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Research report

Which cue to 'want'? Opioid stimulation of central amygdala makes goal-trackers show stronger goal-tracking, just as sign-trackers show stronger sign-tracking

Alexandra G. DiFeliceantonio*, Kent C. Berridge

Department of Psychology, University of Michigan, Ann Arbor, MI 48109, USA

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ABSTRACT

Pavlovian cues that have been paired with reward can gain incentive salience. Drug addicts find drug cues motivationally attractive and binge eaters are attracted by food cues. But the level of incentive salience elicited by a cue re-encounter still varies across time and brain states. In an animal model, cues become attractive and 'wanted' in an 'autoshaping' paradigm, where different targets of incentive salience emerge for different individuals. Some individuals (sign-trackers) find a predictive discrete cue attractive while others find a reward contiguous goal cue more attractive (location where reward arrives: goal-trackers). Here we assessed whether central amygdala mu opioid receptor stimulation enhances the phasic incentive salience of the goal-cue for goal-trackers during moments of predictive cue presence (expressed in both approach and consummatory behaviors to goal cue), just as it enhances the attractiveness of the predictive cue target for sign-trackers. Using detailed video analysis we measured the approaches, nibbles, sniffs, and bites directed at their preferred target for both sign-trackers and goal-trackers. We report that DAMGO microinjections in central amygdala made goal-trackers, like sign-trackers, show phasic increases in appetitive nibbles and sniffs directed at the goal-cue expressed selectively whenever the predictive cue was present. This indicates enhancement of incentive salience attributed by both goal trackers and sign-trackers, but attributed in different directions: each to their own target cue. For both phenotypes, amygdala opioid stimulation makes the individual's prepotent cue into a stronger motivational magnet at phasic moments triggered by a CS that predicts the reward UCS.

1. Introduction

Reward cues (Pavlovian CSs) can carry incentive salience: eliciting craving for the reward, and making the cues themselves 'wanted', approached and even the target of consummatory acts such as ingestive licks, nibbles, and bites that normally belong to an associated food reward (UCS). Thus food cues can tempt a binge-eater to overindulge or drug cues can trigger relapse in a drug addict [1–4], and such cues can attract appetitive-consummatory behaviors acting as 'motivational magnets' [5–8].

However, reward cues are not always attractive, but rather vary across time in motivation potency. A cue's power to trigger temptation fluctuates especially when encountered in different physiological-brain states (e.g., drug intoxication, stress, hunger, satiety) [9–11]. Particular activations in mesocorticolimbic brain states, we will suggest, are why particular cue encounters may make addicts relapse into excessive consumption even after the same cue has been successfully resisted many times before [10–13]. Some brain activations may also focus 'wanting' more narrowly onto a single target, as well as elevating intensity [19].

Useful individual differences in the target of incentive salience have been found in autoshaping or "sign-tracking" experiments in rats [14,15], which model the 'motivational magnet' feature of incentive salience for Pavlovian cues. In one version of autoshaping, phasic presentation of a lever CS (CS+ Lever; sometimes called the sign) always predicts a reward UCS: a sucrose pellet delivered to a dish (CS_{dish}; sometimes called the goal). After learning the Pavlovian CS–UCS association, many individual rodents, fish, pigeons, dogs, and people come to approach and bite the discrete CS+ sign and are known as "sign-trackers" [3,8,16–19]. By contrast, other individuals come to approach the goal location where reward is delivered (CS_{dish}) during the CS+ sign presentation and are known as "goal-trackers" [5,16,19]. Goal-tracking vs. sign-tracking differences emerge in the first few days of Pavlovian training in rats, and remain stable [9,20,21].

This difference in individual phenotype is related to underlying mesolimbic brain traits, but can also be experientially biased by environmental situations such as encountering uncertainty in CS–UCS contingencies, receiving reward UCS directly without needing to approach a goal, receiving amphetamine or related

* Corresponding author at: Department of Psychology, University of Michigan, 530 Church Street, 4027 East Hall, Ann Arbor, MI 48109-1043, USA.
Tel.: +1 734 764 8078; fax: +1 734 763 7480.
E-mail address: agdife@umich.edu (A.G. DiFeliceantonio).

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drugs, or having been previously sensitized by drugs administered weeks earlier [16,22–28]. Some similarity in underlying mechanisms might be recruited in both sign-trackers and goal-trackers to attribute incentive salience to individualized targets, at least when mesocorticolimbic brain systems are in a stimulated state. Stimulation of mu opioid circuits in the central nucleus of the amygdala (CeA) was indicated to achieve that mesocorticolimbic state by an earlier study in our laboratory: producing elevation of incentive salience in both sign trackers and goal trackers, and simultaneously focusing that intense incentive salience onto a single Pavlovian target [21].

CeA has distinct inputs and outputs that distinguish it from other nuclei in amygdala [29,30]. Anatomically, the CeA can be viewed as a serial output nucleus for basolateral amygdala (BLA) [31], as a serial starting point for the extended amygdala complex [32–34], or as a striatal-level structure within macrocircuits that organize cortico-striatal-pallidal networks to generate motivated behavior [35]. Functionally, CeA is a site where mu opioid stimulation via DAMGO microinjection can markedly increase motivation to seek and consume palatable food rewards, and CeA interacts with nucleus accumbens in opioid enhancements of food intake [36–39]. CeA also plays special roles in translating learned Pavlovian information into active motivation [40–45]. For example, stimulation of mu opioid receptors in the central nucleus of the amygdala (CeA) magnifies the ability of cues to trigger incentive motivation toward sucrose or sex incentives, and to act as CS motivational magnets [21,46].

Here we explore further the idea that in autoshaping the two Pavlovian CSs (sign and goal) have potentially distinct roles: acting as (1) the *trigger* to elicit a phasic pulse of intense incentive salience, vs. as (2) the *target* of focused incentive salience attribution (that becomes the most 'wanted' Pavlovian object of desire). That is, CeA opioid stimulation may make sign-trackers 'want' the CS+ Lever more, and similarly make goal-trackers 'want' the CS_{dish} more, each in a phasic pulse when triggered by CS+ encounter [21]. We hypothesize that the CS+ acts as the *trigger* in both sign-trackers and goal-trackers to evoke a temporary surge in the intensity of CeA-amplified incentive salience, which lasts seconds. However, the *target* CS that is attributed with focused incentive salience differs between sign-trackers and goal-trackers during a state of CeA opioid stimulation. For sign-trackers, the target is the same trigger or CS+ Lever that predicts sucrose. By contrast, for goal-trackers the target is the CS_{dish} object/location where the UCS is delivered. Finally, we hypothesize that the breadth of focus for incentive salience attribution on the individualized target is also narrowed by CeA stimulation in a winner-take-all fashion. That is, individualized Pavlovian information-to-motivation links are amplified to make the most 'wanted' target even more intensely attractive after CeA opioid stimulation, while alternative targets may even decline in relative attractiveness.

However, a potential problem for our hypothesis is that goal-trackers may essentially lack incentive salience, as only sign-trackers appear to show high cue-triggered 'wanting' [8,47]. Sign-trackers have been suggested to model addiction-like features of incentive salience much more than goal-trackers [14,15,48,49], whereas goal-trackers might approach their dish using non-'wanting' mechanisms, such as cognitive expectancy mechanisms or via simpler S–R habit mechanisms [9,48]. A potential reconciliation between such evidence and our hypothesis might be achieved if it could be shown that specific mesocorticolimbic states (e.g., CeA opioid stimulation) produce the higher intensities and sharper focus of incentive salience in goal-trackers. Specifically, our hypothesis is that CeA stimulated states cause goal-trackers to show pulses of high incentive salience that are equal in intensity to sign-trackers, though focused on a different target: the dish.

To test this hypothesis, it is necessary that goal-trackers in a state of mesocorticolimbic activation show the full cue-triggered sequence of motivated appetitive-consummatory behaviors that characterizes a 'motivational magnet.' For a sucrose pellet UCS, these are sequences of approach, nibble, sniff, grasps and bite behaviors directed to the metal object (CS_{dish} or CS+ Lever). That sequence was not completely confirmed for goal-trackers in the earlier Mahler and Berridge study because the opaque metal wall of the goal dish precluded a clear camera view of actions inside, so that it was not possible to observe a goal-tracker's mouth performing nibble, sniff and bite behaviors in the dish [21].

Here we aimed to more stringently test whether CeA stimulation enhances incentive salience using an additional close-up camera focused on the inside surface of the dish. This measured the full appetitive-consummatory sequences of approach, nibbles, sniffs, grasps and bites of the CS_{dish} in goal-trackers. We also aimed to more closely examine the winner-take-all aspect of narrower focusing on a single target induced by CeA DAMGO, in individuals that show nearly balanced mixtures of goal-tracking vs. sign-tracking, as well as in the more extreme phenotypes. Our results here confirm that CeA stimulation does make goal-trackers approach and 'consume' their metal CS_{dish} more, and in more focused fashion. The intensity and focus of the enhancement in goal-trackers' behavior is comparable to sign-trackers' enhanced behavior toward CS+ Lever, consistent with the trigger vs. target hypothesis for Pavlovian incentive salience.

2. Materials and methods

2.1. Subjects

Sprague Dawley rats ($n = 28$; female) weighing 280–340 g at the start of the experiment were pair housed on a reverse light/dark cycle. Water was provided ad libitum; food was provided ad libitum except during weeks containing autoshaping training or test sessions, when rats were restricted to 90% free feeding weight and fed about 12 g of standard laboratory chow daily after each training session. All experiments were conducted in accordance with protocols approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

2.2. Surgery

Rats were anesthetized with ketamine (80 mg/kg), xylazine (7 mg/kg), and atropine (0.04 mg/kg). The central nucleus of the amygdala was targeted by placing bilateral cannula aimed at (CeA) AP ≈ −2, ML ≈ 4, DV ≈ −5.8, following [21] and [46]. Placement coordinates for cranial cannulae were calculated based on Paxinos and Watson [50], and lowered into place with a stereotaxic apparatus (Kopf Instruments). Each rat was surgically implanted with chronic, bilateral, 14 mm microinjection guide cannulae (23 g) positioned 2 mm above the target CeA sites. Cannulae were anchored to the skull with bone screws and acrylic cement, and steel stylets were inserted to prevent occlusion. All rats were given chloramphenicol sodium succinate (60 mg/kg) to prevent infection and carprofen (5 mg/kg) to provide pain relief. Carprofen was administered again 24 h post-surgery.

2.3. Microinjections

Prior to tests, steel stylets were removed and cleaned, and 16 mm microinjectors were inserted into the guide cannula, pre-measured so that microinjector tips extended 2 mm below guides, while the rat was gently cradled against the experimenter by hand. Microinjections of DAMGO (Sigma–Aldrich) or vehicle were controlled by a syringe pump which delivered 0.2 μL over 90 s. DAMGO injections were 0.1 μg of DAMGO dissolved in 0.2 μL of aCSF vehicle; control vehicle injections were of aCSF alone in the same rats, in counterbalanced order. Microinjector tips were left in for 1 extra minute to allow for drug diffusion. Before any test rats were given one "sham" injection of 0.2 μL vehicle to habituate them to the microinjection process.

2.4. Behavioral autoshaping

Autoshaping training and testing was carried out in one of eight operant chambers (Med Associates) controlled by Med PC software, containing two retractable levers on opposite sides of a food receptacle. Each rat was always assigned to the same chamber for training and testing. Insertion of a lever on one side was designated as the CS+ sign that predicted sucrose pellet UCS delivery with 100% correlation. This CS+ Lever was a 4.5 cm × 2 cm retractable metal lever with a light emitting diode on its ventral surface. As CS+, the lever was inserted into the chamber

through the wall for 8 s and accompanied by a 2.9 KHz tone. The CS+ was followed immediately by UCS presentation (delivery of sucrose pellet). Another lever was always present and designated as CS− because it bore no relation to UCS. Presses on the CS− lever were taken as measures of generalization or nonspecific motor activity. Sucrose UCS pellets (45 mg) were presented in a metal dish 3 cm^2 at the bottom center of the same wall with the levers. The dish in which sucrose was delivered will be referred to from now on as CS_{dish} because the sight of the dish upon head insertion was the stimulus and action most contiguously paired in time and space with oral receipt of the UCS.

2.5. Training

Prior to Pavlovian CS–UCS pairings rats received 1 day of magazine training when 20 sucrose pellets were dropped into the CS_{dish}, approximately one pellet every 90 s. Autoshaping (CS+ paired with UCS) training sessions started the next day. Each Pavlovian session began with the illumination of the red house light and insertion of the control CS− lever at the beginning of the trial. Subsequent 8-s CS+ Lever presentations were always paired associatively with a UCS reward presentation under a Pavlovian contingency. Then the CS+ Lever was retracted and a UCS sucrose pellet was immediately presented in the CS_{dish}. Each autoshaping session lasted about 40 min and consisted of 25 CS+ and UCS pairings with a variable intertrial interval of ~90 s.

Rats received 5 days of Pavlovian training prior to tests that began on the 6th day. By the 3rd day of training, every rat began to respond to the CS+ onset with an approach–consummatory CR predominantly focused toward either the CS+ Lever itself (in which case the rat was classified as a sign-tracker) or toward the CS_{dish} (in which case the rat was classified as a goal-tracker). The criterion for classification as 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 goal-tracker was to approach, nibble, sniff, grasp and bite the dish at least three times more frequently than the lever during CS+ presentations, and additionally 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). All rats were successfully classified as either sign or goal-tracker by day 5.

2.6. Test for effects of opioid activation of CeA

Effects of DAMGO stimulation in CeA were tested by a within-subject comparison to the same rat's behavior on vehicle (control) on days 6 and 8 (in counterbalanced order across rats with 48 h in between). That is, on day 6 a rat received either vehicle or DAMGO (0.1 μg/0.2 μL) microinjection before the autoshaping session. On day 8 the other microinjection was administered (drug or vehicle) and the test was repeated.

2.7. Behavioral video scoring

Rats were always videotaped from two angles by separate cameras. One 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. This allowed scoring of both sign-tracking approaches and goal-tracking approaches, as well as scoring of consummatory behaviors in sign-trackers. A second camera was directed from the side toward the inner surface of the CS_{dish} to provide a close up view of the rat's face and mouth movements when inside the dish. Both videos were analyzed off line in slow motion (1/10th to ½ actual speed) by an observer blind to experimental conditions. For each trial, the 8 s before and 8 s during the 5th, 10th, 15th, 20th, and 25th presentation of CS+ were selected for comparison [21]. Scored behaviors were *latency to first contact* (time to first cue contact), *look* at the cue (orienting towards the cue by moving the head or forequarters toward it, without bodily approaching it), *nibble and sniff* the cue (contact of the nose or mouth on lever or dish, combined with rapid short (<0.5 s) rhythmic 1–2 Hz bobbing movements of the head and nose (sniff), and of jaw, tongue, and/or teeth (nibble), similar to movements of normal eating of UCS), and *bite* the cue (jaw closing and contact by maxillary and mandibular incisors, often while grasping the object with one or both paws, similar to movements that bite the actual UCS sucrose pellet). Finally, presses of the lever and photo beam entries into the food dish were also automatically recorded.

2.8. Statistical analysis

Within-subject ANOVAs comparing drug and vehicle days were performed for the cue and pre-cue periods. Significant differences for individual dependent variables in autoshaping were determined by Bonferroni corrected *t*-tests. To avoid distortions in percentage change calculations arising from any zero baselines, a constant value of 1 was added to each rat's behavioral score for both CS_{dish} and CS+ Lever. Nominal data was assessed using the related samples McNemar's test.

2.9. Histology

Rats were sacrificed immediately after the final day of testing by administration of a sodium pentobarbital overdose. Rats were decapitated and the brains were extracted and fixed in 10% paraformaldehyde solution for 1–2 days followed by a 25% sucrose solution in 0.1 M NaPB for 2–3 days before slicing. 60 μm slices through the CeA were taken from each rat, mounted, dried, and stained with cresyl violet. Microinjection center was determined for each bilateral injection site and slides were compared with the stereotaxic atlas [50] to determine placement in the CeA. Those rats with placements outside the CeA ($n = 9$) fell instead in the interstitial nucleus of the posterior limb of the anterior commissure (IPAC) and 1 in the basomedial nucleus of the amygdala.

3. Results

3.1. Overview: mu opioid stimulation of central amygdala potently enhanced approach and appetitive-consummatory actions of goal-trackers toward dish but of sign-trackers toward lever

In goal-trackers, DAMGO microinjections in CeA potently increased the number of appetitive-consummatory sequences directed toward their $CS_{dish.}$ The increase was selective to moments when the CS+ Lever was physically present. The increased sequences were always initiated by CS+-triggered approaches to the dish, followed by nibbles and sniffs of the dish rim and internal surface. In sign-trackers, these CeA DAMGO enhancements were matched by increased numbers of approaches and appetitive-consummatory sequences directed to the CS+ Lever. That is, CeA DAMGO similarly intensified the motivated cue-triggered behaviors that each phenotype directed at their own prepotent Pavlovian target.

In more detail, for goal-trackers, DAMGO in CeA selectively increased the number of CS+-triggered approaches, nibbles and sniffs to the goal CS_{dish} by 150%, compared to vehicle microinjection effects in the same rats. Simultaneously, CeA DAMGO conversely decreased goal-trackers' already low rate of approach to the CS+ Lever when it was present, indicating that the increase in the number of already-dominant goal tracking responses was at the expense of already-weaker sign-tracking responses ($F_{(4,8)} = 7.3$, $p < .01$; $F_{(1,11)} = 8.639$, $p < .05$). Similarly but conversely, sign-trackers increased their approaches, nibbles and sniffs of the prepotent CS+ Lever by 230% more under DAMGO compared to vehicle conditions, while oppositely decreasing their already low level of approaches to the goal or CS_{dish} (overall $F_{(3,4)} = 11.1$, $p < .05$; $F_{(1,6)} = 11.791$, $p < .05$). For both sign-trackers and goal-trackers, CeA-induced effects on these appetitive-consummatory behaviors were manifest only when the CS+ Lever was physically present, and never in intervening baseline intervals when the lever was absent, regardless of target for approach. That is, even for goal-trackers whose dish target was always present, CeA selectively enhanced phasic elevations of approach and consumption-related behaviors toward the dish only during the CS+ presentations, and did not enhance lower baseline levels of approaches or consummatory actions toward the dish during longer intervals in the absence of CS+ (goal-trackers: $F_{(4,8)} = 1.1$, $p > .1$). Likewise, sign-trackers only enhanced approach/consummatory sequences during CS+ presentation, but that was perhaps less surprising since consummatory actions could not be directed to an absent lever; however, we note that DAMGO also failed to enhance sign-tracker's baseline levels of approach to the location in the chamber where the CS+ appeared, which in principle could still have been enhanced in lever absence ($F_{(3,4)} = 1.27$, $p > .1$) (Fig. 1). Finally, a sequence was usually terminated by opening the mouth and dipping the head in the CS_{dish} (for goal trackers) or grasping, biting and depressing the CS+ Lever (for sign trackers).

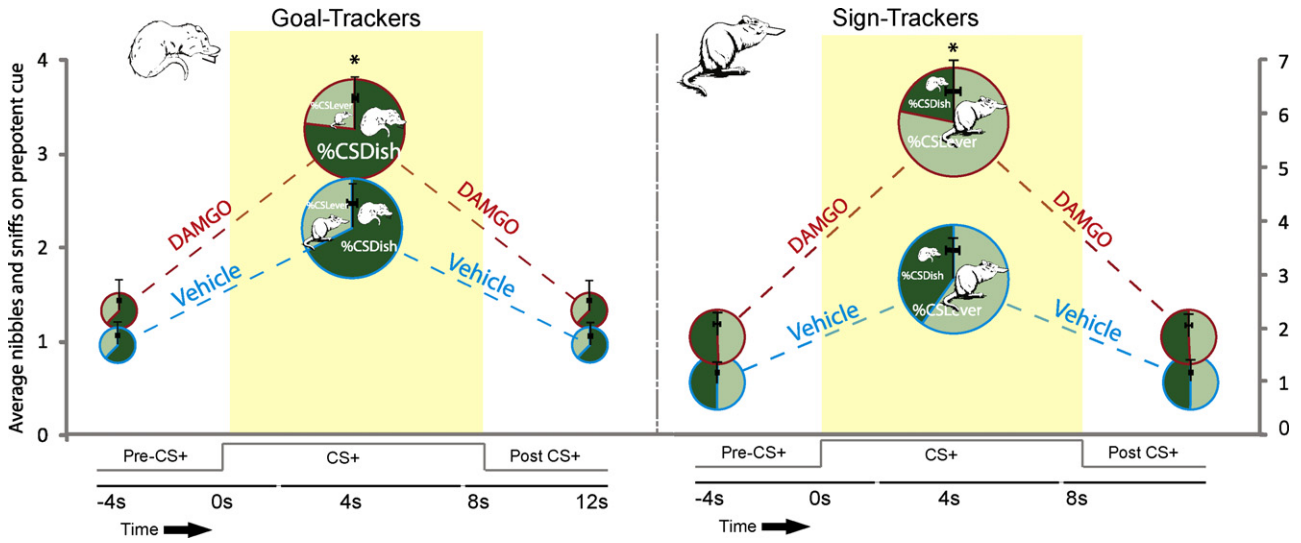

Fig. 1. *CeA DAMGO enhances focus.* CeA DAMGO microinjection amplifies and focuses appetitive-consummatory behaviors directed toward the prepotent cue for both sign-trackers and goal-trackers. The number of approaches, nibbles and sniffs directed at the prepotent cue is increased. Likewise, the proportion of all approaches, nibbles and sniffs directed toward the prepotent cue is increased after DAMGO microinjection, while the proportion directed toward the nonprepotent CS is simultaneously decreased. Yellow background indicates periods when the CS+ is physically present; white backgrounds indicate before and after CS+ presentations; *indicates $p < .05$. Pie-graph circles show the proportion of appetitive-consummatory behaviors directed by that phenotype to CS+ Lever (sign) vs. CS_{dish} (goal). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Approach to target

DAMGO enhanced approach to each rat's individualized target CS, as reflected in (a) the alacrity or speed with the target CS was reached, and (b) the ability of the CS target as a motivational magnet to pull all cue-triggered approaches exclusively to itself. In terms of approach latency, microinjection in CeA made both sign-trackers and goal-trackers approach their prepotent cue more quickly. Overall, rats reached their target within 1–3 s after trigger onset of CS+ Lever under vehicle conditions. While it is difficult to move much faster than a 2 s latency, it is possible to speed up longer >2 s latencies toward the <2 s floor. Here we found that all rats which took over 2 s to reach their targets after vehicle microinjection (mean ± SEM = 2.5 ± 0.15 s latency) speeded up and reached their target in less than 1.5 s after DAMGO in CeA (mean ± SEM = 1.4 ± 0.33 s, $t_{(6)} = 3.7$, $p < .05$).

Similarly, the strength of a motivational magnet can be assessed by the ability of a target CS to fully capture all appetitive approach behavior toward itself during whenever the CS+ Lever or trigger is presented, and so to eliminate the defection of any approaches toward the alternative CS. Exclusivity of appetitive capture by a target CS can be dichotomized as either 100% complete capture (i.e., the rat exclusively approaches only its prepotent CS, and does not defect at all to the nonprepotent cue while triggers are present), or as incomplete capture (i.e., the rat approaches both CSs at least once during a trial). In principle, prepotent capture could also fail completely (so that the rat approached only the nonprepotent CS during a trigger), but that happened on less than 6% of vehicle cue presentations and was never observed after DAMGO in CeA. Instead the motivational magnet strength of the target CS essentially varied between 100% complete capture vs. incomplete, and DAMGO selectively strengthened the target CS's completeness of capture for goal-trackers and sign-trackers. For rats overall, the incidence of 100% complete capture by the prepotent target CS, or sessions in which the rat never defected even once to the non-prepotent CS during any recorded trigger presentation, rose from 9/19 rats (47%) under vehicle to 15/19 rats (79%) under DAMGO (McNemar's test, $p < .05$). Even for goal-trackers considered separately, completeness of capture rose from 5/12 rats (41%) under vehicle to 11/12 rats (92%) under DAMGO (McNemar's test, $p < .05$). Accordingly, the probability of any visit by the rat to the nonprepotent cue during a trial fell from 29% under vehicle (this 29% includes approximately 5% contributed by 2 rats that exclusively approached their nonprepotent CS on few stimulus presentation) to just 6% under DAMGO ($F_{(1,18)} = 6.0$, $p < .05$). Similarly, the probability of visiting both CSs during a trigger fell from 24% under vehicle to 6% under DAMGO for all rats ($F_{(1,18)} = 6.4$, $p < .05$).

This DAMGO pattern of endowing the prepotent CS with ability to completely capture 100% of all cue-triggered appetitive behavior suggests DAMGO made the prepotent target CS into a stronger "motivational magnet" for both goal-trackers and sign trackers. In summary, when a rat's CeA was in an opioid-stimulated state its prepotent target CS attracted approach more quickly to itself as soon as the trigger stimulus appeared, and more exclusively to itself (i.e., away from the alternative nonprepotent CS). These appetitive features applied to goal-trackers as well as sign-trackers.

3.3. Focus on target

In terms of relative focus of incentive salience between the two CSs, for all rats, opioid stimulation by DAMGO in CeA increased the proportion of approaches, nibbles and sniffs directed by each rat to its already prepotent CS, while simultaneously decreasing the already low proportion of nibbles and sniffs toward its nonprepotent CS ($F_{(11,8)} = 5.8$, $p = .01$). In more detail, 71% of all nibbles and sniffs by goal-trackers were directed at their prepotent CS_{dish} under vehicle conditions, and that proportion rose under DAMGO stimulation to 77% at the CS_{dish} ($t_{(11)} = -2.52$, $p < .05$). Simultaneously, the proportion of responses directed by goal-trackers toward their non-prepotent CS+ Lever fell from 29% under vehicle to 23% under DAMGO (Fig. 1). Conversely, for sign-trackers 68% of nibbles and sniffs were directed at their prepotent CS+ Lever under vehicle, and that proportion increased to 78% under DAMGO stimulation ($F_{(11,8)} = 5.8$, $p = .01$). Simultaneously, the proportion of responses directed by sign-trackers toward their non-prepotent CS_{dish} fell from 32% under vehicle to 22% under DAMGO in CeA. This narrowing of focus on the individual's own prepotent target, occurring at the detriment of attraction to the alternative nonprepotent cue, is

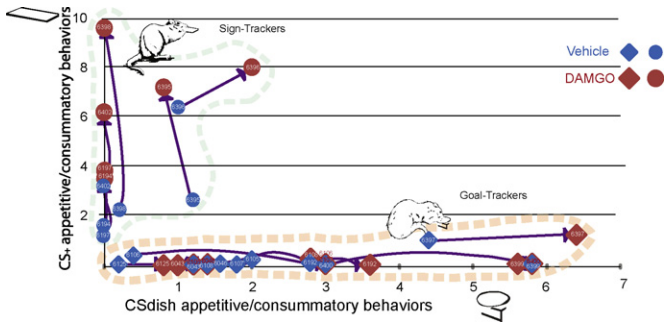

Fig. 2. *Individualized enhancements of prepotent target.* DAMGO microinjection into the central nucleus of the amygdala makes individual sign-trackers into more intense sign-trackers, but makes individual goal-trackers into more intense goal-trackers. In this scatterplot, each individual rat is represented by two dots (within-subject comparison of CeA conditions): a blue dot in vehicle condition and a connected red dot in DAMGO condition. Sign-trackers are circles and goal-trackers are diamonds. Vertical axis plots the number of sign-tracking behaviors toward CS+ Lever (sign). Horizontal axis plots the intensity of goal-tracking behaviors toward CS_{dish} (goal). DAMGO always intensifies the pre-existing preference of an individual that was already prepotent. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

compatible with a "winner take all" property of incentive salience enhancement induced by CeA opioid stimulation. Under DAMGO, all rats more nearly ignored their nonprepotent cue, instead focusing the increase in approaches, nibbles and sniffs only toward their prepotent cue, whichever stimulus that was for an individual rat. In short, CeA DAMGO microinjections made the sign-trackers better sign-trackers and made goal-trackers better goal-trackers. This can be seen most clearly by plotting each rat individually for its number of nibbles and sniffs directed at the CS_{dish}, and simultaneously the number directed at the CS+ Lever on each trial. DAMGO stimulation shifts each animal towards a more extreme preference. This demonstrates an enhancement of focus and intensity for both goal-trackers and sign-trackers (Fig. 2).

3.4. In the middle: individuals that balance targets still enhance only the prepotent one

The 'winner-take-all' feature applied even to relatively balanced individuals that showed substantial attraction to both CSs. One way to see this is to assess every individual separately, to trace the effect of CeA DAMGO on individualized response patterns. Every individual's signature can be plotted as a point in a space defined by two orthogonal axes of goal-tracking strength vs. sign-tracking strength, and the effect of CeA stimulation is visible as moving the individual to a second point location (Fig. 2). This individual-by-individual analysis revealed that DAMGO virtually always enhanced appetitive-consummatory response sequences only toward each individual's own prepotent target. Even if the initial bias approached balance as closely as 60:40, only responses toward the prepotent CS (e.g., 60) were enhanced by DAMGO. Thus, the prepotent CS is a stronger 'motivational magnet' for every individual under CeA DAMGO stimulation, while the weaker CS typically gets weaker.

A more collective way to assess that 'winner-take-all' feature is to isolate for statistical analysis the intermediate one-third of the population, which typically shows mixed sign-tracking and goal-tracking responses in more nearly equal proportions (compared to the extreme one-third of goal trackers, or the opposite one-third of extreme sign-trackers) [14,15] (Fig. 2). Split into three groups of one-third each, the middle group divided their CS+-triggered responses under vehicle in roughly 60:40 proportions between targets (some preferring the CS+ Lever and others the CS_{dish}). DAMGO microinjection into CeA selectively enhanced only the prepotent target even for this middle group, which increased its preference ratio to 75:25 ($F_{(1,5)} = 7.8$, $p < .05$). The absolute number of nibbles and sniffs for this middle group directed to the prepotent target also more than doubled from 1.2 under vehicle conditions to 3.1 after DAMGO stimulation ($F_{(1,5)} = 7.7$, $p < .05$). Conversely the number of responses directed at the nonprepotent cue trended downward, if anything for this group (.3 to .2, n.s.). Taken together, these results show that the attribution of 'wanting' becomes more narrowly focused on a single Pavlovian target for all individuals, as well as intensified in level, after DAMGO microinjection in CeA.

3.5. Temporal pattern phasic enhancements of approach and consummatory behaviors

In terms of response timing, enhancements of nibbles and sniffs on the prepotent cue were always limited to phasic bouts lasting only 8 s for both sign-trackers and goal-trackers, each bout triggered by the insertion of CS+ Lever, lasting for its duration, and terminating almost immediately when the lever was retracted ($F_{(5,7)} = 31.9$, $p < .001$). For goal-trackers, DAMGO microinjection in CeA amplified the number of cue-triggered approaches and consummatory actions toward the CS_{dish} by over 50% during each CS+ presentation, but did not alter the low baseline level in the absence of the CS+ Lever (drug × cue interaction $F_{(1,11)} = 5.4$, $p = .04$; Fig. 3). Likewise DAMGO microinjections in CeA doubled the number of sign-trackers' approach and consummatory CRs to CS+ Lever (5.6 per 8 s presentation) while not altering the baseline number of approaches to the same location when CS+ was absent ($F_{(3,4)} = 1.27$, $p > .1$). These patterns demonstrate that approaches and consummatory acts were always temporally locked to the insertion of the CS+ Lever, and that CeA stimulation enhancement was similarly time-locked and triggered by presentations of CS+ Lever, even for goal-trackers, for which the prepotent target CS_{dish} was always present.

3.6. CeA DAMGO intensifies microstructure of appetitive-consummatory behavior at prepotent target

A more fine-grained behavioral (frame-by-frame to 1/5 speed) video analysis of the detailed microstructure pattern of nibble-and-sniff movements directed toward the prepotent CS suggested that motivated behaviors also became more frenzied after CeA stimulation, and in the same way for goal-trackers and sign-trackers. To show the DAMGO change, the nibble-and-sniff behavior was choreographed in a randomly selected subset of animals under both vehicle and CeA DAMGO trials using a visual notation system [21] (Fig. 4). DAMGO in CeA increased the temporal rate of early phase nibble and sniff movements to the prepotent CS that normally began an appetitive-consummatory sequence, as well as the number of those actions. As a consequence, the bout of intensified nibbles and sniffs endured several seconds longer and so postponed the occurrence of slower bite movements that typically ended an 8 s sequence (Fig. 4; duration of nibble-sniff bout before first bite vehicle vs. DAMGO $F_{(1,12)} = 5.9$, $p < .05$). Increases in rate, number, and bout duration of these rapid early phase sniff-nibble movements gave a more frenzied appearance to consummatory CRs under DAMGO. That pattern also suggested that incentive salience enhancement particularly promoted appetitive behavior and consummatory *initiation*, without necessarily potentiating late-phase consummatory termination acts involving the bite and swallow movements of actual UCS ingestion. In other words, the DAMGO stimulation of CeA appeared to make the metal cue take on food-like incentive properties, but did not make the rats mistake the lever or dish object for food.

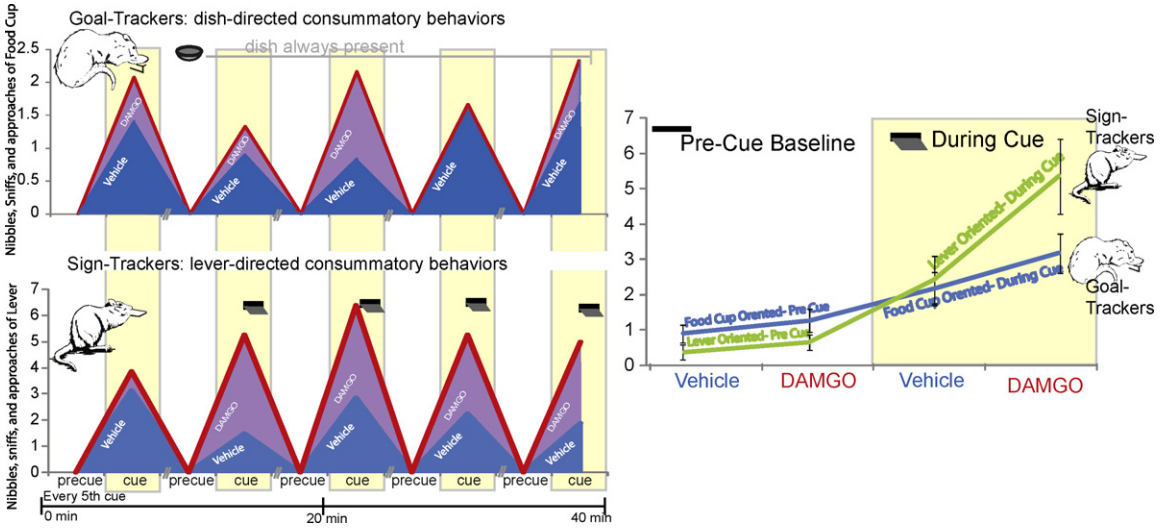

Fig. 3. *Cue Locked Enhancement.* DAMGO microinjection increases nibbles and sniffs to the prepotent cue specifically during trigger presentation moments (CS+ Lever insertions into chamber; yellow backgrounds) only, but not during inter-cue intervals. This cue-locked increase is similar for both sign-trackers and goal-trackers, even though the CS_{dish} is always present for goal-trackers. On vehicle nibbles and sniffs increase during the cue periods ($p < .05$) and on DAMGO this cue-locked increase is greatly enhanced (drug × cue $p < .05$). Left: temporal pattern of behaviors over successive CS+ Lever presentations and baseline intervals during the 40 min test session for goal-trackers (top) and sign-trackers (bottom). Pre-cue nibbles and sniffs were subtracted from all values depicted to normalize baseline levels. Right: total approaches, nibbles and sniffs to each CS during baseline intervals vs. during CS+ presentations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

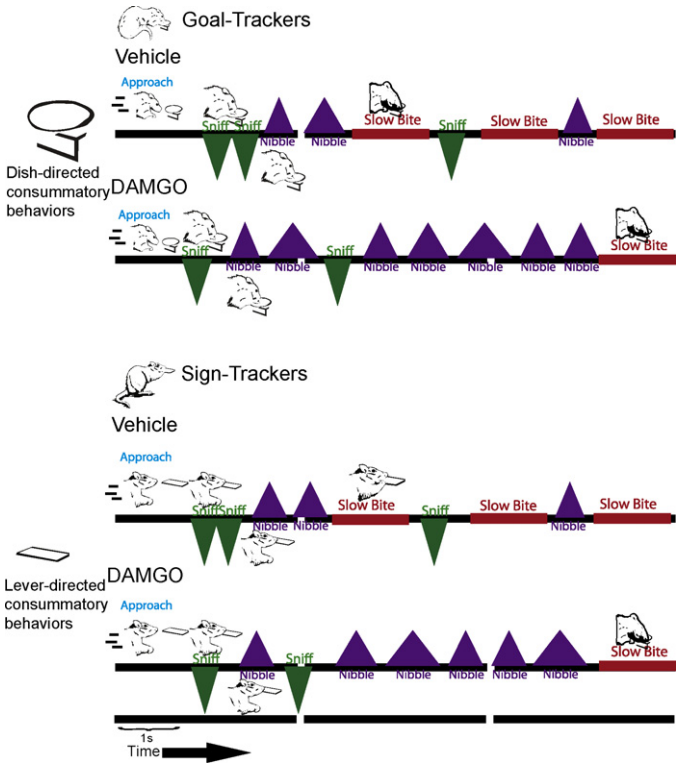

Fig. 4. *Topography of Behavior.* DAMGO shifts the individual choreography or microstructure of each rat's response to more anticipatory nibbles and sniffs and less terminal slow bites. Consequently, latency to the first slow bite is increased after DAMGO microinjection. Each choreograph shows a 'typical' instance compiled from several actual rats. Time proceeds from left to right during 8 s presentation of CS+. Green downward triangles denote individual CS sniff actions; purple upwards triangles denote nibble actions; red bars denote slower consummatory bites typically seen in later phases of actual ingestion. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.7. Anatomical specificity of DAMGO enhancement in CeA

Microinjection cannulae tips that produced enhancements were located bilaterally well within the CeA in 19 rats (out of 25; Fig. 5). In order to assess whether DAMGO was likely to be contained within the borders of CeA, we applied the earlier observation by Mahler and Berridge that DAMGO microinjections in CeA filled a tissue volume of approximately 0.43 mm^3 surrounding the microinjection tip (based on radius of Fos plumes produced at the same dose used here) [21]. If a similar radius applied here, we estimated that 90% of the entire DAMGO impact volume would have been

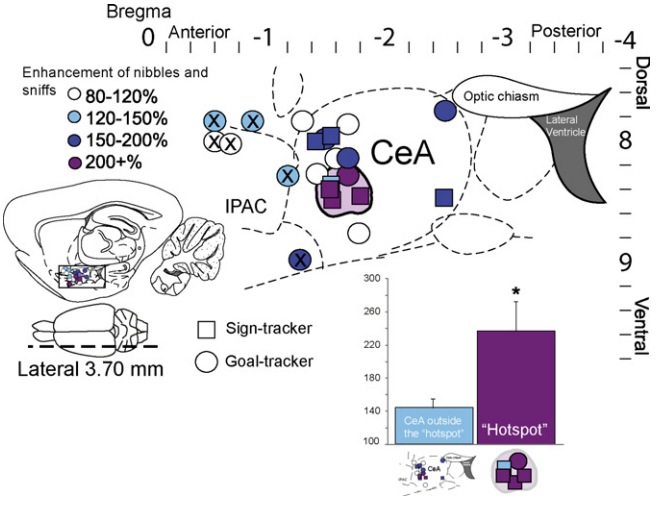

Fig. 5. *Localization of DAMGO effects.* The center of each microinjection cannula placement is represented as a circular point. The enhancement effect of DAMGO on that rat's CS+ Lever-triggered 'motivational magnet' attraction toward its individualized target CS (lever or dish) is color coded and represented as % change from vehicle control level in the same rat. A DAMGO "hotspot" of maximal effect is highlighted in anterior CeA, defined as a contiguous cluster of anatomical placements that produced enhancements >200%. DAMGO placements outside of CeA did never increased nibbles and sniffs on the prepotent cue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

contained inside CeA for 15 out of the 19 rats that had tips within CeA. Over 75% of the DAMGO impact volume would have been contained within CeA for another two rats, and over 50% contained within CeA for the final two rats. By contrast, we observed that other rats with placements outside the CeA (e.g., in IPAC) would not have entered CeA, and did not express a DAMGO enhancement of their prepotent target, by contrast to the 19 placements contained within CeA that did ($F_{(2,4)} = 2.8$, $p < .1$). Thus we are confident that DAMGO enhancement effects observed here were mediated essentially by receptors within CeA.

Further, a more precise localization of function for incentive salience enhancement in a subregion of CeA was potentially indicated by a spatial clustering of the most effective sites in a mid-anterior subregion of CeA, compared to other sites in CeA (Fig. 5). Sites where DAMGO enhancements exceeded >200% in the number of CS+-triggered approaches, nibbles and sniffs to the individual's pre-potent target (e.g., more than twice the vehicle-control level for the same rat) all fell into this restricted mid-anterior subregion (Fig. 5). To quantitatively probe this function localization, a hotspot was tentatively defined anatomically by outlining the outer border of the cluster of contiguous placements where DAMGO enhancements exceeded >200% over vehicle, and comparing the magnitude of increase for sites inside the hotspot vs. outside the hotspot but still in CeA. Hotspot sites produced a DAMGO enhancement that averaged 236% in behavioral magnitude whereas other CeA sites that fell outside this hotspot averaged only 145% enhancement ($t_{(18)} = -3.9$, $p < .05$). Although the number of sites here is too small to draw a firm conclusion, we note that a similar anatomical mid-anterior clustering of the most potent sites in CeA was also found by Mahler and Berridge [21], suggesting that this anterior CeA hotspot may well be real for CS motivational magnet enhancement.

3.8. Sign-tracker vs. goal-tracker differences in absence of CeA DAMGO stimulation

Do goal-trackers ordinarily attribute less incentive salience to their prepotent target than sign-trackers do to theirs, in absence of special mesocorticolimbic stimulation? Under vehicle conditions, our goal-trackers may have shown a slightly lower intensity cue-triggered increase in incentive salience than sign-trackers at least in one sense. That is, goal-trackers had a lower cue-triggered relative increase when incentive salience was calculated as a percentage increase in approaches, nibbles and sniffs to the prepotent target triggered by the CS+ presentation, over immediately prior pre CS+ Levels (goal-trackers = 163%, sign-trackers = 339%; $F_{(1,17)} = 6.873$, $p < .05$). By contrast, administration of DAMGO raised both groups to higher and equal levels of relative CS+-triggered increase (drug × phenotype $F_{(11,8)} = 2.15$, $p > .05$). However, while the lower relative increase under vehicle may reflect lower CS+-triggered levels of incentive salience in goal-trackers than in sign-trackers under vehicle condition [9], it also reflects higher pre CS+ baseline levels in goal-trackers (ST = .1 per 8 s, GT = .9 per 8 s; $F_{(1,17)} = 5.402$, $p < .05$), and the absolute levels of cue-evoked nibbles and sniffs did not statistically differ between sign- and goal-trackers here under vehicle conditions (ST = 2.45 per 8 s, GT = 2.18 per 8 s; $F_{(1,17)} = .106$, $p > .1$). Still, we agree with the proposition of Robinson and colleagues that goal-trackers may ordinarily attribute lower incentive salience than sign-trackers [8,9,48] (when in an ordinary mesocorticolimbic state involving no physiological stimulation), and we note that even under vehicle condition our goal-trackers had a mild physiological state of hunger that could induce mesocorticolimbic reactivity because all of our rats were maintained at roughly 90% ad libitum body weight (fed 12–15 g chow daily to keep them at that weight). By contrast, studies by Robinson and colleagues typically have tested rats in a completely sated state of permanent ad libitum access to chow [9,48]. Recent pilot data from our lab suggests that such differences between mildly hungry and fully sated testing may matter for incentive salience, and that the phenotype difference between goal-trackers vs. sign-trackers may best be observed when rats are fully sated. Using similar autoshaping procedures, studies in our lab have found that fully sated goal-trackers show significantly slower latencies of CS-triggered approaches to CS_{dish} than fully sated sign-trackers to CS+ Lever, and that hunger enhances approach for both phenotypes (ST = 1.5 ± 28, GT = 3 ± .67; $p < .05$; personal observations, Springstead and DiFeliceantonio).

4. Discussion

4.1. Central nucleus of the amygdala focuses incentive salience

Goal-trackers and sign-trackers ordinarily differ in their targets of incentive salience, so that it has been suggested that sign-trackers may uniquely attribute incentive salience to discrete CSs for reward in ways relevant to addiction [8,9,14,15,48]. Our results add a degree of richness to this picture by confirming that goal-trackers can achieve similar high intensities of incentive salience pulses, though focused on a different type of Pavlovian CS target, especially when their brains are in a state of mesocorticolimbic activation induced by mu opioid stimulation in CeA.

Our results indicate that goal-trackers in a state of mesolimbic activation attribute intense incentive salience to their own prepotent CS_{dish} target. This occurs as a phasic pulse that makes the dish into a 'motivational magnet' as strong as the CS+ Lever is to sign-trackers, during moments while the CS+ trigger is present (here about 8 s each presentation). That is, mu opioid stimulation of CeA enhanced incentive salience levels that each phenotype attributed to its own prepotent Pavlovian target. Our results also suggest that both sign-trackers and goal-trackers share the same trigger stimulus, namely presentation of the CS+ Lever.

After DAMGO microinjection in CeA, goal-trackers were more intensely attracted to the delivery CS_{dish} where sucrose arrives (goal), specifically at moments when the CS+ Lever was present. DAMGO in CeA enhanced the number of goal-trackers' approaches and number of appetitive-consummatory nibble and sniff sequences toward the CS_{dish} target, each time the CS+ Lever appeared, without at all enhancing those motivated behaviors towards the same object during intervening baseline periods when the CS+ was absent. Sign-trackers, after DAMGO in CeA, were similarly attracted at those same CS+ Lever moments to their prepotent sucrose-predicting CS+ cue (sign), so that the CS+ Lever acted as both their trigger and target. The enhancement of attraction to the prepotent cue was accompanied by a simultaneous reduction of attractiveness of the alternative cue. In short, this "winner take all" pattern of incentive salience was always limited to one CS target, corresponding to the individual's own prepotent Pavlovian stimulus, at the expense of the other CS.

4.2. Synergy of incentive salience generation

A synergy between mesocorticolimbic state and trigger presence in generating incentive salience is revealed by the need for two simultaneous conditions: CS+ Lever presence (phasic trigger; present for only 8 s per occurrence) and CeA opioid stimulation (which presumably was relatively constant during the 40-min test). Simultaneous necessity of CS+ presence and stimulated brain state has been computationally modeled for incentive salience enhancement by Zhang et al. [11] as: $V(S_t) = r(r_t, K) + \gamma V(S_{t+1})$. In that Zhang model $V(S_t)$ is the intensity of incentive salience triggered at the moment (t) when the trigger CS+ (S) appears. Here the target of the pulse of 'wanting' was selectively always the individual's own

prepotent target CS_{dish} or CS+ Lever, but the trigger was always CS+ Lever, reflecting the r_t carried by its Pavlovian correlation with sucrose UCS in the past. That r_t essentially corresponds to a memory cache formed by previous reward encounters and prediction errors, drawing on a temporal difference model of reward learning [51]. Mesocorticolimbic reactivity, which is influenced by CeA state, is represented by K, a multiplicative gain factor that interacts with r_t at the moment of CS+ Lever encounter. Stimulation of CeA mu opioid receptors by DAMGO here can be understood as having elevated $K \gg 1$, thus dynamically elevating the multiplied product of $V(S_t)$ to produce excessive incentive salience at those particular moments. Applying this model to our results, the cached memory value was not changed intrinsically by opioid stimulation of CeA during the test, but the reactivity was heightened of mesocorticolimbic circuits that phasically generate 'wanting' to the r_t association, and attributes the incentive salience directionally toward the Pavlovian prepotent target. The rise in intensity of target "wanting" was triggered each time the CS+ was inserted and was revealed in more frenzied appetitive and consummatory behaviors directed at the prepotent target.

4.3. CS trigger vs. CS target roles

A continuing puzzle is why target and trigger are separate objects for goal-trackers, but not for sign-trackers. For sign-trackers, the situation is most intuitive. The CS+ Lever presentation is always the most *predictive* event for reward delivery, being correlated in event probability with the UCS. Each CS+ (lever insertion and sound) was followed 8 s later by a UCS, and the UCS never occurred without being preceded by the CS+. That predictive relationship makes the CS+ Lever the trigger (and at least for sign-trackers, also the target).

By comparison, the CS_{dish} was less informatively predictive, being always present and therefore associated both with UCS and with its absence. However, the CS_{dish} was the Pavlovian CS with closest spatial and temporal contiguity to the UCS. That is, the dish was always the last thing the rat saw or felt before tasting sucrose reward, because the rat's head was always inserted into that dish at the moment of pellet ingestion. This dish-in-the-face as stimulus complex was paired almost simultaneously with the hedonic taste of sucrose. CS–UCS contiguity has long been recognized as important to facilitating Pavlovian associations, and contiguity may remain important in controlling the target for goal-trackers even when contingency dominates the associative correlation that generates r_t as a phasic prediction from the trigger [19,52–54].

Contiguity of CS with UCS may become even more important for incentive salience attribution when mesocorticolimbic circuits are pharmacologically stimulated [10,55]. For example, previous studies reported that opioid or dopamine stimulation of the nucleus accumbens selectively enhanced incentive salience of a UCS-contiguous CS2 stimulus, but not of a UCS-predictive CS1. Contiguity dominance applied to all rats when tested in a Pavlovian CS–CS–UCS series, in which a UCS-predictive CS1 was followed by a UCS-contiguous CS2, [10,55]. Here, DAMGO in CeA of goal-tracking individuals selectively enhanced attributions of high incentive salience to that contiguous CS_{dish}, target alone, just as it enhanced the attributions by sign-trackers to their own prepotent cue, the predictive CS+ Lever.

4.4. CeA modulation of corticolimbic circuitry

What features of CeA allow its opioid stimulation to both magnify incentive salience intensity and narrow the target focus of attribution even more than usual to a single Pavlovian CS? Opioid circuits in central nucleus of amygdala may particularly aid the translation of previously learned information, in the form of a static Pavlovian CS–UCS reward association, into dynamic incentive salience that motivates behavior at the moment when CS is subsequently re-encountered [21,35,42,56,57]. Thus CeA is in an excellent position to modulate 'wanting' of Pavlovian CSs.

The central nucleus of amygdala receives distinct inputs that might be important to reward processing, including gustatory inputs from the parabrachial nucleus in pons, and has important outputs, including indirect modulation of mesolimbic dopamine neurons in the ventral tegmentum [58,59]. CeA also has been suggested to be embedded within the larger extended amygdala macrosystem [30,32,33,60], the lateral (or central) division of which begins in CeA and connects to the bed nucleus of stria terminalis (BNST), sublenticular extended amygdala (SLEA) and interstitial posterior limb of the anterior commissure (IPAC) [34]. The extended amygdala system shares special features with caudal portions of the medial shell of the nucleus accumbens [33,61]. The CeA also can be viewed in light of macrocircuit concepts described by Swanson [33,35], in which CeA is a striatal-level component (GABAergic), receiving inputs from the basolateral nucleus of amygdala (BLA) as a cortical-level component (glutamatergic), and sending outputs to BNST, SLEA and IPAC as pallidal-level components (GABAergic). A striatal-level status may be especially noteworthy for CeA's status as an incentive salience generator, in that other several other striatal-level structures also can generate intense enhancements of incentive salience when neurochemically stimulated [10,55,62,63]. These include nucleus accumbens (ventral striatum) and even regions of neostriatum (dorsal striatum). Thus, CeA having striatal-level features may be important to its capacity for opioid stimulation to intensify CS 'wanting'.

In analyses of emotional learning, CeA has often been considered to be an output relay for BLA [31]. Comparing BLA to CeA, BLA inputs have been indicated to be especially important for pure Pavlovian learning functions such as formation of specific cue-reward associations or learning of new positive cognitive incentive values, whereas the CeA may be more involved in the active translation of learned information into motivation and generating incentive salience at moments of CS re-encounter [44,64–66].

Opioid neurotransmission in CeA appears to be especially important to dynamic amplification and focusing of incentive salience that makes a Pavlovian cue into a motivational magnet. Endogenously, CeA neurons receive mu opioid stimulation from local enkephalin neurons of amygdala and from B-endorphin axons projecting from the hypothalamic arcuate nucleus [67–69]. DAMGO microinjection in CeA may mimic such endogenous opioid sources, increasing fos gene transcription in CeA neurons [21]. Opioid stimulation may promote GABAergic disinhibition of output structures [70,71], to modulate and stimulate mesocorticolimbic dopamine circuits, via indirect projections such as to the lateral hypothalamus and peduncular pontine nucleus which in turn project to VTA [33,58,72–74]. Here, DAMGO microinjections into CeA may well have potentiated mesolimbic dopamine circuits to nucleus accumbens as a step in amplifying the intense bouts of incentive salience observed in appetitive-consummatory behavior [10,62,75].

4.5. Sign-trackers' and goal-trackers' phenotypes: differences and similarities

Our finding of similarities for CeA enhancement of incentive salience in sign-trackers and goal-trackers does not deny that sign-trackers ordinarily differ from goal-trackers in many important neurobiological and psychological ways. For example, Flagel, Robinson and colleagues have shown that sign-trackers have higher tonic levels of mRNA for dopamine D1 receptors in the nucleus accumbens, whereas goal-trackers have higher mRNA for D2 receptors, tyrosine hydroxylase, and the dopamine transporter in the

ventral tegmental area (VTA) [8]. Psychologically, the same group has reported that only sign-trackers assign incentive salience to the CS+ Lever, and that sign-trackers ordinarily assign high intensities of incentive salience to their target CS but goal-trackers do not [8,9]. For example, Flagel and colleagues reported that sign-trackers show higher dopamine elevations than goal-trackers in nucleus accumbens to CS+ Lever presentations [9]. Behaviorally, only sign-trackers learn to perform a new instrumental response to obtain CS+ presentation (i.e., instrumental conditioned reinforcement) [9,14,76]. Such observations have led to suggestions that sign-trackers attribute high levels of incentive salience, whereas goal-trackers rely upon non-'wanting' psychological processes of S–R habit or of cognitive expectations [9,76]. In conformance with that situation, we agree that non-hungry and pharmacologically non-stimulated goal-tracking rats may attribute less incentive salience than sign-trackers.

Our findings apply especially to heightened states of mesocorticolimbic reactivity, induced here by CeA opioid stimulation, which generate intense levels of incentive motivation. We conclude that, when in a heightened mesocorticolimbic state, goal-trackers and sign-trackers showed intense and comparably high elevations of incentive salience, narrowly attributed to their own particular target. Those pulses of intense incentive salience attribution to the target phasically came and went with the presence of the shared CS+ trigger, while simultaneously reducing the attractiveness of the competing alternative target in the same moments. This capacity for similarity in intense incentive salience states may also be related to why some psychological, pharmacological, or neurobiological manipulations are able to shift potential goal-trackers to become sign-trackers, or vice versa [22–24].

4.6. Clinical implications

Brain mechanisms that generate intense levels of incentive motivation may be especially relevant to addiction. Addiction and related compulsive pursuit disorders involve intense motivations that often have two important features: incentive specificity and temptation fluctuation. The first feature is that the 'wanted' target is usually specific. At moments of peak urge, a particular incentive may be 'wanted' much more than anything else. Drug addicts mostly 'want' drug rewards, and some addicts may even 'want' a particular drug, whereas binge eaters 'want' food, and perhaps a particular food. Other compulsive motivations have their own specific targets and triggers (sex, gambling, shopping, etc.). The focusing of incentive salience attributed to a prepotent target, at the expense of other competing targets, here made the one stimulus more 'wanted' above all else. Conceivably, related CeA circuits might similarly be involved in sharpening the focus of 'wanting' on a single incentive target in intense compulsive disorders like drug addiction and binge eating.

The second feature in drug addiction and other compulsive motivations is temporal fluctuation in the cue's temptation power: a reward cue may be resisted many times successfully, only to elicit overpowering attraction on a subsequent encounter that triggers relapse. Why does the same Pavlovian CS+ trigger greater temptation on some occasions than on others? Our data suggest that one factor is the mesocorticolimbic reactivity state at the moment of cue re-encounter, which modulates the intensity of incentive salience that is triggered. Mesocorticolimbic reactivity can be enhanced by mu opioid activation of CeA related circuitry, by mesolimbic dopamine or opioid stimulation of nucleus accumbens, and by drug-induced sensitization of those mesocorticolimbic circuits, all of which exploit the motivational plasticity of mesocorticolimbic circuits that evolved for natural appetite states [10,13,21,62,75,77]. All may similarly amplify the intensity of phasic pulses of incentive salience triggered by a predictive CS+. We suggest that temporal fluctuation of mesocorticolimbic circuit states involving CeA opioid activation could dynamically amplify incentive salience attributed to a previously resisted CS at a particularly intense moment of temptation, creating a more powerful 'wanting' for its reward that could drive relapse in maladaptive drug addiction, binge eating and related addiction-like disorders.

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