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Dorsolateral neostriatum contribution to incentive salience: Opioid or dopamine stimulation makes one reward cue more motivationally attractive than another

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Abstract

Pavlovian cues for rewards can become attractive incentives: approached and ‘wanted’ as the rewards themselves. The motivational attractiveness of a previously learned cue is not fixed, but can be dynamically amplified during re-encounter by simultaneous activation of brain limbic circuitry. Here we report that opioid or dopamine microinjections in the dorsolateral quadrant of the neostriatum (DLS) of rats selectively amplify attraction toward a previously learned Pavlovian cue in an individualized fashion, at the expense of a competing cue. In an autoshaping (sign-tracking vs goal-tracking) paradigm, microinjection of the mu opioid receptor agonist (DAMGO) or dopamine indirect agonist (amphetamine) in DLS of sign-tracker individuals selectively enhanced their sign-tracking attraction toward the reward-predictive lever cue. By contrast, DAMGO or amphetamine in DLS of goal-trackers selectively enhanced prepotent attraction toward the reward-proximal cue of sucrose dish. Amphetamine also enhanced goal-tracking in some sign-tracker individuals (if they ever defected to the dish even once). That DLS enhancement of cue attraction was due to stronger motivation, not stronger habits was suggested by: 1) sign-trackers flexibly followed their cue to a new location when the lever was suddenly moved after DLS DAMGO microinjection, and 2) DAMGO in DLS also made sign-trackers work harder on a new instrumental nose-poke response required to earn presentations of their Pavlovian lever cue (instrumental conditioned reinforcement). Altogether, our results suggest that DLS circuitry can enhance the incentive salience of a Pavlovian reward cue, selectively making that cue a stronger motivational magnet.

Keywords

rat; motivation; striatum; autoshaping

Introduction

The neostriatum is traditionally considered a motor structure, and the dorsolateral quadrant of striatum (DLS) is viewed to mediate movement and habit functions (e.g., action sequencing and habit automaticity) (Packard & Knowlton, 2002b; Balleine *et al.*, 2007; Tang *et al.*, 2007; Yin, 2010; Bornstein & Daw, 2011; Everitt & Robbins, 2013; Smith & Graybiel, 2013; Kalueff *et al.*, 2016). However, neostriatum also is increasingly recognized to have motivation-related functions, including potentially DLS and other dorsal regions of neostriatum (Palmiter, 2008; Wise, 2009; DiFeliceantonio *et al.*, 2012; Kravitz *et al.*, 2012). For example, dorsal neostriatum is activated by drug cues in human addicts (i.e., Pavlovian conditioned stimuli or CSs), and by food cues in obese and binge eaters (Volkow *et al.*, 2002; Stice *et al.*, 2008; Nummenmaa *et al.*, 2012; Jastreboff *et al.*, 2013). Similarly in rats, dorsal neostriatum levels of enkephalin surge when palatable food is encountered, and opioid stimulation in dorsal neostriatum causes increases in eating behavior (DiFeliceantonio *et al.*, 2012). Further, DLS lesions in rats disrupt the ability of Pavlovian cues to trigger peaks of increased effort to obtain reward in Pavlovian-instrumental transfer (PIT) studies (Corbit & Janak, 2007), potentially indicating a need for DLS in cue-triggered incentive salience or ‘wanting’ for reward.

The intensity of incentive salience triggered by a Pavlovian reward cue can be amplified by relevant physiological appetites and by localized neurobiological stimulations of limbic circuitry (Robinson & Berridge, 1993; Mahler & Berridge, 2009; DiFeliceantonio & Berridge, 2012; Hickey & van Zoest, 2012; Robinson & Berridge, 2013). For example, dopamine and opioid stimulations in limbic structures, such as nucleus accumbens or central amygdala, are especially effective at amplifying the incentive salience of a previously-learned Pavlovian reward cue (Wyvell & Berridge, 2001; Smith *et al.*, 2011; DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2012; Pecina & Berridge, 2013; Robinson *et al.*, 2014a).

Incentive salience can make a Pavlovian cue become attractive and ‘wanted’. Amplification of incentive salience raises a cue's attractiveness, making it into a stronger ‘motivational magnet’ that elicits approach and even consummatory behaviors. In animal studies, this can be studied via the Pavlovian autoshaping paradigm, which reveals individual differences in attraction toward reward cues as either sign-tracking or goal-tracking responses (Robinson & Flagel, 2009; Robinson *et al.*, 2014b). For example, insertion of a metal lever into a chamber's wall serves as a Pavlovian conditioned stimulus (CS+) or reward-correlated sign that predicts delivery of a sucrose pellet into a dish as unconditioned stimulus (UCS) (Zener, 1937; Jenkins & Moore, 1973; Boakes *et al.*, 1978; Flagel *et al.*, 2009; Meyer *et al.*, 2012). After learning the CS-UCS association, some individual rats (called sign-trackers) approach the CS+ and avidly sniff and nibble the lever cue. By contrast, other individuals (called goal-trackers) respond to CS+ insertion by instead approaching and nibbling the reward-contiguous dish where sucrose is actually received.

Here we test whether DLS has the capacity to amplify the incentive salience of a particular reward cue in an individually-tailored fashion, when neurochemically activated by either

local opioid stimulation (via DAMGO microinjection) or local dopamine stimulation (via amphetamine microinjection).

Materials and Methods

Overview

We compared the effect of DAMGO versus amphetamine microinjections in DLS, or alternatively in the dorsomedial neostriatum (DMS), on CS+ incentive salience. Incentive salience of Pavlovian cues was assessed by measuring changes in sign-tracking versus goal-tracking behaviors after rats had learned the CS-UCS association. Lever cue presentation (CS+Lever), which predicts sucrose, is a CS+ *trigger* potentially able elevate incentive salience, especially when limbic circuitry is simultaneously pharmacologically stimulated (Berridge, 2012). Sign-trackers and goal-trackers differ in their target of incentive salience (Robinson *et al.*, 2014b). For sign-trackers, the CS+Lever becomes the target of motivation as well as the trigger, so that sign-trackers approach, sniff and nibble the predictive lever cue. By contrast, for goal-trackers, especially in states of mesocorticolimbic pharmacological stimulation, the more ‘wanted’ target becomes the goal dish (CS_{dish}) where sucrose UCS is obtained, even though the temporal trigger for the phasic surge in target attraction remains the CS+Lever (Mahler & Berridge, 2009; DiFeliceantonio & Berridge, 2012).

We assessed whether DLS stimulation amplified motivation versus an automatic motor habit by asking if DLS enhancement of sign-tracking impeded flexible switching of CS+ approach to a new location, when the location of the target CS+Lever was suddenly changed (Carr & Watson, 1908). We further confirmed that DAMGO microinjection in DLS indeed enhanced CS+ motivation, making the cue more ‘wanted’, by showing DLS opioid stimulation also increased instrumental conditioned reinforcement: sign-tracking rats acquired a new nose poke response and performed it at higher levels to earn presentations of the CS+Lever.

In order to help map localization of function within dorsal neostriatum, Fos plume maps of behavioral effects were constructed, following procedures described by previous studies (Pecina & Berridge, 2005; Richard & Berridge, 2011). First, the diameter of drug impact on neuronal function was assessed by measuring local drug-induced elevation (relative to vehicle microinjections) in Fos protein expression in cells surrounding a drug microinjection (‘Fos plume’). The mean size of measured Fos plume diameters was then used to set the size of function symbols in neostriatum maps. The site of each rat's microinjection was identified histologically after the experiments. Each rat's incentive salience effects of DAMGO, measured by changes in sign-tracking or goal-tracking behavior produced by drug microinjections relative to its vehicle-control trial, were mapped onto its anatomical site using color-coded symbols on brain atlas representations of neostriatum (Paxinos & Watson, 2007).

Methods details

Subjects: Female Sprague Dawley rats bred in house, were between 4-5 months old, and between 280-350 grams body weight at the start of the experiment (n=81 total: n=28 for

sign-tracking/goal-tracking tests; n=14 for moved-cue tests; n=38 for instrumental conditioned reinforcement tests). Females were used in order to allow comparisons to data from previous articles from our lab reporting enhancements in female rats of sign-tracking vs goal-tracking or by microinjections of DAMGO or amphetamine (e.g., in amygdala) (Mahler & Berridge, 2009; DiFeliceantonio & Berridge, 2012; Robinson & Berridge, 2013; Robinson *et al.*, 2014a; Robinson *et al.*, 2015b). Pavlovian sign-tracking and goal-tracking responses remain stable across the estrous cycle in females (Pitchers *et al.*, 2015). Another reason why females were used was to conform to recent NIH directives to not rely on male-only groups (Clayton & Collins, 2014). An explicit comparison across sexes would have required doubling the number of rats, and so was not included here but could be done by future studies. Rats were pair-housed on a reverse light/dark cycle. Water was always provided *ad libitum*; food was also provided *ad libitum* except during autoshaping training or test weeks, when rats were restricted to 90% free feeding weight (about 14g of standard laboratory chow provided daily after each training session). Before surgery, all rats received 2-4 handling sessions of 10-min each to acclimate them to being held and moved to the laboratory. All experiments were conducted in accordance with protocols approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

Surgery: Rats were anesthetized with ketamine (80 mg/kg), xylazine (7 mg/kg), and atropine (0.04 mg/kg). To prevent infection, chloramphenicol sodium succinate (60 mg/kg) was administered, and carprofen given immediately after surgery (5 mg/kg) as an analgesic. Carprofen and chloramphenicol doses were repeated again 24 h later. Rats were allowed 5-7 days to recover from surgery before any training or testing began.

Chronic bilateral 14 mm (23 ga) guide cannulae were implanted aimed at sites in the dorsolateral neostriatum (individually staggered sites between AP +0 to 2.5, ML $\pm$ 3.0 to 4.0, DV -3.5 to -4.5, all relative to bregma with head secured in flat skull position), or alternatively at control sites in dorsomedial neostriatum as an anatomical comparison (between AP +0 to 2.5, ML $\pm$ 1.8, and DV -3.5 to -4.5) (Paxinos & Watson, 2007). Guide cannulae tips were aimed 2 mm above intended target injection sites, because the actual microinjectors for drugs extended a further 2 mm below guide cannulae into the intended site. Guide cannulae were anchored to the skull with bone screws and acrylic cement. Steel stylets were inserted into guide cannulae to prevent occlusion, and removed only prior to microinjections. Anatomical sites were confirmed histologically after each experiment.

Microinjections and drugs: Prior to all behavioral tests, stylets were removed and guide cannulae were cleaned, before 16mm microinjectors were inserted into the guide cannulae. Microinjections of [D-Ala², N-MePhe⁴, Gly-ol]-enkephalin (DAMGO; Sigma; 973 μ M concentration at 0.25 μ g/0.5 μ L dose), amphetamine (116.5 mM concentration at 10 μ g/0.5 μ L dose), or vehicle alone (artificial CSF; Harvard Apparatus) were controlled by a syringe pump which delivered 0.5 μ L over a 2-min period. Microinjection cannulae were left in place for an additional 1-min following the microinjection to allow drug diffusion. Each rat also received a “sham” microinjection of vehicle on the day prior to first test to habituate to the procedure.

Behavioral Autoshaping Training: Autoshaping training procedures were the same as in our previous studies (Mahler & Berridge, 2009; DiFeliceantonio & Berridge, 2012). In brief, autoshaping training and testing was always carried out in the same operant chamber for a particular rat, containing two retractable levers on opposite sides of a food receptacle (Med Associates), controlled by Med PC software. Rats first received a session of magazine training consisting of 20 sucrose pellets being delivered into the food dish. Pavlovian autoshaping training (CS+ paired with UCS) began the second day. Training sessions began with illumination of the red house light. Each UCS presentation was preceded by insertion of the CS+Lever, which carried a light emitting diode on its ventral surface and was accompanied by an auditory 2.9 KHz tone. The CS+Lever/tone presentation lasted 8s, after which the lever was retracted back through the wall, and was immediately followed by delivery of one sucrose pellet into the goal dish (UCS; Test Diet). Twenty-five CS+ UCS pairs were presented on a 90s variable inter-trial interval schedule during the 40 minute session. A control lever was always present in the chamber. A camera was positioned under the transparent floor of the autoshaping chamber to provide a clear view of the rat's entire head and body wherever it was in the chamber, and allowed visual scoring of approaches to lever or dish, as well as scoring of consummatory sniffing, licking and biting of the lever. A second camera was directed from the side toward the inner surface of the CS_{dish} to allow scoring of consummatory sniffing, nibbles or biting of the dish. Video recorded behaviors were scored later offline by the experimenter blind to drug condition. Lever depressions and beam-break entries into the dish were also recorded automatically.

Training sessions were repeated over 5 consecutive days. By the 3rd training day, every rat began to respond to the CS+ onset with an approach-consummatory CR predominantly focused toward either the CS+Lever or the CS_{dish}. The criterion for classification as a predominant sign-tracker was to approach, nibble, sniff, grasp and bite the CS+Lever at least three times more frequently than they did the sucrose dish during CS+ presentations on day 5. The criterion for classification as predominant goal-tracker was a) to approach, nibble, sniff, grasp and bite the dish at least three times more frequently than the lever during CS+ presentations, and additionally b) to approach the CS_{dish} three times more frequently when the CS+Lever was present than in intervening baseline periods when the CS+ was absent (to ensure that CS+Lever presentation was the trigger for a phasic elevation in goal-tracking behavior, since the dish was present during baseline periods as well as during CS+ and UCS presentations). All rats' phenotypes were discernible by day 3.

Autoshaping Testing: After a sham microinjection on day 6, behavioral testing was conducted on days 7 and 9. Each rat received either a DAMGO or vehicle microinjection immediately prior to the autoshaping test on day 7 (n=13) and received the other microinjection on the repeated test on day 9 (order counter-balanced across rats) following a day of rest. A separate group of identically-trained rats (n=15) similarly received either an amphetamine microinjection or vehicle microinjection in counterbalanced order. After microinjections, rats were immediately placed in their usual autoshaping chamber. Each test consisted of 25 CS-UCS presentations identical to training.

Behavioral Video Scoring: Autoshaping: Videos from the two cameras were analyzed off line in slow motion (1/10th to 1/2 actual speeds) by an observer blind to experimental conditions. For each trial, the 8 seconds before and 8 seconds during the 5th, 10th, 15th, 20th, and 25th presentation of the CS+ were selected for comparison (Mahler & Berridge, 2009). Appetitive behaviors scored were: *look* at the cue (orienting towards the cue by moving the head or forequarters toward it, without bodily approaching it), and *approach* the cue to make contact or within 1 cm. Consummatory behaviors towards the CS+Lever or dish (similar to ingestive movements directed at UCS sucrose pellets) were scored: *sniff* the cue (contact of the nose and rhythmic nose flaring movements with either lever or dish), *nibble* the cue (short contacts (<0.5 sec) of mouth or teeth on lever or dish, combined with rapid opening and closing movements of jaw and rhythmic 1-2 Hz bobbing movements of the head), and slow *bite* of the cue (jaw closing and contact by maxillary and mandibular incisors for >0.5 sec, often while grasping the cue lever or dish with one or both paws).

Free intake paradigm testing: To assess effects of DAMGO microinjections DLS on food and water intake, rats received free access to palatable milk chocolate candies (M&Ms) in a 1-hr intake test after receiving microinjections (DiFeliceantonio & Berridge, 2012). DAMGO or vehicle administration was counterbalanced across days with 48 h between each testing session. Prior to any test, rats were habituated for 4 days to clear plastic tub cages with ~3 cm of corn cob bedding, 20 g of pre-weighed M&Ms, 20 g of pre-weighed chow. Water was always available through a drinking spout. At the end of each intake test, any remaining M&M's or chow were counted and re-weighed, and videoed eating behavior was scored at a later date offline.

Ingestive intake videos were scored by experimenters blind to the experimental condition of each rat. Seconds spent engaging in the following behaviors were recorded: eating M&Ms (actual chewing and consumption), eating chow pellets (similar consumption behaviors), drinking from spout, and noningestive chewing on non-food bedding, cage, or spout. Also, sniffing M&Ms (anticipatory sniffs and approaches, without actual eating), sniffing chow, grooming, cage crossing, and rearing were additionally recorded as single events each time they occurred (Richard & Berridge, 2011).

Histology and Fos plume measurement: Rats were euthanized immediately after the final day of testing by a sodium pentobarbital overdose. Rats that had been behaviorally tested were decapitated and the brains were extracted and fixed in 10% paraformaldehyde solution for 1-2 days followed by a 25% sucrose solution in 0.1M NaPB for 2-3 days before slicing. 60 micron slices through the neostriatum were taken from each rat, mounted, dried, and stained with cresyl violet. Microinjection center was determined for each bilateral injection site mapped onto a stereotaxic atlas (Paxinos & Watson, 2007) to locate placement within the dorsolateral or dorsomedial neostriatum. Rats used for Fos analysis were anesthetized and transcardially perfused 90 min after bilateral microinjection of vehicle (dorsomedial n = 8; dorsolateral n = 6), DAMGO (dorsomedial n = 10; dorsolateral n = 9), or normal (no injection, dorsomedial n = 2; dorsolateral n = 2). Brains were extracted, frozen, and sliced at 40 µm. Brain slices were processed for Fos-like immunoreactivity using normal donkey serum, goat anti-c-fos (1:500; Santa Cruz Biotechnology), and donkey anti-goat Alexa Fluor

488 (excitation=488nm, emission=519nm; Invitrogen) (Reynolds & Berridge, 2008). Sections were mounted, air-dried, and coverslipped with Prolong Gold antifade reagent (Invitrogen). The radius and intensity of plumes of c-Fos positive cells surrounding the microinjection ("Fos plumes") site were mapped as described previously (Pecina & Berridge, 2005; Richard & Berridge, 2011).

Mapping localization of function via Fos plumes: Localization of function within dorsal neostriatum was mapped by assigning behavioral effects produced at each identified site to its corresponding brain atlas location, using color-coded symbols. The size of the symbols was based on the average maximum radius of "Fos plumes" observed surrounding the site of DAMGO microinjections (elevation over vehicle-induced levels). Fos plumes were measured in separate rats after a single microinjection in order to capture maximal spread, and avoid plume shrinkage. That was because a series of repeated microinjections in behaviorally tested animals has been shown to diminish the size of Fos plume induced by a final drug microinjection (Richard & Berridge, 2011; Castro & Berridge, 2014), and an underestimate of Fos plume size could give rise to overly-precise estimates of localization. All information beyond symbol size contained in maps was obtained from behaviorally tested rats (symbol sites and behavioral color intensities).

Moved cue experiment: The 'moved lever' experiment was assessed in separate rats (n=14) after identical autoshaping training to ask whether DAMGO DLS stimulation amplified perseveration of a habitual motor ritual of approach movements toward the old CS+ location, or instead enhanced flexible attraction to the moved cue's new location. Rats were trained as above, receiving 5 days of 25 CS+ UCS pairings per day, and sign-trackers vs goal-trackers were identified on the 5th day. A between-subjects design was used for testing on day 7, so that each rat could encounter the new location cue shift for the very first time under either DAMGO or vehicle. Sign-trackers arbitrarily received either DAMGO or vehicle microinjections in DLS, and immediately placed in the autoshaping chamber. On this day, the CS+Lever was inserted into the box in a new location, on the opposite wall from its accustomed location (Fig. 6B). The old location was left empty on this day. Video recorded approaches and movement sequences toward the old location versus new location were scored. Additionally, a choreography of approach movements and trajectories was scored, both for the last two videotaped training days to the old location, and the test day with the new location to allow identification and comparison of any movement rituals. During the moved cue test, responses to the first 3 presentations of the lever were selected for video analysis in order to capture initial reactions to the shift of CS+Lever to its novel location.

Instrumental conditioned reinforcement testing: Another way to assess whether DAMGO microinjection in DLS enhances motivated 'wanting' for CS+ is to test for enhancement of instrumental conditioned reinforcement, which the willingness to work more to obtain the cue by itself. After 5 days of autoshaping training, identified sign-trackers were microinjected on test day with either DAMGO or vehicle in DLS, immediately before being placed in an operant chamber for a test of instrumental conditioned reinforcement. In this test, the operant chamber contained two novel nose-poke ports, located on either side of a retractable lever port (the lever was kept retracted until earned by nose pokes in the

designated port hole) (Fig. 7). One nose port was randomly assigned as “active”, so that pokes into it produced the CS+Lever (FR1). The other port was inactive, serving merely as a control stimulus option for exploratory responses, and pokes in it were recorded but produced no consequence. Each poke into the active nose port resulted in a 2s presentation of the Pavlovian CS+Lever and its associated tone (no sucrose was ever delivered, and the previous dish was absent from the chamber). Each conditioned reinforcement session lasted 30 minutes.

Statistical Analysis: Within-subject repeated measures analyses of variance (ANOVA) compared drug and vehicle, while anatomical site placement within dorsolateral versus dorsomedial regions of neostriatum, and sign-tracker vs. goal-tracker phenotype were between-subjects variables. Between-subject multivariate and univariate ANOVAs were used to assess the moved cue and conditioned reinforcement tests. All post-hoc t-test presented were corrected for multiple comparisons using the Bonferroni correction. Measures of effect size, where appropriate, are presented as partial eta squared and Cohen's d.

Results

Sign-trackers versus goal-trackers

Sign-trackers were identified as individuals that approached the CS+Lever and nibbled it at least >3 times more than they approached the dish on Day 3, during the 8-sec lever presentations (Meyer *et al.*, 2012). We distinguished two types of sign-trackers. Several DLS sign-tracker individuals never approached the dish even once while the CS+Lever was present on vehicle microinjection test trials, and therefore were considered to be *exclusive* sign-trackers. Other sign-trackers went reliably to the CS+Lever as soon as it appeared, but occasionally defected to the dish while the CS+Lever was still extended: typically these rats went first to the lever, and then went to the dish before the lever was retracted (whereas exclusive sign-trackers waited until the lever was retracted before moving to the dish to obtain sucrose). Conversely, goal-trackers were identified as individuals that approached, sniffed and nibbled the ‘goal’ or metal dish where sucrose pellets were delivered (CS_{dish}) >3 times more than lever, when CS+Lever was present on Day 3, and continued to increase goal tracking over subsequent training days. Finally, rats were classified as intermediate or mixed if they failed to prefer either cue by >3 times than the other cue, and so showed nearly equal numbers of conditioned approaches to both CS+Lever and CS_{dish} while the lever was extended.

DAMGO in dorsolateral neostriatum enhances attraction to a prepotent cue

Bilateral microinjections of DAMGO in dorsolateral neostriatum (DLS; 0.25µg/0.5µL) made all rats become even more intensely attracted to their prepotent cue (CS+Lever for sign-trackers; CS_{dish} for goal-trackers; Figure 1A & 2) All rats approached their prepotent cue faster after DAMGO microinjections in DLS than after vehicle microinjections (omnibus: $F_{(2,12)}=17.87$, $p<0.001$, $\eta^2=0.764$; latency: $F_{(1,12)}=6.84$, $p=.023$, $\eta^2=0.363$; Figure 1A). Once the lever or dish was reached, DAMGO made each rat also display nearly twice as many sniff, grasp and nibble consummatory acts to their prepotent metal CS+ than on control vehicle trials ($F_{(1,12)}=22.3$, $p<.001$, $\eta^2=0.651$; Figure 1A). . Split by phenotype,

for sign-trackers, DAMGO microinjection in DLS selectively sped up approach to the CS+Lever compared to vehicle-control trials in the same rats (omnibus $F_{(2,3)}=4.972$, $p=.12$, $\eta^2=0.768$; $F_{(1,4)}=2.4$, $p=.19$, $\eta^2=0.375$; Figure 1B), without changing approach to the CS_{dish} cue. Once the lever was reached, DAMGO also made sign-trackers emit more consummatory sniffs and nibbles toward the metal lever than on vehicle control trials ($F_{(1,4)}=12.9$, $p=.023$, $\eta^2=0.764$; Figures 1B and 2). For goal trackers, DAMGO microinjections in DLS instead did not change approaches toward the CS+Lever, but instead marginally sped up (decreased latency) goal-trackers' approach to the CS_{dish} (omnibus $F_{(2,6)}=19.37$, $p=0.002$, $\eta^2=0.866$; latency: $F_{(1,7)}=4.64$, $p=.07$, $\eta^2=0.389$; Figure 1c), and increased the number of consummatory sniffs and nibbles on the dish by about 20% over vehicle control levels ($F_{(1,7)}=16.047$, $p=.005$, $\eta^2=0.696$; Figures 1C and 2). By comparison, DAMGO in DLS never enhanced consummatory actions toward the alternative nonpreferred cue for any rat (all actions towards non-preferred cue: $t_{(12)}=0.989$, $p>.05$). Therefore, whichever CS object was already prepotent for an individual rat, opioid stimulation in DLS made that particular prepotent CS become more attractive and more able to elicit ingestive-style nibbles and sniffs.

CS+ as temporal trigger

The temporal pattern of DLS opioid enhancement of cue attraction was always time-locked to the presentations of the CS+Lever, regardless of phenotype: approach was triggered by the lever's appearance through the wall, continued for as long as the lever was present, and declined when it was retracted (Figure 1). Even for goal-trackers, whose CS_{dish} was always physically available, the DAMGO-enhanced peaks in CS_{dish} approach were phasically bound to the presence of CS+Lever, and faded almost immediately after lever disappeared (Figure 1C). We have previously suggested that such temporal patterns reveal the distinction between a CS as *trigger* as versus as *target* for incentive salience attributions under limbic states of pharmacological stimulation (DiFeliceantonio & Berridge, 2012). That is, the reward-predictive CS+Lever was always the temporal *trigger* of enhanced incentive salience pulses for all rats, sign-trackers and goal-trackers alike. The same CS+Lever was also the *target* attributed with incentive salience for sign-trackers, which became their stronger motivational magnet. However, for goal-trackers, their target CS was the reward-proximal CS_{dish}, which became the enhanced motivational magnet, and phasically more 'wanted' for goal trackers during moments of triggering CS+Lever presence. Further supporting this trigger vs target interpretation, even goal-tracker rats under DLS DAMGO never nibbled and sniffed their preferred goal dish more during inter-stimulus intervals when the lever was absent ($t_{(11)}=0.072$, $p>.5$).

Directional selectivity of DLS enhanced approach

A different aspect of the 'motivational magnet' strength of a CS attributed with incentive salience is its ability to reduce attraction to other competing CSs, narrowing the focus of 'wanting' to itself (Mahler & Berridge, 2009; DiFeliceantonio & Berridge, 2012). DAMGO microinjections in DLS enhanced this motivational magnet focus for each rat's prepotent cue (CS+Lever or CS_{dish}): DAMGO in DLS cut in half attraction to the alternative cue (12% vehicle to 6% DAMGO; $t_{(12)}=6.85$, $p=.024$, Cohen's $d=3.95$; cue approach probability

calculated according to Meyer et al., 2011; rats displayed individually in Figure 2), while at the same time enhancing approach to the individual's prepotent cue as described above.

Sites in dorsomedial neostriatum failed to enhance CS attraction

By contrast to DLS, sites in dorsomedial striatum (DMS) for DAMGO microinjections never altered cue approach probability for either sign-trackers or goal-trackers, and did not intensify prepotent attraction nor did it shift rats between the two cues (vehicle= 98.46, DAMGO=95.38; $F_{(3,10)}=2.34$, $p>.1$, n.s.). Similarly, DAMGO at DMS sites failed to alter approach speed, or latency to reach the preferred CS+Lever or CS_{dish} for either group of rats (latency, vehicle= 1.2, DAMGO=1.5, n.s.). Finally, DAMGO microinjections at sites in DMS failed to alter the number of consummatory nibbles and sniffs in sign-trackers (CS+Lever) or goal trackers (CS_{dish}) once a rat reached its prepotent cue (vehicle=6.0, DAMGO= 5.9, n.s.). Consequently, dorsolateral neostriatum sites differed from dorsomedial neostriatum sites for CS+ enhancement effects as an anatomical determinant (Drug*Placement: $F_{(2,22)}=4.85$, $p=.010$, $\eta^2=0.397$). DMS sites failed to enhance cue attraction to Pavlovian conditioned stimuli associated with sucrose reward, despite our previous finding that DAMGO microinjections in DMS potently stimulate eating and intake of unconditioned sweet food itself (DiFeliceantonio *et al.*, 2012).

Unconditioned food intake was not changed by DAMGO in DLS

Finally, DAMGO microinjections in DLS failed to increase the amount of food eaten in the 1-hr free intake test. Under vehicle conditions rats ate 8.25gs of M&Ms and ate 8.48gs after DAMGO microinjection ($t_{(12)}=0.223$, $p>0.5$). The lack of enhancement was likely not due to a ceiling effect as we have previously observed large increases in eating behavior and consumption to over 15gs of M&Ms after DAMGO in the anteromedial dorsal neostriatum (anterior DMS) from similar 8g baselines (DiFeliceantonio *et al.*, 2012). DAMGO microinjection in DLS also did not alter any other behavioral measure collected during the free intake test. Rears (vehicle=127.1, DAMGO= 110.9), cage crosses (vehicle = 59.3, DAMGO= 63.1), and grooming events (vehicle= 7.4, DAMGO= 7.2) did not increase after DAMGO injections ($F_{(3,7)}=1.12$, $p=.402$). A few rats' videos were corrupted and unscorable ($n=3$), resulting in the slightly lower number reported for these measures.

Amphetamine in DLS shifted goal-trackers and sign-trackers toward goal bias (except exclusive sign-trackers)

Amphetamine microinjections in DLS (10 μ g/0.5 μ L) selectively potentiated goal tracking responses in most rats, both in all goal-trackers, and also even in any predominant sign-trackers which had made a single goal-tracking approach to the CS_{dish} while the CS+Lever still remained extended, as is typical of many sign-trackers (omnibus $F_{(2,8)}= 5.273$, $p=0.03$, $\eta^2= 0.569$, Figure 3). We called these rats 'nonexclusive sign-trackers' (6 of 10 predominant sign-trackers) to distinguish them from 'exclusive sign-trackers' that always remained focused on the CS+Lever throughout its entire 8-sec presentations, and never deflected at all to the dish while the still lever remained present (4 of 10 predominant sign-trackers). For both goal-trackers and nonexclusive sign-trackers (Figure 3A, 3B;) amphetamine microinjection in DLS sped up approach specifically to the goal CS_{dish} by 30% (latency to reach dish after amphetamine=3.1 sec, vehicle = 4.5 sec; $F_{(1,9)}=7.83$,

$p=0.048$, $\eta^2=0.367$), while simultaneously also slowing the first approach to the alternative CS+Lever for nonexclusive sign-trackers (latency amphetamine=2.6 sec, vehicle=2.0 sec; $p>0.05$). Once the goal dish was reached by goal-trackers and non-exclusive sign-trackers rats, DLS microinjection of amphetamine also doubled the number of consummatory grasps, sniffs, and nibbles directed toward the metal dish (though sucrose UCS was not yet delivered during the 8-sec CS+Lever presentation; $F_{(1,9)}=5.4$, $p=0.011$, $\eta^2=0.528$). However, amphetamine in DLS never actually reduced nonexclusive sign-trackers' probability of eventual approach to their CS+Lever (although it did slow their approach): probability of sign-tracking still remained at 100% in all predominant sign-trackers after amphetamine (Figure 4). Thus goal-tracking in nonexclusive sign-trackers was enhanced in a noncompetitive fashion by DLS dopamine stimulation, facilitating CS_{dish} approach while slowing approach to CS+Lever, but never actually pulling all triggered behavior toward the CS_{dish} at the expense of the CS+Lever.

By contrast, in 'exclusive sign-trackers' (the 4 of 10 sign-trackers), amphetamine microinjection in DLS did not enhance goal-tracking responses at all. Instead, amphetamine in DLS of exclusive sign-trackers slightly enhanced sign-tracking: increasing by about 10% approaches to the CS+Lever, and similarly increasing nibbles, sniffs, and bites (vehicle=8.65, amphetamine = 9.65, $F_{(1,3)}=8.13$, $p=.065$, $\eta^2=0.730$, Figure 3C). These exclusive sign-trackers never approached the CS_{dish} while the CS+Lever remained physically present after DLS amphetamine microinjections, just as after vehicle microinjections. In other words, for exclusive sign-trackers, the effect of DLS amphetamine appeared similar to that of DLS DAMGO: both neurochemical forms of DLS stimulation selectively enhanced their prepotent (exclusively prepotent for these rats) response of sign-tracking. In sum, dopamine stimulation of DLS selectively enhanced attraction toward a single CS+ in an individualized fashion (Figure 4). The CS+Lever became a stronger motivational magnet for exclusive sign-trackers, but the CS_{dish} became a stronger motivational magnet for goal-trackers (again similar to DLS DAMGO), and for nonexclusive sign-trackers (a CS switching effect different from DLS DAMGO, which had potentiated prepotent sign-tracking responses in all sign-trackers, whether exclusive or nonexclusive).

Mapping localization of CS enhancement in DLS

Anatomical analysis of sites revealed that only the lateral half of the dorsal neostriatum (DLS) contained sites that produced enhancements of cue attraction after DAMGO or amphetamine microinjections as described above (Figure 5).

For mapping of behavioral effects, a microinjection site was classified to be within DLS if >75% of its total Fos plume volume was estimated to be contained inside the lateral half of dorsal neostriatum (Figure 5). In DLS, DAMGO microinjections produced Fos plumes of similar intensity and size, about 0.2 mm in radius. DAMGO Fos plumes each contained a small center of intense 200-400% Fos protein elevation (compared to vehicle microinjections as baseline; 0.13mm radius, volume=0.009mm³), and a larger surrounding sphere of moderate 150-199% elevation above normal Fos levels (0.2mm radius 0.033mm³ volume). These Fos plume sizes were used to make maps in Figure 5, and were similar to those previously reported for DAMGO microinjections in dorsomedial neostriatum and

nucleus accumbens s at the same doses (Pecina & Berridge, 2005; DiFeliceantonio *et al.*, 2012; Castro & Berridge, 2014).

The most effective sites were clustered in DLS (Figure 5). For example, individual enhancements by DAMGO in DLS often doubled or nearly tripled a rat's approaches to its prepotent cue and likewise doubled consummatory nibbles directed toward the metal lever or dish. Effective sites filled most of the anterior-posterior extent of DLS, stretching from the far rostral tip of DLS (approximately +2.5 mm anterior to bregma) to at least two-thirds through the posterior extent of DLS to at least 0 bregma, as far posterior as our sites reached in this study. Dorsoventrally, motivationally enhancing DLS sites extended from the dorsal surface of lateral neostriatum to at least 1 mm below the surface (more ventrally at very lateral placements, though that is partly because the dorsal surface of neostriatum also curves ventrally as it moves laterally). Beyond that ventral point, deeper sites were generally silent in motivational effects, or if anything, slightly suppressive (respectively, white or gray circles in Figure 5). However, mediolaterally, it was clear that virtually all sites in the medial half of dorsal neostriatum failed to enhance attraction to Pavlovian cues. Sites in DMS (i.e., within 2.5 mm of midline) were nearly unanimously silent in enhancing either sign-tracking or goal-tracking, sharply contrasting to the efficacy of sites in DLS in enhancing Pavlovian incentive salience.

Rigid habit or flexible motivation?

Did DLS DAMGO enhance prepotent CS approach as a ritualized or automatic habit of pre-programmed movements? Or by making the cue into a stronger motivational magnet, which was attractive independent of any habitual rituals used to approach? S-R habits often become automatic, and ritualized into predictable or stereotyped sequences of movement (Dickinson & Balleine, 1990; Yin *et al.*, 2004; Belin-Rauscent *et al.*, 2012; Murray *et al.*, 2012; Dolan & Dayan, 2013; Smith & Graybiel, 2013). Consequently, habits can famously persevere even when guiding stimuli are suddenly moved (Carr & Watson, 1908). DLS in particular has been implicated in control of such stereotyped movement sequences, both for instinctive ritualized action patterns and for learned habits, DLS neurons change firing and alter stimulus processing over the course of habit learning as movement patterns become more automatic (Graybiel, 2008; Kalueff *et al.*, 2016; Smith & Graybiel, 2016).

Sign-tracking was focused on here, because it was more feasible to move the CS+Lever to a different slot located on an opposite wall than to move the sucrose dish for goal-trackers. Similar to Carr & Watson (1908), we predicted that if DLS opioid stimulation potentiates an automatic stimulus-response habit or motor ritual, DLS DAMGO microinjection would make rats persist in repeating their pre-established motor ritual, and continue to approach the original location. Conversely, we predicted if DLS opioid stimulation makes sign-trackers motivationally more attracted toward the CS+Lever, then after DAMGO microinjection sign-tracker rats should flexibly abandon their old location and ritual, and follow the lever to a new location, even if the switch required a change in movement pattern.

We observed that rats did tend to each develop a particular ritualized approach pattern, a potential habit that was shared by virtually all sign-trackers, and was quite evident by the final day of training (Figure 6A). This sign-tracking ritual followed a sequence that used

predictable movements: 1) Before the lever emerged, a rat typically sat within 3 cm of the goal dish (typically, the rat had just obtained a sucrose pellet from the dish on a previous presentation); 2) when the CS+Lever appeared in the chamber about 5 cm away from the rat, the rat first typically turned 30-90 degrees toward the CS+Lever; 3) next, the rat took an initial small-stride forelimb step toward the CS+Lever (<2 cm stride), followed immediately by 4) a corresponding small hind step pulling up the hind leg toward the shifted forequarters (<2 cm step), and then 5) repeated alternating series of small #3 forelimb & #4 hind limb steps until the head was within 1 cm of the CS+Lever) from that lever-proximal position, 6) the rat merely leaned its head forward without moving the legs to make mouth (lip and tongue) contact with the CS+Lever, often accompanied by a forelimb grasp of the lever), and finally initiated 7) repetitive consummatory sniffing and nibbling sequences on the metal lever as long as it remained present (8-sec duration; Figure 6A).

Microinjections of either DAMGO or vehicle were made bilaterally in DLS of sign-trackers (between-subject comparison; randomly assigned) immediately before the location of the CS +Lever was shifted to the opposite side of the cage (a previously empty slot in the opposite wall). In the crucial test, we focused on the first three CS+Lever presentations in new location after DLS microinjections, when the CS+Lever now emerged on the opposite wall from where it had previously been.

Results showed that rats uniformly abandoned their potentially habitual pattern of approach on the very first presentation of the newly-located CS+Lever after DAMGO (or vehicle) microinjections in DLS. Instead of returning to the old location after DAMGO microinjection, all sign-trackers went directly to the new location that was in the opposite direction from before, and further away. Opioid stimulation of DLS did not cause rats to perseverate in visits to the old location: instead, rats actually visited their old location fewer times than rats that received vehicle microinjections (DAMGO=0.68±0.3 visits to old location per session; vehicle = 0.78±0.6; $p>0.05$, Figure 6B and 6E). Similarly, if anything DLS DAMGO rats tended to reach the new cue location slightly faster than rats that received vehicle microinjections (though this was only a trend, and speed did not quite reach statistical significance; DAMGO latency=4.2 sec; vehicle latency = 5.13, $p>0.05$, Figure 6B and 6D).

Further, in terms of movement microstructure, rats changed their ritualized movement pattern after DLS DAMGO. In the new movement pattern, rats typically 1) turned 90 – 180 degrees in opposite direction away from their previous direction of turn 2) took a longer initial forelimb step than previously (toward the >5 cm more distant location), 3) and took a correspondingly longer initial hindlimb step (>5 cm), and then 4) repeated steps # 2 and #3 several more times until reaching the new location (Figure 6B). Finally, once the CS+Lever was reached in its new location after DAMGO in DLS, rats trended toward emitting more consummatory nibbles on the metal lever than control vehicle rats (Vehicle = 2.79±1.2, DAMGO = 3.85±0.96, $p>0.05$; Figure 6B and C). In short, DAMGO did not cause rats to perseverate to the old location, suggesting it did not strengthen a ritualized motor habit. Instead, they shifted flexibly, abandoning their old movement ritual with greater alacrity, adopting a new and different movement pattern, and directing more ingestive-consummatory

actions toward the metal object in its new location – all consistent with the idea that DLS DAMGO had strengthened the CS+Lever as an attractive motivational magnet.

DLS opioid stimulation enhances motivation to earn CS+Lever on new instrumental task (conditioned reinforcer)

An independent way of assessing whether DLS stimulation enhances ‘wanting’, is to test for a different feature of incentive salience: instrumental conditioned reinforcement (Berridge & Robinson, 2003). Our conditioned reinforcement results showed that DAMGO microinjection in DLS did indeed make sign-trackers ‘want’ to obtain the CS+Lever cue more, in the sense of working harder on a new nose-poke instrumental task to earn brief presentations of the CS+Lever. Rats that received DAMGO in DLS worked 150% harder in their very first session on the newly-learned nose-poke task to earn presentations of the Pavlovian CS+Lever, compared to control rats that received vehicle microinjections (38 ± 7.5 vehicle; 57.1 ± 5.54 DAMGO; $F_{(1,19)} = 4.6$, $p = .045$, $\eta^2 = 0.195$). DLS DAMGO rats poked specifically in the ‘active’ porthole that earned the CS+Lever, and did not poke in the control inactive hole (inactive pokes $F_{(1,19)} = 0$, $p = .992$; nose poke type*drug interaction $F_{(1,38)} = 4.229$, $p = .047$, $\eta^2 = 0.1$; Figure 7).

By contrast to DLS enhancement of conditioned reinforcement, DAMGO at sites in the dorsomedial neostriatum failed make rats perform more nose-pokes to earn CS+Lever presentations compared to vehicle ($F_{(2,6)} = 0.675$, $p = .544$). DAMGO microinjections in DMS had no effect on number of responses for either the active hole or inactive hole (CS+Lever hole: vehicle = 53.87, DAMGO = 56.37, vehicle = 33.12, DAMGO = 25.37 $F_{(1,7)} = 0.071$, $p = 0.789$; control hole $F_{(1,7)} = 0.905$, $p = 0.375$). This pattern seemed to confirm that DLS, but not DMS, supports opioid enhancement of incentive salience, at least for a Pavlovian CS+ that predicts sucrose reward.

Discussion

Our results suggest that dorsolateral neostriatum (DLS) contributes to amplifying incentive motivation triggered by Pavlovian reward cues, beyond its traditional role as ‘motor striatum’ chiefly involved in the regulation of movement, habits, and sequences. Here, local mu opioid or dopamine stimulation in DLS selectively magnified the motivational attractiveness of one particular reward-cue for each rat, interacting with its pre-existing sign-tracking vs. goal-tracking individual phenotype. Our results indicate that opioid or dopamine stimulation in DLS can specifically enhance Pavlovian incentive salience in a focused manner for a single target CS.

Cue enhancements by mu opioid vs dopamine stimulation in DLS

In virtually all sign-trackers, mu opioid stimulation in DLS via DAMGO microinjection magnified attraction specifically toward their prepotent CS+Lever (or ‘sign’ predicting reward UCS), producing faster approach, higher probability of approach, and more consummatory sniff-nibble reactions to the metal lever cue once reached. By contrast, in goal-trackers DLS DAMGO instead magnified attraction and consummatory reactions toward their prepotent CS_{dish} (the ‘goal’). Thus, in both goal-trackers and sign-trackers,

DAMGO enhanced attraction to the individual's prepotent Pavlovian cue, and simultaneously pulled attraction away from the other competing cue. Also, for both goal-trackers and sign-trackers, the temporal trigger for enhanced cue attraction was always presentation of the CS+Lever. Even for goal-trackers, the CS+Lever triggered phasic increases in attraction to the CS_{dish}, even though the dish always remained physically present while the lever was absent.

Dopamine stimulation in DLS by amphetamine microinjection also enhanced attraction toward one particular cue, but with a stronger goal-tracking bias towards the CS_{dish} in a larger population of rats. This bias was strong enough to actually switch the maximally attractive target for some sign-trackers, from lever to dish, as well as enhance dish attraction for all goal-trackers. That is, any sign-tracker individuals that were not entirely exclusive in CS+Lever attraction under vehicle (meaning that they defected to the dish before the CS+Lever was retracted on vehicle test day) switched targets when tested under DLS amphetamine. Only exclusive sign-trackers, which never defected to the dish while CS+Lever was present on vehicle trials, instead showed enhancement of prepotent sign-tracking responses toward the lever after amphetamine microinjection in DLS (i.e., similar to the effect in all sign-trackers of DLS DAMGO microinjection). Once again, regardless of which cue was the target, the dopamine-induced enhancement of attraction was always temporally bound to moments when the triggering CS+Lever was physically present, and never occurred during intervening no-lever periods (even when the CS_{dish} was target, which remained always available). Finally, for both dopamine and opioid stimulation of DLS, enhanced CS attraction was accompanied by an increase in consummatory nibbles and sniffs directed toward the metal target cue once it was reached.

Motivational nature of sign-tracking versus goal-tracking

Sign-tracking has been suggested specifically to reflect incentive salience attributed to the predictive CS+Lever (Robinson *et al.*, 2014b). Goal-tracking, by contrast, could in principle be mediated by other psychological processes: for example a) cognitive act-outcome expectations that sucrose would be obtained in the dish, and b) automatic stimulus-response habits of approach movements that were previously elicited during CSUCS pairings. Accordingly, Robinson, Flagel and colleagues have reported that normal sign-tracking is more closely associated with endogenous dopamine responses in nucleus accumbens than normal goal-tracking (Flagel *et al.*, 2011; Saunders & Robinson, 2012; Robinson *et al.*, 2014b). They suggest that goal-tracking is instead mediated predominantly by the alternative psychological mechanisms in normal rats.

It may be true that goal-tracking under normal conditions is less purely mediated by incentive salience than sign-tracking. However, states of mesocorticolimbic stimulation, induced by DAMGO microinjections in structures such as central amygdala or induced after some cases of amphetamine sensitization, can enhance goal-tracking as a motivated response by amplifying incentive salience, which becomes targeted on the CS_{dish} (Mahler & Berridge, 2009; Simon *et al.*, 2009; Holden & Peoples, 2010; DiFeliceantonio & Berridge, 2012; Robinson *et al.*, 2015a). In those stimulated mesocorticolimbic states, the CS_{dish} can become a more 'wanted' motivational magnet for goal-trackers, similar to the CS+ Lever for sign-

trackers. In both cases, the trigger for the phasic pulse of higher incentive salience remains presentation of the CS+Lever.

Even though the CS_{dish} is less predictively correlated than the CS+Lever to sucrose, a rat typically experiences CS_{dish} as a contiguous cue immediately prior to sugar consumption. Early animal learning studies indicated that contiguous CSs may also often elicit motivated responses, similar to predictive CSs (Zener, 1937; Rescorla & Cunningham, 1979; Delamater & Holland, 2008). Pavlov emphasized the CS-UCS closeness factor in his 'law of temporal contiguity,' and subsequently both contiguity and correlation have been viewed as important in Pavlovian learning, each emphasized to varying degrees by different investigators (Pavlov, 1927; Mackintosh, 1974; LoLordo, 1979; Gallistel & Balsam, 2014; Rescorla, 1972). The idea that goal-tracker individuals can become highly motivated under at least some conditions also may be compatible with the recent report that goal-trackers can actually show higher relapse in cocaine seeking than sign-trackers when triggered by contextual cues (rather than by a discrete Pavlovian CS) (Saunders *et al.*, 2014).

Our results indicate that opioid/dopamine stimulation in DLS can enhance intensification of motivated goal-tracking as well as sign-tracking, similarly to some mesocorticolimbic stimulations. In particular, our pattern of enhanced prepotent cue attraction observed after DAMGO microinjections in DLS was nearly identical to that previously reported for DAMGO microinjections in the central nucleus of amygdala (Mahler and Berridge, 2009; DiFeliceantonio and Berridge, 2012). That similarity raises the possibility that DAMGO microinjection in DLS and central amygdala might activate different nodes of the same functional circuit, resulting in similarly enhanced Pavlovian motivation. This possibility also seems consistent with related evidence for functional interaction between DLS and central amygdala, from studies of surprise-induced attention and conditioned orienting (Han *et al.*, 1997; Esber *et al.*, 2015).

Phasic nature of DLS enhancements of Pavlovian attraction: CS+Lever as trigger

Why was the enhancement of incentive salience tied, phasically, to presentations of the CS +Lever, when DAMGO or amphetamine activation of DLS would have remained relatively constant throughout the entire session? For incentive salience enhancements, the pharmacological DLS stimulation would have acted as a physiological state (kappa) (Zhang *et al.*, 2009), similar to an appetite or satiety state (Robinson and Berridge, 2013). Even though a pharmacological/physiological kappa state may last many minutes or hours, its most potent effects on motivated behaviors are much shorter and cue-linked, because incentive salience generation involves an interaction of such neurobiological kappa states with phasic encounters of Pavlovian reward cues. Thus DAMGO or amphetamine microinjection in DLS specifically and multiplicatively amplified the CS+Lever cue's ability to trigger a phasic surge in incentive motivation. The temporal trigger remained the same regardless of whether the amplified incentive salience focused on the CS+Lever or CS_{dish} as target. By contrast, when the lever was retracted, the multiplicative interaction had no triggering CS+Lever association to amplify, which is why DLS stimulation alone would not directly drive an increase in motivated approach (not even to dish) when the lever was missing (Mahler and Berridge, 2009; DiFeliceantonio and Berridge, 2012).

DLS stimulation: stronger motor habit or amplified incentive salience?

DLS is often posited to contribute to stimulus-response habits (Packard & Knowlton, 2002b; Aldridge *et al.*, 2004; Balleine *et al.*, 2007; Tang *et al.*, 2007; Yin, 2010; Bornstein & Daw, 2011; Everitt & Robbins, 2013; Smith & Graybiel, 2013). So can DLS-induced strengthening of a habit explain the behaviors observed here? An answer to this question is made more difficult by the observation that there actually is no widespread agreement in neuroscience on the defining positive features of a habit. Indeed, habits are often described more in terms of features lacked (i.e., not goal-directed; not flexible; not motivated; etc.) rather than in terms of positive features that allow the habit to be recognized as such.

Automaticity was a useful defining feature of habit in classical and behaviorist psychology of a century ago (James, 1890; Carr & Watson, 1908; Guthrie, 1935). The defining feature of a habit was its automatic stereotypy of a sequential pattern: a ritual that could be performed without attention, indeed being especially liable to surface when attention was distracted, and which always appeared in the same, invariable, behavioral sequence. Automaticity and sequential stereotypy is also emphasized by some modern neuroscientists who study DLS mediation of movement and action rituals (Graybiel, 2008; Kalueff *et al.*, 2016). For example, Graybiel (2008) suggested “habits are sequential, repetitive, motor, or cognitive behaviors elicited by external or internal triggers that, once released, can go to completion without constant conscious oversight.” (p. 361).

Automaticity and ritualized sequential stereotypy was the particular feature exploited by Carr and Watson (1908) to expose well-learned habits in their classic ‘kerplunk!’ experiment, and was also the feature used by our moved cue experiment. In our study, after opioid DLS stimulation, sign-trackers did not rigidly perseverate in their habitual movement sequence to the old location when the CS+Lever was suddenly moved, but instead switched immediately to the new location and adopted a new movement pattern to go there. Further, amphetamine microinjection in DLS actually switched nonexclusive sign-trackers from their characteristic sign-tracking response to instead mostly goal-tracking while the lever was present: altering their most preferred target stimulus as well as their previous movement sequence.

Alternatively, a habit may also be motivated – in which case it is no longer merely a habit. Adding motivational compulsion in the form of amplified incentive salience of attractive stimuli could make their approach more intense and difficult to control. This may be similar to the proposal by Everitt, Robbins, and colleagues that DLS can endow addictive habits with a compulsive ‘must do!’ feature to persist in the face of punishment (Belin *et al.*, 2009; Everitt & Robbins, 2016). We suggest compulsion necessarily adds a motivational process: only motivation can give a ‘must!’ feature to a habit which otherwise would be merely ‘done’ automatically as a routine. Our suggestion is similar to a distinction made between mere automatic rituals vs. compulsively motivated obsessions in OCD (Boyer & Lienard, 2008).

Our results may be the first to demonstrate that DLS circuitry is actually capable of intensifying motivated attraction to a learned target stimulus, with several signature features of incentive salience. First, the increased attraction was flexible, easily adjusting when the

target moved. Next, DAMGO and amphetamine in DLS also amplified the targeted cue's ability to elicit ingestive-type consummatory actions, so the rat more intensely sniffed and nibbled the cue as though the metal CS object was the sucrose UCS itself. Further, the target for maximal incentive salience flexibly switched to a different stimulus in the case of amphetamine microinjection in DLS that made nonexclusive sign-trackers become stronger goal-trackers. A switch from lever to dish as target is more evidence against the habit explanation, demonstrating flexibility in attractive stimulus as well as in motor approach. Finally, DLS-enhancement of CS 'wanting' was evident even in the absence of any physical target stimulus: DAMGO microinjection in DLS of sign-trackers enhanced instrumental conditioned reinforcement, magnifying effort on a newly-learned response to earn brief presentations of their absent CS+Lever. Although conditioned reinforcement can be alternatively explained by other processes (e.g., response 'stamping in' or related reinforcement processes), being more willing to work to gain a 'wanted' cue is another feature predicted by enhanced incentive salience that involves no possible habit of stimulus-response approach (Robinson and Berridge, 1993). Overall, we interpret this pattern of observations to indicate that DLS opioid/dopamine stimulation, interacting with individualized phenotypes, made a targeted CS become more motivationally 'wanted', more attractive yet flexibly approached, more avidly nibbled as though it were sucrose when reached, and more highly sought when missing.

Opioid versus dopamine roles in DLS

Opioid and dopamine stimulations in DLS enhanced CS incentive salience in ways that were related, but not identical. To understand the difference between motivation effects of opioid versus dopamine stimulation in DLS, it may be relevant to note that mu opioid receptors are expressed post-synaptically on medium spiny neurons primarily in patches or striosomes in DLS and other neostriatum regions, and have been suggested to tune neuronal responses to dopamine and other neuromodulators (Pert *et al.*, 1976; Herkenham & Pert, 1980; Gerfen, 1984; Banghart *et al.*, 2015), whereas dopamine receptors occur on neurons in the matrix compartment as well as in patches/striosomes (Besson *et al.*, 1988; Graybiel, 1990; Crittenden & Graybiel, 2011). Patch neurons in DLS also receive inputs primarily from limbic regions of prefrontal cortex, including prelimbic and anterior cingulate regions, whereas matrix neurons receive inputs predominantly from motor cortex and somatosensory cortex (Ragsdale & Graybiel, 1990; Eblen & Graybiel, 1995; Kincaid & Wilson, 1996; Levesque & Parent, 1998; Haber *et al.*, 2006). Regarding output targets, patch neurons project more directly to midbrain dopamine neurons in substantia nigra pars compacta, whereas matrix neurons project to the pars reticulata (Crittenden & Graybiel, 2011; Fujiyama *et al.*, 2011). Therefore, one possibility is that mu opioid receptor stimulation by DAMGO microinjection in DLS altered neuronal function in patches more particularly than in matrix, and in turn preferentially modulated direct nigrostriatal projections. By contrast, endogenous local dopamine release from amphetamine microinjections in DLS would more equally modulate patch and matrix neurons together, as well as impact presynaptic terminals of ascending dopamine neurons and of corticolimbic glutamate neurons that also express dopamine receptors (Gerfen, 1984; Packard & Knowlton, 2002a; Crittenden & Graybiel, 2011). Finally, our DLS amphetamine enhancement of approach biased toward goal-tracking (at least, in all but exclusive sign-trackers) seems conceptually similar to effects reported for

systemic amphetamine administration or for some cases of amphetamine-induced sensitization that also enhanced goal-tracking (Simon *et al.*, 2009; Holden & Peoples, 2010), potentially indicating a shared dopamine bias toward goal-tracking (although see also (Doremus-Fitzwater & Spear, 2011; Robinson *et al.*, 2015a) for sign-tracking enhancements by dopamine or by amphetamine-induced sensitization).

Anatomical localization and neurochemical mechanisms of motivation amplification

Neuroanatomically, only sites located in the lateral half of the most dorsal 25% of neostriatum supported opioid/dopamine enhancements of cue attraction here (i.e., sites in DLS). By contrast, all sites in the medial half of dorsal neostriatum failed to enhance cue attraction (i.e., sites in DMS). Within DLS, both anterior and posterior sites appeared equally effective at enhancing Pavlovian incentive salience for sign-trackers and goal-trackers. Our finding that DMS sites failed to enhance Pavlovian cue attraction to a target CS contrasts with our previous report that that DAMGO microinjections in the anterior DMS powerfully increased intake of palatable food as UCS (anterior DMS surges of endogenous enkephalin also were triggered by eating a palatable sweet food UCS in that study) (DiFeliceantonio *et al.*, 2012). Here, DLS opioid stimulation did not promote food UCS consumption. Thus, DLS enhancements of incentive salience of a Pavlovian CS seem distinct from DMS enhancement of motivation to consume a food UCS. This double dissociation between DLS enhancement of cue-triggered incentive motivation directed towards learned Pavlovian CSs, versus anterior DMS enhancement of UCS-targeted motivation directed towards unconditioned foods may also be consistent with reports that DMS lesions impair goal-directed behavior guided by cognitive memories of food UCS value, operating in act-outcome representations to guide actions (Yin *et al.*, 2005). Conversely, a DLS role in Pavlovian motivation is consistent with the disruption of cue-triggered ‘wanting’ to obtain reward after DLS lesions in Pavlovian Instrumental Transfer (PIT) tests (Corbit & Janak, 2007). DLS lesions also disrupt other cue-triggered reactions, some of which might involve motivational components, as well as disrupting other complex sequences of instinctive action (Yin *et al.*, 2006; Belin & Everitt, 2008; Murray *et al.*, 2012; Lucantonio *et al.*, 2014; Kalueff *et al.*, 2016).

Conclusion: DLS modulates cue-triggered incentive motivation

Within DLS, local mu opioid or dopamine stimulation specifically enhanced attraction toward one particular Pavlovian reward cue in an individually tailored fashion. This is consistent with a ‘winner-take-all’ enhancement of that cue’s incentive salience, making it specifically more highly ‘wanted’ and attractive, at the expense of the competing reward cue. A motivational role for DLS in enhancing Pavlovian incentive salience seems consistent with other reports of motivation effects caused by dorsal neostriatum manipulations including optogenetic self-stimulation of dorsal neostriatal D1-expressing neurons (Kravitz *et al.*, 2012) and rescue of eating behavior in aphagic dopamine-deficient mice by dorsal neostriatal dopamine replacement (Palmiter, 2008). A role for DLS in cue-triggered incentive motivation is also consistent with many reports of dorsal neostriatal activity increases evoked by reward cues in both animals and humans (Ito *et al.*, 2002; Volkow *et al.*, 2002; Stice *et al.*, 2008; Nummenmaa *et al.*, 2012; Jastreboff *et al.*, 2013; Li *et al.*, 2015). Applied to addiction, an incentive salience role for DLS circuitry in making a particular

reward CS+ more attractive would increase the danger of cue-triggered addictive relapse, especially when craving, pursuit, and consumption were pulled toward a targeted CS as a suddenly more powerful motivational magnet.

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Abbreviations

AMPH	amphetamine
ANOVA	analysis of variance
CS	conditioned stimulus
DAMGO	[D-Ala ² , N-MePhe ⁴ , Gly-ol]-enkephalin
DLS	dorsolateral neostriatum
DMS	dorsomedial neostriatum
UCS	unconditioned stimulus

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Figure 1. Dorsolateral neostriatal DAMGO enhances prepotent attraction

A. Dorsolateral DAMGO microinjection amplifies attraction toward, and narrows the focus on, an individual's prepotent cue. B. Sign-trackers show stronger and more selective appetitive approach and consummatory nibble/sniff behaviors targeted at the CS+Lever. C goal-trackers show stronger CS+-triggered attraction toward the CS_{Dish}. Background indicates 8-sec periods when the CS+Lever was physically present; white backgrounds indicate baseline periods before and after CS+; * indicates $p < 0.05$. Pie-graph centers show the total number, approach, and consummatory behaviors during those periods, and the

proportions indicate the ratio of responses directed toward individual's pre-diagnosed prepotent cue versus the alternative cue.

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Figure 2. DLS opioid stimulation enhances CS motivational magnet in an individually-tailored fashion

Effects of DAMGO microinjection in DLS enhances CS+Lever attraction for sign-tracker individuals but enhances CSdish attraction for goal-tracker individuals. Every individual rat is represented by two dots: a solid dot in its baseline vehicle condition and a connected striped dot in DAMGO condition. Sign-trackers are shown as diamonds, and goal-trackers are shown as circles. Vertical axis indicates sign-tracking approaches and nibbles toward CS+Lever (sign). Horizontal axis indicates goal-tracking approaches and nibbles toward CS_{dish} (goal) during CS+Lever presentations. DAMGO always intensifies the motivational attraction toward an individual's own pre-existing most attractive CS.

Figure 3. DLS dopamine stimulation increases CS_{dish} responses in mixed sign-trackers and goal-trackers but not exclusive sign-trackers

Dorsolateral amphetamine microinjection amplifies attraction toward the CS_{dish}. A. Mixed sign-trackers show relative unchanged responding on the CS+Lever, but an enhanced proportion of responses on the CS_{dish}. B. Goal-trackers nibble and sniff the CS_{dish} more after amphetamine microinjection. C. Exclusive sign-trackers continue to show no defections to the CS_{dish} and nibble and sniff their CS+Lever more after amphetamine microinjection. * indicates $p < 0.05$. Pie-graph centers show the total number, approach, and consummatory

behaviors during those periods, and the proportions indicate the ratio of responses directed toward individual's pre-diagnosed prepotent cue versus the alternative cue.

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Figure 4. DLS dopamine stimulation individually biases attraction toward CS_{dish}
Every individual rat is represented by two dots: a solid dot in its baseline vehicle condition and a connected striped dot in amphetamine condition. Amphetamine microinjection into the dorsolateral neostriatum enhanced attraction toward CS_{dish} (goal) in predominant goal-trackers (circles) and even in predominant but nonexclusive sign-trackers that ever deflected to CS_{dish} during CS+Lever presentation under vehicle condition (squares). However, in exclusive sign-trackers (diamonds), which never deflected to dish under vehicle, amphetamine in DLS instead enhanced prepotent attraction toward CS+Lever.

Figure 5. Localization of function for CS motivation enhancements in dorsolateral neostriatum
The behavioral effects of each microinjection site are represented in 3 anatomical planes. The size of the symbol represents the spread of maximal drug impact, as measured by Fos plume diameters (shown at bottom center). The color of each symbol represents the intensity of behavioral effects on CS motivational attraction at that site, as described in text. The ventral distance from top was measured from the dorsal most portion of striatum directly above each site, thus showing position in dorsal level of neostriatum. Dorsolateral sites of neostriatum shown in purple effectively enhanced cue-triggered incentive salience of CS +Lever or CS_{dish} (depending on individual phenotype, as described in results). By comparison, dorsomedial neostriatum sites failed to enhance Pavlovian attraction (white or gray).

Figure 6. DLS DAMGO enhances flexible following of a moved CS+

A. On training days prior to shift of CS+Lever position, sign-trackers developed a ritualized and predictable pattern of approach movements toward the original position. B. DAMGO or vehicle microinjection in DLS was given immediately prior to shift in the lever location to opposite side of chamber. Bar graph shows that DAMGO in DLS facilitated the shift to the new location: if anything DLS opioid stimulation reduced approach to the old location, and enhanced speed of approach to the new lever location. DLS DAMGO also increased consummatory nibbles on the CS+Lever in its new location. C-E. Here, nibbles and sniffs on

the new lever, latency to the new lever, and nibbles and nsiffs on the old lever location are shown individually for each cue (1-3) under vehicle (solid) and DAMGO (dashed).

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Figure 7. DLS opioid stimulation enhances ‘wanting’ to earn CS+: Instrumental conditioned reinforcement

DAMGO microinjections in DLS enhanced acquisition and performance of a new instrumental nosepoke response, which earned brief presentations of the CS+Lever as an instrumental conditioned reinforcer (in absence of any sucrose UCS). DAMGO (dashed line) in DLS increased nose pokes specifically into the hole that earned CS+Lever presentations (compared to vehicle control performance in the same rats) but not into the control hole that earned nothing.