behavior generation along a rostrocaudal gradient. The effect of combined D_1/D_2 receptor antagonism on muscimol-induced behavior was analyzed using a three-way ANOVA (muscimol by dopamine antagonists by placement). If a significant effect was found, rats were split according to placement location, and both two-way ANOVAs (muscimol by dopamine antagonists) and one-way ANOVAs with pairwise comparisons, using Sidak corrections for multiple comparisons, were additionally used. For the analysis of binomial

data (distress vocalizations, escape attempts, bite attempts), rats were split into rostral and caudal groups, and McNemar's test was used.

Results

Statistical analysis was performed using data from sixteen of the eighteen rats that completed the entire testing regime. Two rats were excluded from statistical analysis upon determining that microinjection cannulae placements did not aim at the intended target brain structure, the nucleus accumbens shell, but were instead found to extend to the nucleus accumbens core. Nine rats were confirmed to have cannulae placements targeting rostral nucleus accumbens shell, and seven rats were confirmed to have placements targeting caudal nucleus accumbens shell.

Muscimol alone elevates eating or fearful behaviors in medial shell along a rostrocaudal gradient

For the purpose of this analysis, rats were usually divided into two groups based on whether location of microinjection site placement resided in rostral or caudal accumbens shell. In accordance with previous findings, the administration of the $GABA_A$ agonist muscimol to medial accumbens shell led to significant increases in the generation of motivated behaviors (Faure et al., 2010; Reynolds & Berridge, 2001, 2002). When muscimol alone was infused into rostral accumbens shell, rats exhibited robust feeding behaviors and a three-fold increase in time spent eating when compared with vehicle (cumulative time spent eating, main effect of muscimol at rostral sites, $F(1, 8) = 20.53$, $p = 0.002$; eating, interaction of muscimol by placement, $F(1, 14) = 14.51$, $p = 0.002$; see Figures 2A and 4A), as well as a three-fold increase in measured food intake, consistent with appetitive behavioral observations (food intake grams consumed, rostral shell, mean of 6.03 ± 2.32 g under muscimol vs. 2.05 ± 1.34 g under vehicle; food intake, main

effect of muscimol at rostral sites, $F(1, 8) = 17.59$, $p = 0.001$; see Figure 2B). The infusion of muscimol alone to caudal shell, conversely, elicited a 60-fold increase in defensive treading behavior when compared with vehicle (cumulative time spent treading, main effect of muscimol at caudal sites, $F(1, 6) = 13.53$, $p = 0.010$; treading, interaction of muscimol by placement, $F(1, 14) = 6.15$, $p = 0.026$; see Figures 2C and 4B). Muscimol in caudal shell led to no increase in eating behavior and was even found to repress drinking behavior to a level over twenty times less than that of vehicle (cumulative time spent drinking, main effect of muscimol at caudal sites, $F(1, 6) = 6.19$, $p = 0.047$). It was further noted that there was a smaller but significant twenty-fold elevation in defensive treading behavior in rostral rats (treading, main effect of muscimol at rostral sites, $F(1, 8) = 8.62$, $p = 0.019$), also consistent with previous findings (Reynolds & Berridge, 2001).

In addition, the number of distress vocalizations and number of escape attempts made in reaction to pick-up at the conclusion of testing significantly increased in rats receiving muscimol when compared with vehicle alone (both p values < 0.001; see Figures 3A, 3B, and 4C). The number of bite attempts also increased under muscimol condition; however, this increase was not significant ($p = 0.250$; see Figure 3C), most likely due to the small number of rats that exhibited a bite attempt (3 rats on muscimol). Furthermore, rats were given a *total* distress score based on the number of defensive reactions expressed upon pick-up and severity of distress observed. The mean total distress score significantly increased in rats under muscimol when compared with vehicle (total distress score, main effect of muscimol, $F(1, 14) = 81.02$, $p < 0.001$).

Muscimol alone enhances general activity

Muscimol strongly elevated performance of certain activities in both rostral and caudal rats, noting that none of these effects were dependent on rostrocaudal location. Locomotor

activity, measured by number of cage crosses, was tripled in animals given muscimol when compared with vehicle (crosses, mean of 121.44 ± 84.83 crosses under muscimol vs. 44.81 ± 26.30 crosses under vehicle; main effect of muscimol, $F(1, 15) = 26.46$, $p < 0.001$). This effect was not site specific (number of crosses, interaction of muscimol by placement, $F(1, 14) = 2.56$, $p = 0.132$). Other behaviors that were significantly affected in both rostral and caudal rats include an increase in the amount of times rats sniffed food in the cage (number of food sniffs, main effect of muscimol, $F(1, 15) = 12.74$, $p = 0.003$) and an elevation of burrowing behavior (number of burrows, main effect of muscimol, $F(1, 15) = 5.68$, $p = 0.032$), both of which more than doubled in rats that received muscimol when compared with vehicle alone. Furthermore, the increase in motivated behaviors and activity in muscimol rats was complemented by a significant decrease in amount of time spent sleeping to less than half the amount observed under vehicle (sleeping, main effect of muscimol, $F(1, 15) = 31.80$, $p < 0.001$). Similar to locomotor activity, none of these effects were determined to be site specific (interaction of muscimol by placement, food sniffs, $F(1, 14) = 1.07$, $p = 0.319$; burrows, $F(1, 14) = 0.72$, $p = 0.411$; sleep duration, $F(1, 14) = 2.86$, $p = 0.113$). Other behaviors, including food carries, grooming, and rearing were not significantly affected by muscimol (main effect of muscimol, food carries, $F(1, 15) = 2.51$, $p = 0.135$; grooming, $F(1, 15) = 3.49$, $p = 0.083$; rears, $F(1, 15) = 3.39$, $p = 0.087$).

Presence of a local dopamine blockade does not affect muscimol-elicited feeding or fear

Although it was found that inducing a local dopamine blockade via a mixture of D_1 and D_2 antagonists prevents expression of glutamate-elicited appetitive behaviors in rostral shell and fearful behaviors in caudal shell (Faure et al., 2008), a similar local dopamine blockade in this experiment had no significant effects on GABA-generated eating or fearful behaviors. In rostral rats receiving the mixture of muscimol plus D_1/D_2 antagonists, the amount of time spent eating

did not change significantly from that of the muscimol alone condition despite a slight decrease in mean performance; expression of eating was elevated far above vehicle levels in both muscimol and mixture conditions (rostral shell, eating duration, mean of 696.11 ± 457.15 s under muscimol vs. 463.89 ± 367.24 s under mixture vs. 233.00 ± 182.65 s under vehicle; rostral shell, eating, interaction of muscimol by D_1/D_2 antagonists, $F(1, 8) < 0.01$, $p = 0.996$; see Figures 2A and 4A). The number of food sniffs also did not change significantly in the mixture condition when compared with muscimol alone (rostral shell, food sniffs, mean of 22.11 ± 26.35 under muscimol vs. 8.00 ± 3.16 under mixture; rostral shell, food sniffs, interaction of muscimol by D_1/D_2 antagonists, $F(1, 8) = 0.37$, $p = 0.559$). Similarly, caudal rats receiving the mixture condition showed a marginal decrease in mean expression of defensive treading that also was not significant (caudal shell, treading, mean of 161.43 ± 131.97 s under muscimol vs. 133.71 ± 91.60 s under mixture; caudal shell, treading, interaction of muscimol by D_1/D_2 antagonists, $F(1, 6) = 0.86$, $p = 0.389$; see Figures 2C and 4B). Effects of an induced dopamine blockade on muscimol-generated eating, drinking, and treading were examined in relation to specific site placement, and there were no significant changes in the cumulative durations of these parameters with respect to either rostral or caudal site placement (interaction of muscimol by D_1/D_2 antagonists by placement, all F values < 1).

Distressed responses of the rats to pick-up upon completion of testing were also quantified and analyzed to determine whether a local dopamine blockade would affect muscimol-elicited fearful reactions. There were no significant changes in the amount of vocalizations, escape attempts, or bite attempts observed under the mixture of muscimol plus dopamine antagonists when compared with muscimol alone (vocalizations, $p = 0.375$; escape attempts, $p = 0.180$; bite attempts, $p = 1.000$; see Figures 3A, 3B, and 3C). On the other hand, a

minor, yet significant, suppression of *total* distress score was detected in rats receiving the mixture of muscimol plus dopamine antagonists from that of rats under muscimol alone (total distress score, interaction of muscimol by D_1/D_2 antagonists, $F(1, 14) = 8.90$, $p = 0.010$).

Muscimol-generated locomotor activity may be affected by a local dopamine blockade

While muscimol-induced feeding and fearful behaviors were generally unaffected by a locally induced dopamine blockade, the mean time spent drinking under muscimol, which was lower than vehicle expression, was further decreased in rats receiving the mixture, demonstrating an additive effect (drinking, mean of 60.13 ± 55.85 s under vehicle vs. 6.56 ± 10.19 s under muscimol vs. 2.06 ± 4.33 under mixture; drinking, interaction of muscimol by D_1/D_2 antagonists, $F(1, 14) = 15.32$, $p = 0.002$). Similarly, the number of grooming behaviors completed by rats was reduced to an equivalent level in both muscimol and mixture conditions when compared with vehicle (grooming bouts, mean of 8.69 ± 3.50 under vehicle vs. 3.38 ± 2.87 under muscimol vs. 3.94 ± 3.45 under mixture; grooming bouts, interaction of muscimol by D_1/D_2 antagonists, $F(1, 14) = 28.76$, $p < 0.001$). Regardless of these statistical interactions, it is important to note that any significant effects of the local dopamine blockade did not block or reverse any muscimol-induced changes. Rather, the further reduction of activities already suppressed by muscimol in the presence of the dopamine antagonists could be indicative of a possible floor effect, especially considering the inherently low measurements associated with these activities.

Other behavioral measures that had been elevated in the muscimol alone condition were observed to have mean expression reduced by about 50% under the mixture condition combining muscimol with the dopamine antagonists when compared with expression under muscimol alone; however, these decreases were not significant. These behaviors include the number of cage crosses and of food sniffs (crosses, mean of 121.44 ± 84.83 under muscimol vs. 51.75 ± 42.73

under drug mixture condition; food sniffs, mean of 24.31 ± 23.89 under muscimol vs. 11.38 ± 7.17 under drug mixture condition; crosses, interaction of muscimol by D_1/D_2 antagonists, $F(1, 14) = 1.95$, $p = 0.185$; food sniffs, interaction of muscimol by D_1/D_2 antagonists, $F(1, 14) = 0.76$, $p = 0.397$). Conversely, the number of burrows, which significantly increased in the muscimol alone condition in comparison with baseline levels, reached a mean expression level nearly equivalent to that of the muscimol alone condition when the animal was administered the drug mixture (burrows, mean of 5.06 ± 7.41 under muscimol vs. 5.69 ± 9.59 under drug mixture; burrows, interaction of muscimol by D_1/D_2 antagonists, $F(1, 14) = 1.31$, $p = 0.271$).

Infusion of only the D_1/D_2 dopamine antagonist mixture decreases general activity but does not incapacitate animals

In order to determine whether effects of the local dopamine blockade on muscimol-induced behaviors, if they occurred, would be the result of drug interaction rather than simple motor incapacitation or sedation, rats were tested with an exclusive dopamine antagonist condition. Previous observations indicate that administration of SCH-23390 or raclopride into medial accumbens shell does not significantly suppress eating or fearful behaviors below baseline control levels, although the already near absence of vehicle fearful responses should be noted (Faure et al., 2008; Richard & Berridge, 2011). This study similarly found that fearful behavior in the exclusive D_1/D_2 antagonist condition, specifically defensive treading, was not significantly reduced below vehicle levels (treading, main effect of D_1/D_2 antagonists, $F(1, 15) = 3.17$, $p = 0.097$). However, a significant reduction to more than five times below baseline levels was observed for both eating behavior and food intake under the influence of the dopamine antagonists alone (time spent eating, main effect of D_1/D_2 antagonists, $F(1, 15) = 7.85$, $p = 0.014$; food intake grams consumed, mean of 0.25 ± 0.68 g under D_1/D_2 antagonists vs. 1.65 ±

1.28 g under vehicle; food intake, main effect of D_1/D_2 antagonists, $F(1, 15) = 8.67$, $p = 0.012$). Additionally, general locomotion and other normal activities were suppressed under the D_1/D_2 antagonist condition, including drinking time, food sniffs, food carries, grooming bouts, cage crosses, and rears (main effect of D_1/D_2 antagonists, drinking, $F(1, 15) = 21.10$, $p < 0.001$; food sniffs, $F(1, 15) = 12.69$, $p = 0.003$; food carries, $F(1, 15) = 5.93$, $p = 0.029$; grooming bouts, $F(1, 15) = 19.44$, $p < 0.001$; cage crosses, $F(1, 15) = 13.96$, $p = 0.002$; rears, $F(1, 15) = 38.50$, $p < 0.001$).

Nonetheless, total incapacitation due to presence of the dopamine antagonists can generally be ruled out, as the rats demonstrated sufficient locomotion and general activity in the presence of the mixture condition combining muscimol with the D_1/D_2 dopamine antagonists when compared with activity under vehicle (number of crosses, mean of 51.75 ± 42.73 crosses under mixture vs. 44.81 ± 26.30 crosses under vehicle). Furthermore, the elevation in mean duration of time spent sleeping in rats receiving only the D_1/D_2 antagonists intuitively correlates with reduction of other general behaviors; however, the amount of time spent sleeping does not differ significantly from vehicle levels, ruling out debilitating sedation as a blanket effect of the dopamine antagonists (cumulative sleep duration, mean of 1140.00 ± 784.76 s under D_1/D_2 antagonists vs. 688.13 ± 805.45 s under vehicle; sleep, main effect of D_1/D_2 antagonists, $F(1, 15) = 0.37$, $p = 0.554$).

Discussion

Consistent with previous findings (Faure et al., 2010; Reynolds & Berridge, 2001, 2002), the administration of the $GABA_A$ agonist muscimol to nucleus accumbens shell robustly elevated appetitive and fearful behaviors in rats along a rostrocaudal gradient. Here we determined for the first time that muscimol-generated fear and feeding behaviors do not depend on endogenous

local dopamine. The parameters we assessed include duration of eating time, food intake, duration of defensive treading, and distress exhibited upon pick-up in the presence of a local D_1/D_2 receptor blockade combined in the same microinjection with muscimol. Other behaviors and general activities normally affected by muscimol show minor, nonsignificant reductions of mean expression when observed in the concurrent presence of the dopamine antagonists. Such effects point to the existence of independent mechanisms by which GABA and dopamine in accumbens shell affect motivated behaviors, despite partially overlapping behavioral displays.

Previous findings draw attention to the striking similarities and variations that exist between subcortical GABA circuits and corticolimbic glutamate circuits regarding the extent to which each are able to affect emotional processes. Most notably, hyperpolarizing manipulations in the signaling of either neurotransmitter in the nucleus accumbens have been demonstrated to generate an equivalent pattern of appetitive and fearful motivated behaviors dependent on rostrocaudal localization (Faure et al., 2010). However, only GABAergic signaling is additionally able to regulate hedonic processes along a corresponding gradient, whereas glutamate disruption has no effect on hedonic components. The neurobiological differences between GABAergic and glutamatergic processes may provide some explanation as to why subcortical GABA signals are able to exert a stronger and wider range of influence than those of glutamate. One interpretation considers the possibility that hyperpolarization via the agonism of $GABA_A$ receptors (such as that done by muscimol) may induce more powerful inhibition of medium spiny neuron signaling than the antagonism of glutamate receptors and relative hyperpolarization generated from blocking such glutamate signals (such as that done by administration of DNQX) (Faure et al., 2010). Another important factor to consider is the relative location of neurotransmitter receptors on the dendrites of medium spiny neurons

receiving input. GABA receptors appear to lie closer to the soma, whereas ionotropic glutamate receptors lie more distally on the dendritic spines (Chen et al., 1998; Sun & Cassell, 1993). In such signaling, it has been suggested that locations more proximal to the soma and axon hillock may correspond to stronger intensity of propagated signaling (Faure et al., 2010).

Another prominent difference between subcortical GABA controls and corticolimbic glutamate influence considers mechanisms of interaction with endogenous dopamine. Faure and colleagues (2008) determined that the administration of combined local D_1 and D_2 receptor antagonists in combination with DNQX, an AMPA/kainate glutamate receptor antagonist, was able to block the robust eating and fearful behaviors normally generated by glutamate disruptions in medial shell. Since glutamatergic elicitation of motivated behaviors appears to require dopaminergic co-activation at specific sites, restrictions on the range of influence exhibited by top-down glutamatergic circuits may indicate limitations of these signals in comparison to the control exhibited by subcortically generated processes, specifically those involving GABA signals. Furthermore, the subcortical co-release of glutamate by mesolimbic dopamine neurons in the nucleus accumbens draws attention to a synergistic physiological interaction between glutamate and dopamine signaling in the generation of motivated behavior (Faure et al., 2010), whereas none have yet been definitively found between dopamine and GABA. GABAergic processes also demonstrate an ability to mediate hedonic pleasure and disgust (Reynolds & Berridge, 2002), a mechanism that is known to be dopamine-independent (Berridge, 2007) as well as unaffected by glutamate manipulation (Faure et al., 2010).

We chose to specifically induce a local dopamine blockade via a combined D_1/D_2 receptor antagonist mixture to examine whether GABA-induced motivated behavior requires endogenous dopamine based on previous findings that a similar dopamine blockade was

demonstrated to prevent glutamatergic generation (via DNQX administration) of appetitive and fearful behaviors (Faure et al., 2008). It is evident that dopamine's physiological modulation of GABAergic signaling currently remains an inadequately understood topic (Lovinger, 2010). Although investigations have produced evidence indicating presynaptic mechanisms by which dopamine is able to mediate the firing of GABAergic interneurons (Bracci et al., 2002; Centonze et al., 2002; Towers & Hestrin, 2008), not much study has been done on dopamine's modulation of GABA signaling at GABA receptors on medium spiny neurons. One relevant physiological study suggested that GABA-mediated signaling persists independently of dopaminergic influence based on findings of unaltered GABA synaptic currents in dopamine transporter knockdown mice with elevated levels of extracellular dopamine, although the experimenters did observe changes in glutamate receptor-mediated currents (Wu et al., 2007). However, more research must be done before conclusions can be drawn concerning the extent of dopamine's postsynaptic influence on GABAergic signaling.

Our experiment examined the effects of inducing a local dopamine blockade on the GABAergic elicitation of eating behavior and defensive treading. The presence of the combined D_1/D_2 antagonists did not prevent muscimol-induced eating or treading. Despite slightly lower mean expression of these behaviors under the mixture condition when compared with muscimol, such changes were not significantly different from expression under muscimol alone and were likely due to independent inhibitory effects of dopamine blockade rather than an interaction with muscimol specifically. Rostral rats still exhibited voracious eating behavior under the mixture condition, and caudal rats displayed intensely fearful treading and distress reactions. Mean expression of other general activities, normally elevated above baseline levels in the muscimol alone condition, also exhibited minor reductions from that of muscimol alone when rats were

administered the drug mixture condition, although no significance was found for these decreases either. Such behavioral measures include number of food sniffs, food carries, cage crosses, burrow-treads, and rears during the testing period. These results provide support for the proposed independence of GABA-generated appetitive and fearful motivated behaviors from dopaminergic mediation. In contrast to the pronounced effects of a local dopamine blockade on similar behaviors generated by glutamatergic disruptions (Faure et al., 2008), any small, insignificant changes we found in mean expression of behaviors seem even less likely to be indicative of possible mechanisms of dopaminergic modulation of GABA-induced behaviors.

Our findings do present some statistically significant interaction between muscimol and the dopamine antagonists in relation to two other behaviors. It is noteworthy that while the administration of muscimol normally reduces the duration of time spent drinking to below baseline level, the presence of the dopamine blockade in the mixture condition was observed to further lower the mean drinking duration to a level below that of the muscimol alone condition, demonstrating a possible additive effect. Furthermore, the mean number of grooming behaviors performed under the drug mixture condition was nearly equivalent to that under muscimol alone, both of which lie well below vehicle levels. It is important to note, however, that neither of these instances demonstrated inhibition nor reversal of muscimol-induced behavior due to the presence of the dopamine antagonists.

We did observe one instance where local dopamine blockade significantly suppressed muscimol-induced fearful reactions. The *total* distress score of the rats in reaction to pick-up at the end of the testing session, which takes into account the total number of fearful actions and the intensity of such actions performed, exhibits a small, yet significant, reduction. However, no performance of a single reaction (vocalization, escape attempt, or bite attempt) changed

significantly itself. Rather, it appears as though the decreased intensity of distress displayed by the rats accounts for the predominant change in total distress score between muscimol and mixture conditions. Another justification for this observation could be attributed to the general decrease of motor activity observed in rats receiving dopamine antagonists, contributing to fewer escape attempts and, therefore, a lower total distress score. This one significant statistic suggests that although muscimol-generated behaviors appear to exhibit dopamine independence, there may yet exist some potential mechanisms of interaction. This minor suppression of muscimol-induced fear, however, stands in stark contrast to DNQX-induced fearful behaviors, which are nearly or completely eliminated by a similar dopamine blockade (Faure et al., 2008; Richard & Berridge, 2011).

It has been well established that muscimol administration to rostral nucleus accumbens shell leads to substantial increases in food intake and appetitive behavior (Faure et al., 2010; Reynolds & Berridge, 2001, 2002). However, while measures of food intake and eating duration are often considered important indicators of appetitive motivation, suggestions have been proposed implicating other potential mechanisms of GABAergic influence, such as the direct generation of feeding motor patterns, rather than mediation of motivational state (Kelley, Baldo, Pratt, & Will, 2005). Wirtshafter and Stratford (2010) took a closer look at the effects of different accumbens shell manipulations in order to discern the underlying behavior-directing mechanisms by which they affect feeding and arousal in rats, specifically examining muscimol and amphetamine manipulations. In an effort to determine whether motor-related or motivation-related factors underlie the food-seeking behavior associated with GABAergic and dopaminergic circuits, the experimenters chose to utilize a progressive ratio schedule as a means of discerning motivated behavior from simple arousal or activation of feeding behavior. Muscimol and

amphetamine were both demonstrated to similarly increase breaking point on a progressive ratio performance, implicating more complex effects exerted by both neural circuits on motivation rather than the simple activation of motor action patterns (Wirtshafter & Stratford, 2010). However, only muscimol additionally led to an increase in food intake, whereas amphetamine had no significant effect on intake. This puzzling observation suggests that each circuit possesses distinct functional effects that may be able to contribute to similar behavioral manifestations. In a more recent experiment, Stratford and Wirtshafter (2012) examined food-reinforced lever pressing in non-deprived rats, and their findings implicate a role for muscimol in the specific enhancement of goal-directed behavior in contrast with their observations of amphetamine's general behavioral activation.

The exact role and targets of dopamine in reward-related processes have been debated for decades; however, the establishment that dopamine possesses some significant function in reward is predominantly agreed upon. Although dopamine plays a role in the ability to energize activity and feeding, it is important to also examine its influence on incentive-directed behavior because there is much evidence indicating that dopaminergic processes are involved in reward beyond simple motor activation (Wise, 2006). The original notion regarding dopamine's psychological role in reward, known as the hedonia hypothesis, asserts that dopamine mediates the pleasurable aspects of reward and associates its neurotransmission with the positive reinforcing effects of rewarding stimuli (Wise, Spindler, Dewit, & Gerber, 1978). Much evidence has since risen in opposition to this theory (Berridge, 2007). Another prevalent theory asserts that dopamine functions in reward processes by mediating learning about rewards. The reward learning hypothesis implicates the firing of dopaminergic neurons as a crucial signal associated with learning about rewards, although several branches of this theory exist speculating

whether its role is mainly to stamp-in associations via reinforcement, to mediate learning of habits, or to teach prediction of rewards. While the reward learning hypothesis represents a more compelling theory than the hedonia hypothesis, evidence has demonstrated that genetically modified mice lacking endogenous dopamine were still able to learn without dopamine in an appetitive T-maze task if they had been given caffeine (Robinson, Sandstrom, Denenberg, & Palmiter, 2005). Therefore, the presence of dopamine was not needed for the learning to occur, although dopamine was deemed necessary for these mice to act on what they had learned and actively seek rewards.

The third major hypothesis concerning dopamine's role in reward is largely referred to as the incentive salience theory and addresses the missing link binding the value of reward-related stimuli with the expression of actions needed to attain such rewards (Montague, Hyman, & Cohen, 2004). Originated by Berridge and colleagues, this theory dissociates the "wanting" of rewards from subjective "liking" of them and the learning about such likes (Berridge, 2007). Incentive salience integrates previously learned associations with the current state of the organism at the exact moment the stimulus is encountered and motivates the subsequent approach and consumption of rewards. While each theory has made important contributions to current understanding of dopamine's role in reward, the best evidence currently seems to reside with the incentive salience hypothesis, although the exact functions of dopamine continue to remain under debate.

In our experiment, muscimol alone generated intense appetitive and defensive motivated behaviors. In contrast, the local D_1/D_2 dopamine receptor antagonists alone lowered eating duration, treading duration, and other general activity when compared with vehicle expression. It is intriguing how the presence of the dopamine antagonists failed to alter muscimol-elicited

feeding or fearful behaviors significantly in this study. While variations in eating and treading behaviors have been closely associated with changes in motivational state, investigators have also considered that an inherent arousal state or a state involving perception of effort expenditure may exist and represent conditions targeted by dopaminergic manipulations rather than the modulation of goal-directed motivation (Wirtshafter & Stratford, 2010). In the presence of a robust motivational state, such as that induced via muscimol injections, dopaminergic modulation of arousal may not have significant effects, if any, on goal-seeking behavior, aligning with our experimental observations. Suggestions have been made that GABA-induced behaviors and dopamine-modulated activity may stem from different sets of cells within the same population or from the same cells with different activity patterns (Stratford & Wirtshafter, 2012), and it has been demonstrated that GABAergic and dopaminergic manipulations can produce similar behavioral manifestations (Wirtshafter & Stratford, 2010). However, evidence continues to accumulate identifying distinctions between these two neurological circuits and pointing toward independence between their mechanisms of function.

The suppression of general activity level observed in rats receiving the dopamine antagonists exclusively is important to note; however, incapacitation from the D_1/D_2 receptor antagonists can be ruled out as a confounding factor in this experiment. It is evident that the rats in this experiment were not debilitated under the mixture condition combining muscimol with the dopamine antagonists because most of the locomotive behaviors performed in this state were expressed at a level comparable to that under muscimol alone, even when a particular behavior had been significantly suppressed under the dopamine antagonists alone. Previous findings have also demonstrated that administering the same dose of these D_1/D_2 receptor antagonists failed to produce significant changes from baseline eating levels (Faure et al., 2008; Richard & Berridge,

2011), and while our results appear slightly different in this respect, such evidence provides support that the rats did not experience detrimental motor impairments from the presence of the dopamine blockade that would suppress ability to engage in appetitive behaviors.

Richard and Berridge (2011) investigated the effects of a local dopamine blockade on the glutamatergic generation of fear and feeding behaviors and assessed the specifications of mesolimbic dopamine D_1 and D_2 receptor signaling in motivation. Their study revealed an exclusive role for D_1 receptor signaling related to the rostral enhancement of appetitive actions and eating and a simultaneous function for D_1 and D_2 receptor transmission in modulation of the elicitation of fearful behaviors in caudal shell. Our experiment utilized a combined D_1 plus D_2 receptor antagonist mixture in order to initially determine whether muscimol-induced behaviors demonstrated dependence on either type of dopamine receptor, and no interactions were found. Although our results imply that administering either dopamine antagonist alone would not have had different effects from those of the combined D_1/D_2 antagonist mixture, studies have pointed to potential interactions between D_1 and D_2 dopamine receptor antagonists, such as synergistic effects when activated simultaneously (Svensson, Carlsson, & Carlsson, 1992). Hence, although it appears unlikely that delivering either the D_1 or D_2 dopamine antagonist alone would have produced divergent results in this experiment, such a possibility remains unknown.

The generation of motivated behaviors, such as those elicited by manipulations of the nucleus accumbens, represents a progressively growing field of research today. These studies not only contribute to our growing knowledge of the mechanisms involved in neurobiology and how they relate to behavior, but they also provide evidence indicating where and how aberrant behaviors may be able to emerge. Psychopathology, addiction, and related mental disorders represent areas of great detriment to society and the individuals affected, and such important

investigations produce findings that provide implications for possible future therapies for these afflictions. By examining motivational processes and uncovering ways by which the generation of motivated behaviors can be mediated, certain mental disorders can be better understood and treated. This investigation demonstrates that GABA-generated motivated behaviors exhibit dopamine independence, a finding that contributes to current knowledge of neural circuits, mechanisms of neurotransmitter interaction, and the mediation of motivated behaviors.

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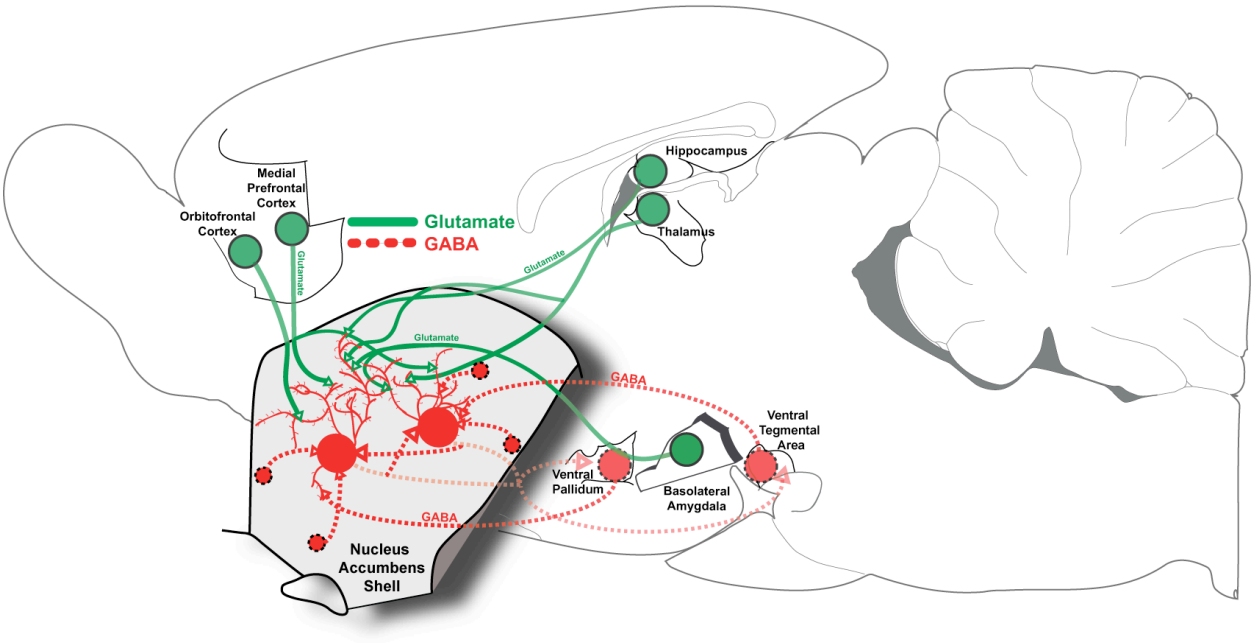

Figure 1. Mesocorticolimbic circuits illustrating glutamatergic (green) and GABAergic (red) inputs involved in nucleus accumbens shell signaling. Diagram shows glutamate signals entering shell from cortical and cortical-like areas including prefrontal cortex, hippocampus, thalamus, and basolateral amygdala, whereas GABA inputs come from subcortical structures, including ventral pallidum and ventral tegmental area, and from instrinsic GABAergic interneurons. Reprinted from Faure et al. (2010) with permission.

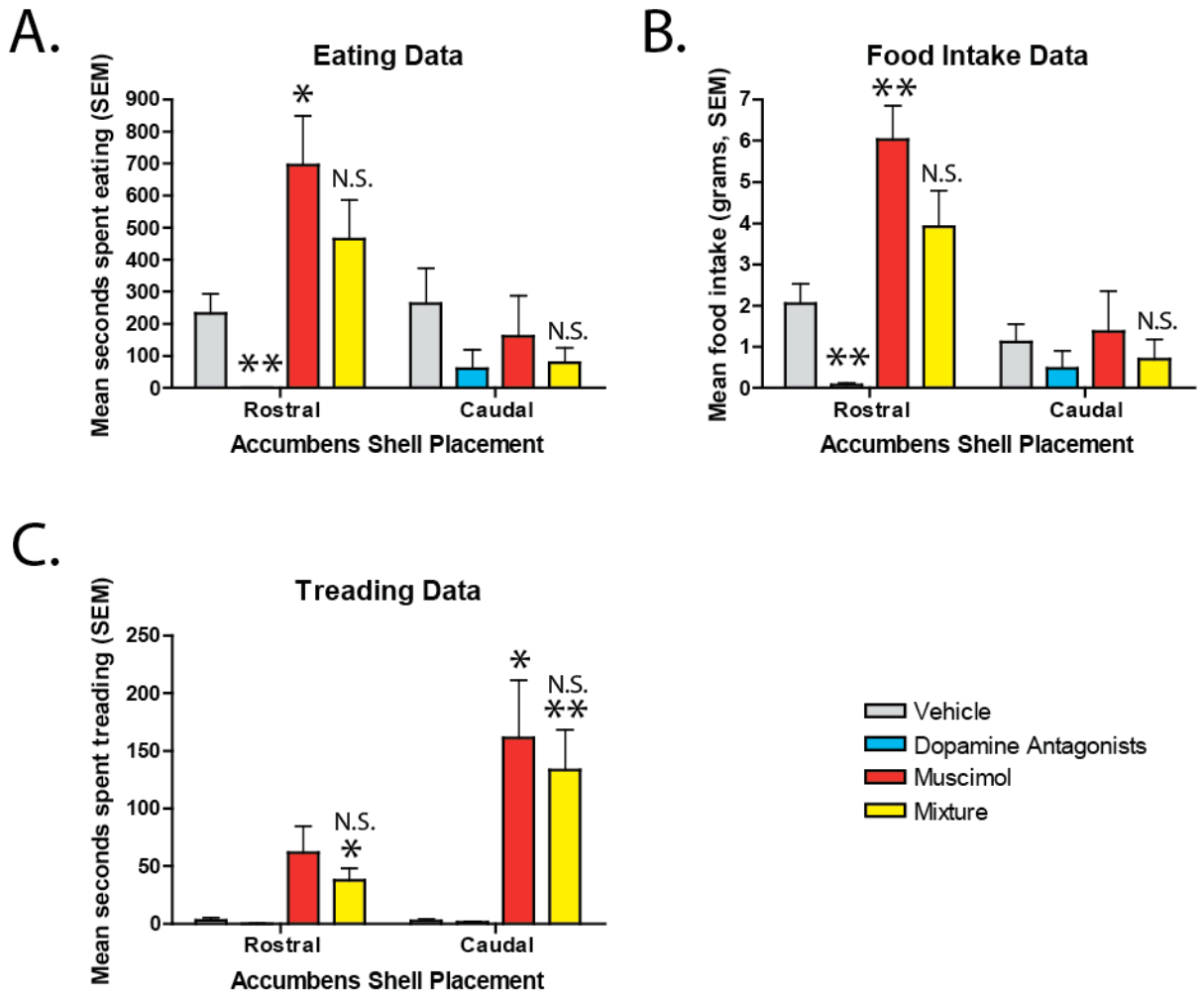

Figure 2. Motivated behaviors under aCSF vehicle, combined D_1/D_2 dopamine receptor antagonists, muscimol, or muscimol + D_1/D_2 antagonists mixture in rostral and caudal nucleus accumbens shell. Magnitudes of time spent eating (A), grams of food intake (B), and time spent treading (C). Error bars represent SEM. *$p < 0.10$, **$p \leq 0.05$ (change from vehicle); N.S. indicates no significant change of mixture condition from muscimol alone ($p > 0.10$).

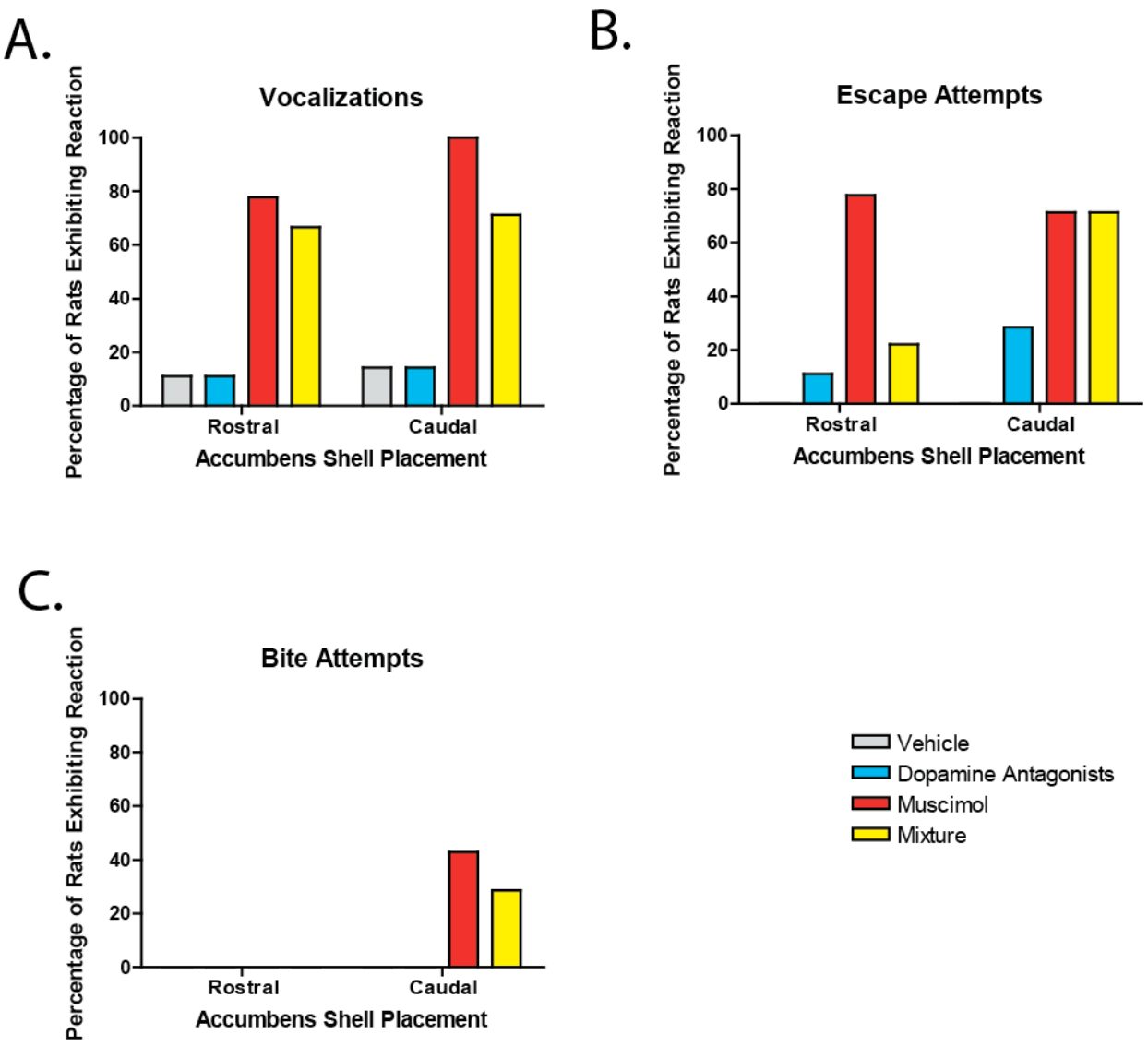

Figure 3. Distress reactions of rats in response to human touch at conclusion of testing under aCSF vehicle, combined D_1/D_2 dopamine receptor antagonists, muscimol, or muscimol + D_1/D_2 antagonists mixture in rostral and caudal nucleus accumbens shell. Percentage of rats displaying instances of vocalizations (A), escape attempts (B), and bite attempts (C).

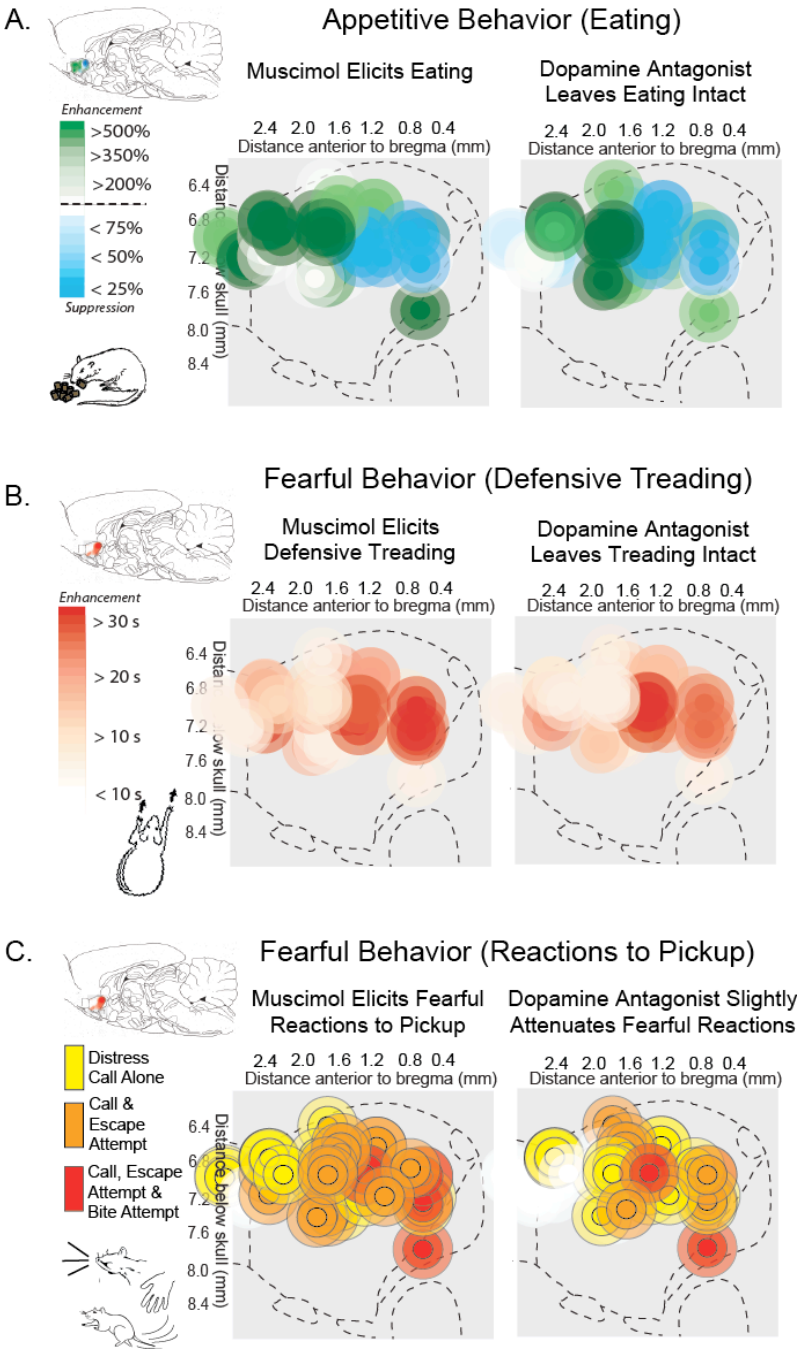

Figure 4. Effects of D_1/D_2 antagonism on muscimol-generated feeding and fearful behaviors. Fos plume maps show a sagittal plane of nucleus accumbens shell, and the colors indicate the amount of eating (A) and defensive treading (B) induced under muscimol (left) or muscimol + D_1/D_2 antagonists (right) as a percentage of behavior expression under vehicle, as well as number of fearful responses (C) exhibited under muscimol (left) or muscimol + D_1/D_2 antagonists (right).