Physiological state gates acquisition and expression of mesolimbic reward prediction signals

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Edited by Richard D. Palmiter, University of Washington, Seattle, WA, and approved January 8, 2016 (received for review October 2, 2015)

Phasic dopamine signaling participates in associative learning by reinforcing associations between outcomes (unconditioned stimulus; US) and their predictors (conditioned stimulus; CS). However, prior work has always engendered these associations with innately rewarding stimuli. Thus, whether dopamine neurons can acquire prediction signals in the absence of appetitive experience and update them when the value of the outcome changes remains unknown. Here, we used sodium depletion to reversibly manipulate the appetitive value of a hypertonic sodium solution while measuring phasic dopamine signaling in rat nucleus accumbens. Dopamine responses to the NaCl US following sodium depletion updated independent of prior experience. In contrast, prediction signals were only acquired through extensive experience with a US that had positive affective value. Once learned, dopamine prediction signals were flexibly expressed in a state-dependent manner. Our results reveal striking differences with respect to how physiological state shapes dopamine signals evoked by outcomes and their predictors.

nucleus accumbens | dopamine | voltammetry | learning | motivation

Reconciling differences between anticipated and experienced outcomes is fundamental for how an organism learns about the world. A key component of temporal difference (TD) learning models is the reward prediction error (RPE) term (1, 2), which is thought to be represented by phasic activity of midbrain dopamine neurons (3–5). Indeed, conditioned stimulus (CS)-related dopamine activity correlates with multiple behavioral indices of learning (6–8), and phasic dopamine signaling is sufficient to drive CS-unconditioned stimulus (US) learning (9).

In much of the supportive empirical work, food- or fluid-restricted animals first experience and then learn to anticipate an innately appetitive US (e.g., sucrose, juice, water). Thus, the US always has an inherent caloric, nutritive, or positive affective value to the organism. Consequently, it is uncertain whether dopamine neurons can acquire CS-US associations without first experiencing the US as a reward. Resolving this question is critical, because the striatal underpinnings of goal-directed behavior may encompass both RPE and experience-independent, model-based strategies (10, 11). One way to delineate dopamine's role in these different learning strategies would be to promote associations between a CS and a neutral or normally avoided US whose affective value could be manipulated and then determine the experience dependency of dopamine CS responses.

Sodium appetite is an ideal platform on which to address this question. Sodium depletion induces a powerful sodium hunger and radically but reversibly alters the rewarding value of hypertonic NaCl solutions (12, 13). The appetite is highly selective for sodium and manifests independent of prior experience with either sodium solutions or sodium deficiency (14, 15). Therefore, sodium appetite facilitates the delivery of a US (hypertonic NaCl) that is rewarding only in a specific physiological state. We measured phasic dopamine signaling in the nucleus accumbens (NAc) of rats while delivering a hypertonic NaCl solution directly into the oral cavity (intraoral) while rats were under different physiological states. We found that dopamine responses to the NaCl US were state-dependent and used this feature to investigate how physiological state influenced acquisition and expression of NaCl CS-US associations. In contrast to the US, dopamine responses to the NaCl CS depended on an interaction between experience and physiological state. Our data suggest that dopamine neurons only signal reward predictions after extensive and direct, state-dependent experience with an appetitive US and, moreover, that reward prediction signals are expressed in a state-dependent manner, a finding most consistent with TD models.

Results

Sodium appetite renders normally avoided hypertonic NaCl positively reinforcing (13). Given the link between phasic dopamine and positive reinforcement (16, 17), we first examined whether sodium appetite regulates the unconditioned dopamine response to hypertonic NaCl. We measured NAc dopamine with fast-scan cyclic voltammetry (FSCV) while delivering brief (4 s) intraoral infusions of 0.45 M NaCl to naive rats. The 0.45 M concentration was selected to maximize the ability to transform a normally avoided US into a powerful appetitive stimulus. We tested four groups of rats in different states of sodium balance: Replete (n = 4), Deplete (n = 5), Re-Replete (n = 4, sodium-depleted but allowed to restore sodium balance for 48 h before

Significance

Associating environmental cues with their outcomes occurs through multiple strategies relying on different neural substrates. Unpredicted reward evokes dopamine release, which also develops to predictive cues, suggesting that predictive dopamine signals arise only after extensive pairings of cues with appetitive outcomes. However, recent work suggests that dopamine may also contribute to model-based learning, which does not require that cues and their appetitive outcomes be experienced in tandem. Taking advantage of the appetitive value of a hypertonic sodium solution, which radically and reversibly changes with physiological state, we show that dopamine differentially encodes hypertonic NaCl depending on sodium balance independent of prior experience. Conversely, dopamine only encoded a NaCl cue after extensive, state-dependent experience, firmly supporting dopamine's role in experience-dependent learning.

Author contributions: J.J.C., G.D.S., J.E.M., and M.F.R. designed research; J.J.C., S.M.F., J.A.M., and J.E.M. performed research; J.J.C., S.M.F., J.A.M., G.D.S., J.E.M., and M.F.R. analyzed data; and J.J.C., J.E.M., and M.F.R. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1519643113/-/DCSupplemental.

testing), and Deplete + Amiloride [$n = 5$, deplete but received 0.45 M NaCl in amiloride (100 μM)]. Intraoral NaCl evoked phasic dopamine release only in Deplete rats (two-way ANOVA: epoch × group interaction: $F_{3,14} = 10.17$, $P < 0.001$; post hoc: Deplete infusion: $P < 0.001$ vs. all comparisons; Fig. 1 *A–C* and *E*). Importantly, a dopamine response was absent in Re-Replete rats, indicating the response depends on physiological state at the time of NaCl exposure. Furthermore, the dopamine response to intraoral NaCl was taste-dependent. Amiloride, which blocks lingual sodium channels and disrupts NaCl intake induced by depletion (Fig. S1), attenuated NaCl-evoked dopamine (Fig. 1*C*; Deplete + Amiloride). The dopamine response was unconditioned because (*i*) it was evident on the first infusion in NaCl naive Deplete rats (Fig. 1*B* and Fig. S2 *A* and *B*) and (*ii*) the sound associated with NaCl delivery (solenoid valve click) in the absence of intraoral NaCl did not evoke dopamine release (Fig. 1*D*). Following depletion, sodium appetite was probed by measuring overnight intake of 0.45 M NaCl (one-way ANOVA: $F_{3,14} = 27.92$, $P < 0.0001$; post hoc: Deplete: $P < 0.05$ vs. all other groups, Re-Replete: $P < 0.05$ vs. Replete and Deplete + Amiloride; Fig. 1*F*). Additional experiments suggested the lateral hypothalamus encoded NaCl taste in a state-dependent manner upstream of ventral tegmental area (VTA) dopamine neurons (Fig. S3).

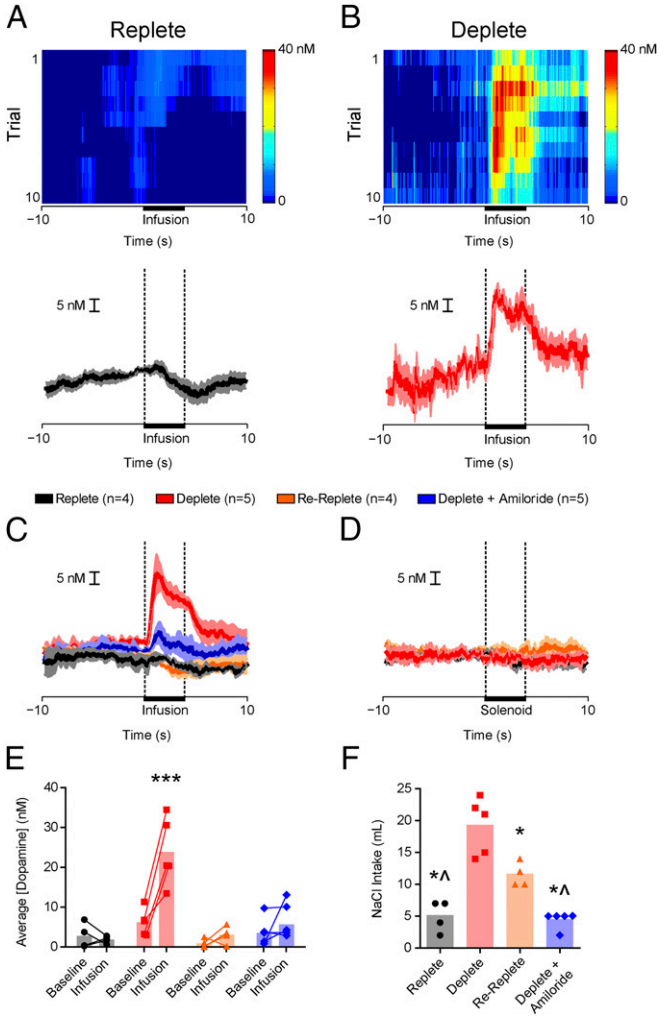

Fig. 1. Phasic dopamine signaling evoked by hypertonic NaCl depends on a taste-by-state interaction. (*A*, *Top*) Trial-by-trial (*y* axis) heat plot from a representative Replete rat depicting NAc dopamine concentration during 20 s (*x* axis) surrounding 4-s intraoral infusions of 0.45 M NaCl. (*A*, *Bottom*) Dopamine concentration from the 10 trials (mean ± SEM). (*B*) Same as in *A* in a Deplete rat. (*C*) Average dopamine concentration (±SEM) evoked by intraoral NaCl across groups (group details are provided in *Results*): Replete ($n = 4$, black), Deplete ($n = 5$, red), Re-Replete ($n = 4$, orange), and Deplete + Amiloride ($n = 5$, blue). (*D*) Average dopamine (±SEM) evoked by solenoid click (no intraoral NaCl delivered). (*E*) Average dopamine concentration during baseline (–4 to 0 s) vs. intraoral infusion (0.1–4 s). There were no differences during baseline, and intraoral NaCl evoked dopamine selectively in Deplete rats (infusion vs. all comparisons, ***$P < 0.001$). (*F*) Overnight NaCl intake following depletion (Deplete, Re-Replete, and Deplete + Amiloride) or control (Replete) treatment. *Significantly different from Deplete (at least $P < 0.05$); ^significantly different from Re-Replete (at least $P < 0.05$). Opaque bars represent the mean.

We then took advantage of the state dependency of the dopamine response to NaCl to investigate how the mesolimbic dopamine system acquires information about outcome-predictive stimuli. Rats with normal sodium balance ($n = 10$) received daily conditioning sessions where a CS (light/lever combination) was presented just before the NaCl US for seven sessions. Rats were then tested under Deplete ($n = 5$) or Replete ($n = 5$) conditions. Recordings were first made during presentations of the CS alone (i.e., in extinction). Our goal was to determine if the CS would evoke a dopamine spike when Deplete rats first experienced the CS while sodium deficient but had yet to experience the NaCl US in the new physiological state. Despite ample experience with the CS-US pairing, the NaCl CS did not evoke phasic dopamine release during extinction in either Deplete or Replete rats (two-way ANOVA: epoch: $F_{2,16} = 0.98$, $P = 0.10$; treatment: $F_{1,16} = 4.0$, $P = 0.07$; interaction: $F_{2,16} = 2.92$, $P = 0.39$; Fig. 2 *A* and *B*). Moreover, neither group exhibited conditioned-approach behavior (Fig. 2*C*). We next began a within-session reinstatement period in which the CS was paired with the NaCl US. The NaCl US evoked phasic dopamine release selectively in Deplete rats during reinstatement (two-way ANOVA: epoch × group interaction: $F_{2,16} = 4.55$, $P < 0.05$; post hoc: Deplete infusion vs. baseline or CS, both $P < 0.01$; Replete, no significant differences; Fig. 2 *D* and *E*). Deplete rats consumed significantly more postsession NaCl than Replete rats (unpaired *t* test: $t_9 = 3.22$, $P < 0.05$; Fig. 2*G*). Thus, even after 7 d of CS-US training while sodium-replete, both NAc dopamine signaling (Fig. 2 *A*, *B*, *D*, and *E*) and the behavior (Fig. 2 *C* and *F*) of Deplete rats closely resembled subjects with no prior CS training with an appetitive US.

The previous experiment suggested that the acquisition of dopamine reward predictions requires that the predicted outcome first be experienced as appetitive. Thus, we tested whether a single day of NaCl CS-US training while rats were sodium deficient would condition dopamine and/or behavioral responses to a NaCl cue. One group of rats was depleted 24 h before a single NaCl CS-US training session ($n = 4$, Trained Deplete), whereas another was depleted and allowed to recover for 48 h before training ($n = 4$, Trained Replete). Twenty-four hours after depletion, all rats were given overnight access to 0.45 M NaCl to confirm sodium appetite. Postdepletion NaCl consumption did not differ between groups (unpaired *t* test: $t_6 = 0.55$, $P = 0.60$). Thus, by the time of the recording session, both groups had equivalent experience with sodium depletion, CS-US training, and NaCl exposure, although one group had CS-US training paired and the other had training unpaired with sodium deficiency. Twenty-four hours before the test session, Trained Replete and Trained Deplete rats were again depleted of sodium. The following day, dopamine measurements were made with FSCV. A single CS-US training session while sodium deficient was insufficient to condition a dopamine response to the NaCl CS (Fig. 3). In contrast, the US evoked phasic dopamine release regardless of training history (two-way ANOVA: epoch: $F_{2,12} = 107.6$, $P < 0.0001$; training history: $F_{1,12} = 0.04$, $P = 0.83$; interaction: $F_{2,12} = 7.13$, $P < 0.01$; post hoc: both groups infusion > baseline, cue epochs, all at least $P < 0.01$, no difference from baseline during cue epoch for either group; Fig. 3 *A* and *B*). In addition, the CS failed to evoke conditioned-approach behavior

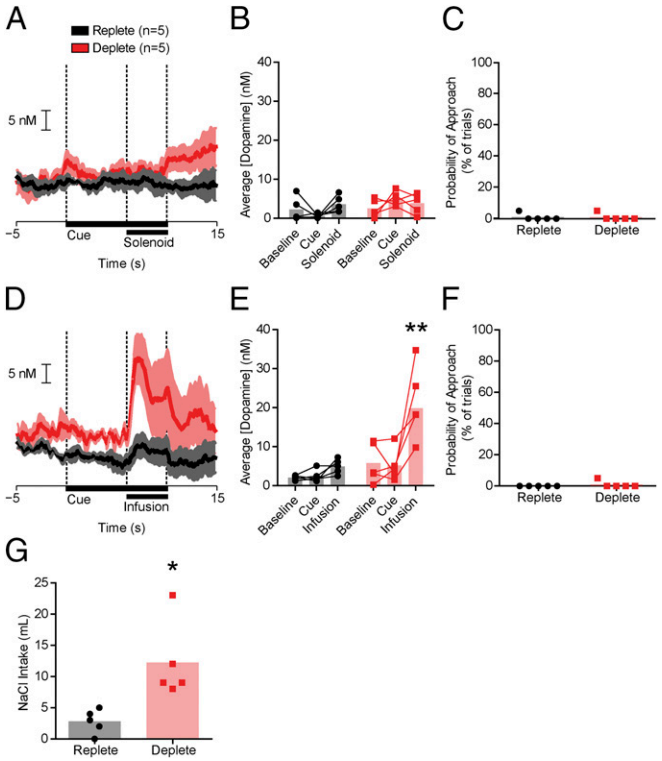

Fig. 2. Seven days of CS-US training while sodium-replete does not condition responses to a sodium CS. (*A*) Average dopamine concentration (±SEM) during extinction for Replete (n = 5, black) and Deplete (n = 5, red) rats. (*B*) Neither the CS nor the solenoid click evoked dopamine during extinction compared with baseline. (*C*) Approach behavior during extinction. (*D*) Average traces of dopamine concentration (±SEM) during reinstatement. (*E*) Intraoral NaCl significantly increased dopamine concentration in Deplete rats. Deplete, infusion vs. baseline or CS (**$P < 0.01$); Replete, no significant differences (all comparisons, $P > 0.05$). (*F*) Approach behavior during reinstatement. (*G*) Postrecording session sodium intake was elevated in deplete rats (*$P < 0.05$). Opaque bars represent the mean.

(Fig. 3*C*). Sodium appetite at the time of dopamine measurements was probed by measuring overnight intake of 0.45 M NaCl, which confirmed a sodium appetite in both groups (unpaired t test: $t_6 = 0.85$, $P = 0.42$; Fig. 3*D*). Thus, a single training session that paired a CS with an appetitive US was insufficient for the development of a dopamine reward prediction signal.

We next explored the possibility that extensive experience with the appetitive features of the predicted US is essential to condition dopamine reward prediction signals. We sodium-depleted two groups of rats four times and conducted four CS-US training sessions. For one group, training was always conducted while sodium deficient (Paired, n = 12). The other group was trained preceding/after recovery from depletion (Unpaired, n = 5; *SI Experimental Procedures*). During training, Paired rats developed preliminary signs of conditioned-approach behavior (Fig. S4). After training, we sodium-depleted a subset of Paired rats (n = 5 of 12) and all Unpaired rats 24 h before the FSCV recording session. The CS evoked dopamine release only in Paired rats, whereas the NaCl US, but not the CS, evoked dopamine release in Unpaired rats (two-way ANOVA epoch × training history interaction: $F_{2,16} = 10.33$, $P < 0.01$; post hoc: Paired, CS vs. baseline or infusion, both $P < 0.01$; Unpaired, infusion vs. baseline or CS, both $P < 0.05$; Fig. 4 *A* and *B*). Only Paired rats exhibited conditioned-approach behavior (unpaired t test: $t_9 = 5.39$, $P < 0.001$; Fig. 4*C*). Both groups consumed NaCl after the recording session, eliminating attribution of these differences to sodium appetite at the time of dopamine measurements (Welch's corrected t test: $t_4 = 0.60$, $P > 0.05$; Fig. 4*D*).

Because physiological state influenced the acquisition of dopamine prediction signals, we next sought to determine whether physiological state would affect their expression. We first tested Paired rats (n = 7, trained as above) in the absence of sodium need (Paired-Replete) and later obtained a second recording from a subset of these same animals while they were sodium-deficient (Paired-Deplete, n = 4 of 7). In absence of sodium need (Paired-Replete), the CS did not evoke dopamine release. However, 2 d later, once sodium appetite was induced (Paired-Deplete), the sodium CS evoked a large dopamine response (two-way ANOVA epoch × state interaction: $F_{2,18} = 6.23$, $P < 0.01$; post hoc: Paired-Replete, no significant differences; Paired-Deplete, CS vs. baseline, $P < 0.01$; Fig. 4 *E* and *F*). In the Paired-Deplete condition, rats tended to show more conditioned-approach behavior compared with Paired-Replete (Mann–Whitney U test, $P = 0.18$; Fig. 4*G*). Moreover, NaCl consumption following the recording session was significantly elevated in the Paired-Deplete condition relative to Paired-Replete condition (Welch's corrected t test: $t_3 = 3.31$, $P < 0.05$; Fig. 4*H*), thereby confirming sodium appetite. Thus, using the same group of rats, we show that the dopamine response to the CS is flexibly expressed based on physiological state.

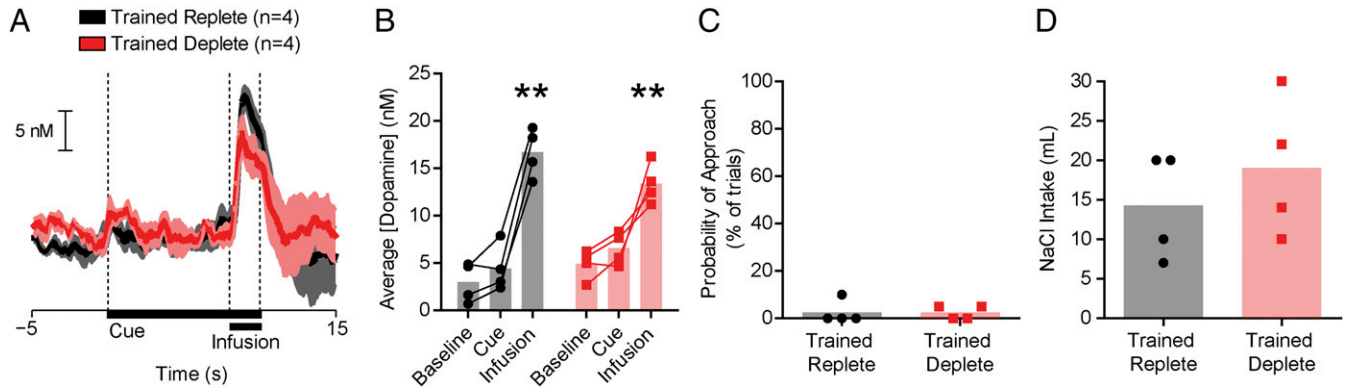

Fig. 3. One day of sodium CS-US training while deplete is insufficient to condition a dopamine response to the sodium CS. (*A*) Average dopamine concentration (±SEM) for Trained Replete (n = 4, black) and Trained Deplete (n = 4, red) rats. (*B*) NaCl US, but not CS, evoked phasic dopamine release regardless of training history. Dopamine concentration during the infusion was significantly greater than both the baseline and cue periods in both groups (**$P < 0.01$). Dopamine concentration during the CS was not greater than baseline for either group. (*C*) Neither group approached the cue lever during the CS. (*D*) Postrecording session NaCl consumption did not differ between groups.

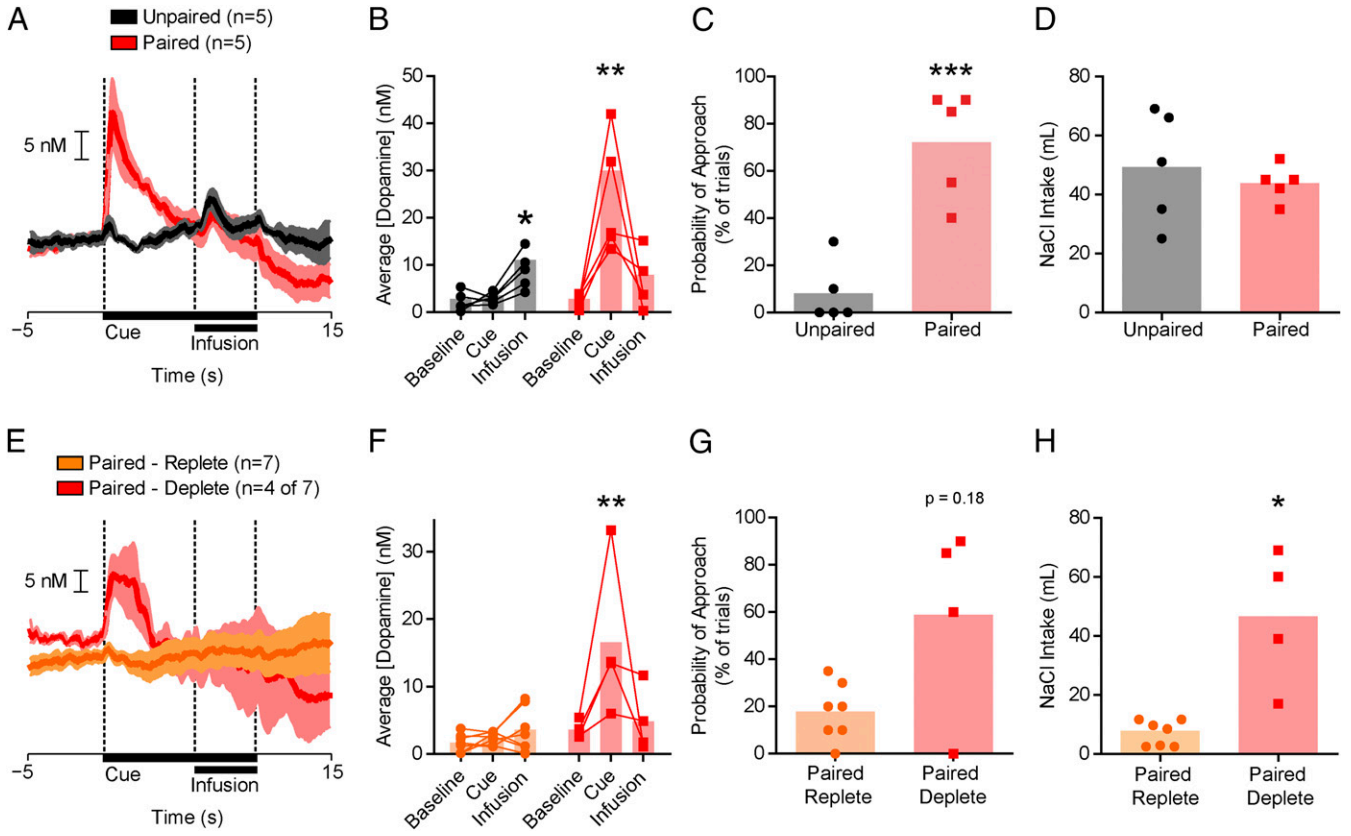

Fig. 4. CS-US associations are acquired and expressed in a state-dependent manner. (*A*) Average dopamine concentration (±SEM) for a subset of Paired ($n = 5$ of 12, red) and Unpaired ($n = 5$, black) rats. (*B*) Sodium CS evoked phasic dopamine release only in Paired rats. Paired, CS vs. baseline or infusion (**$P < 0.01$); Unpaired, infusion vs. baseline or CS (*$P < 0.05$). (*C*) Conditioned-approach behavior for Paired and Unpaired rats (***$P < 0.001$). (*D*) Postrecording session sodium intake did not differ between Paired and Unpaired rats. (*E*) Average dopamine concentration (±SEM) in Paired rats tested while replete (Paired-Replete; $n = 7$ of 12, orange) or deplete (Paired-Deplete; second recording obtained in four of seven rats, red). (*F*) NaCl CS evoked dopamine only in Paired-Deplete rats. Paired-Deplete CS vs. baseline (**$P < 0.01$); Paired-Replete, no significant differences (all comparisons, $P > 0.05$). (*G*) Conditioned-approach behavior for Paired-Replete vs. Paired-Deplete rats ($P = 0.18$). (*H*) Postrecording session NaCl intake for Paired-Replete vs. Paired-Deplete rats (*$P < 0.05$). Opaque bars represent the mean.

Discussion

Appetitive and nonpreferred/aversive stimuli differentially modulate dopamine signaling (18–20). In turn, the presence or absence of a phasic increase in dopamine in response to a primary stimulus can differentially drive learning about predictive cues and reinforce goal-directed behavior (9). We leveraged the fact that the appetitive qualities of a hypertonic NaCl solution strongly depend on physiological state. We found that the NaCl US evoked phasic dopamine release only in Deplete rats and this dopamine release did not require prior US experience (Fig. 1 and Fig. S2). Importantly, the response to the US was taste- and state-dependent. In contrast, the mesolimbic system acquired information about the CS only through extensive and direct, state-dependent experience with the US (Figs. 2–4 *A*–*D*). Once the NaCl CS-US association was learned, the phasic dopamine response to the CS was flexibly expressed according to physiological state (Fig. 4 *E* and *F*). The results have broad implications for how predictive dopamine signals are acquired, updated, and expressed.

The "real-time" responses of dopamine neurons to unconditioned affective stimuli have been visited (21), and revisited (19), yet considerable debate remains (22). Here, using intraoral delivery, we show in naive rats that dopamine release in the NAc core is robustly evoked when NaCl is appetitive but unchanged when it would be avoided. This differential encoding of the same stimulus was independent of prior learning or experience but dependent on physiological state and the ability to detect the sodium ion in solution, both of which are prerequisites for the avid consumption of hypertonic NaCl (23). It is notable that we did not observe a change in dopamine concentration following intraoral NaCl infusions in Replete rats. Previous studies demonstrated that innately or learned aversive stimuli (e.g., quinine, sucrose previously paired with LiCl to induce a conditioned taste aversion) suppress dopamine release (24, 25). However, concentrations of NaCl similar to the concentration used here (0.5 M) evoke a mixture of appetitive and aversive taste reactivity that switches to entirely appetitive following sodium depletion (26). Moreover, whether dopamine neurons encode nonpreferred/noxious/aversive stimuli with decreases, no change, or increases in firing rate may depend on anatomical location in the midbrain (18, 27) and projection target (20, 28). Thus, it remains possible that dopamine terminal fields outside the NAc core may yield different patterns of release. In addition, higher concentrations of NaCl (>0.5 M) may have yielded different results because these concentrations have been shown to recruit amiloride-insensitive taste pathways typically activated by aversive, nonsodium taste stimuli [bitter and sour (29)]. Still, our results demonstrate instant updating of dopamine responses to primary stimuli without need for prior experience. Sodium appetite is a long-studied, striking example of goal-directed behavior. Sodium-deficient animals avidly consume concentrated sodium solutions compared with animals with no sodium deficit. Moreover, the expression of sodium appetite is unlikely to depend on postingestive experience (14). We hypothesize that the ability of sodium taste to drive neuronal responses that support behavioral reinforcement (16, 17) is highly adaptive and helps to ensure

rapid and immediate sodium consumption without need for postingestive learning.

Phasic dopamine responses to reward-predictive cues are arguably a fundamental brain signal, with evidence supporting their existence in mice (19), rats (3), monkeys (18), and humans (30). Cue-evoked dopamine signals serve to invigorate goal-directed behaviors aimed at the impending reward (31). Unlike our results with the NaCl US, dopamine reward prediction signals did not instantaneously update, and therefore did not simply reflect the change in the affective value of the US (Fig. 2). Instead, for dopamine responses to develop to a predictive cue, animals had to experience the CS-US pairing under conditions in which the US was appetitive (Fig. 4). Moreover, the pairings between a CS and an appetitive outcome must be extensive (Fig. 3). Previous work suggested a correlation between cue-evoked dopamine release and the development of conditioned-approach behavior (6). We found a similar relationship that further supports a role for dopamine in promoting learned approach behavior.

Our data reveal striking differences with respect to how previous experience and physiological state interact to modulate dopamine prediction signals. Given that sodium-deficient animals will consume hypertonic NaCl without needing to learn that the solution will relieve their deficit (14), it is notable that following a change in physiological state, we failed to observe instant updating of the value of the cue in either approach behavior or the phasic dopamine response (Fig. 2). The lack of instant behavioral updating contrasts with both an older report (13) and a recent report (11). Importantly, there were many methodological differences between the current work and previous work, including sodium depletion strategies, sex, and NaCl concentration. Given that the higher salt concentrations used in the previous work would also have activated sour and bitter taste receptors (29), taste-mediated, experience-independent learning may not rely on sodium ion transduction; instead, it may rely on other pathways. However, the most striking differences relate to training history. In both previous reports, rats underwent some form of pretraining where they learned cue- or response-outcome associations for a nonsodium US (sucrose, water). It is also critical to note that neither study measured phasic dopamine signaling, and thus cannot speak to dopamine prediction signals. We show that both behavior and the dopamine response to a NaCl CS are flexibly expressed with physiological state, but only after multiple days of training under deplete conditions.

FSCV combined with sodium appetite enabled us to conclude that acquisition of dopamine reward prediction signals is consistent with RPE models rather than model-based strategies. Work in nonhuman primates has shown that dopamine RPEs are modulated by an external context that dictates the likelihood a given trial will be rewarded (32). The authors explained the modulation using a TD model that featured a context parameter. Our data therefore reflect the ability of a subject's internal context (physiological state) to modulate RPE expression once it has been learned. Moreover, we have previously shown that physiological state (e.g., hunger and associated hormones) augments the magnitude of dopamine responses to primary rewards (33) and their predictors (34). Thus, physiological state powerfully augments the magnitude, acquisition, and expression of reward-related responses in the mesolimbic system. A recent study in humans found evidence for both model-based and model-free learning strategies in the striatum (10). Because this work used functional MRI, it was unknown which striatal inputs carried model-based vs. model-free information. Our results strongly suggest that during initial learning, mesolimbic dopamine does not contribute to model-based encoding at the level of the ventral striatum.

In contrast to the experience-based acquisition of CS-US associations, the absence of a dopamine CS response in Paired-Replete rats during their first need-free session (Fig. 4 *E* and *F*) is inconsistent with a standard model-free account of how dopamine reward prediction signals are expressed. One potential explanation is that physiological state acts similar to a discriminative stimulus and "gates" the expression of a learned association. The predictive values of model-free discriminative stimuli are normally learned through experiencing different combinations of states and their associated outcomes. However, Paired-Replete rats never before experienced intraoral sodium infusions or predictive cues in a need-free condition. Thus, physiological state may act as a discriminative stimulus through a model-based process allowing rats to infer the change in value of NaCl upon the first CS exposure in a need-free state, and this inference was reflected by the lack of a dopamine response to the CS in a low-value context.

In sum, our data suggest that differential encoding of primary affective stimuli (here, the same taste stimulus) by dopamine neurons can manifest independent of learning. In contrast, dopamine neurons only acquire cue-outcome associations through direct and extended experience with primary stimuli that have positive affective value. Moreover, once these associations are formed, they are expressed as a function of their current value to the organism. Our findings parallel recent studies in *Drosophila* demonstrating that dopamine neurons facilitate the formation and expression of nutrient-related memories in a state-dependent manner (35, 36), suggesting a highly conserved process. Thus, different physiological states give rise to unique subjective experiences, which can have profound influences on brain substrates of associative learning.

Experimental Procedures

Subjects. Male Sprague–Dawley rats (Charles River) weighing 375–475 g were used. This study reports data obtained from 60 rats. Most attrition resulted from loss of patency of the intraoral cannula or the inability to lower a recording electrode successfully into the NAc on test days. Because we were unable to obtain a recording from these animals, we omitted all of their data from inclusion in the study. Rats were individually housed with lights on from 7:00 AM to 7:00 PM and tested during the light phase. Animal care and use was in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (37), and was approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

Sodium Depletion Protocol. Sodium appetite was induced by two injections of furosemide (10 mg/kg, s.c.; Sigma) spaced 1 h apart. Diuresis was verified by weight loss of $\geq$20 g in the 1 h following the second injection. During the 24-h depletion, rats were housed in wire-bottom cages to prevent consumption of urine and had ad libitum access to a sodium-deficient diet (Teklad Sodium Deficient Diet; Harlan) and distilled water. Vehicle-treated rats were also housed in wire-bottom cages but given regular chow and water. Upon being returned to their home cage, rats were given ad libitum 24-h access to 0.45 M NaCl to restore lost sodium and ad libitum access to normal chow and water. Overnight water and NaCl intake, as well as body weight, were recorded during the 24-h repletion period.

Apparatus. All sessions took place in a standard operant box (Med Associates) inside a sound-insulated chamber. An infusion line from a syringe containing 0.45 M NaCl was attached to a solenoid valve (flow rate = 50 $\mu L \cdot s^{-1}$; The Lee Company) and suspended outside the sound-insulated chamber. The infusion line passed through a commutator (Crist Instruments) and was connected to the rats' intraoral cannula. Before all sessions, the intraoral cannula was flushed with distilled water to ensure patency. For experiments involving amiloride, intraoral cannulas were first flushed with 100 μM amiloride in distilled water to ensure epithelial sodium channels were blocked in advance of the first intraoral infusion.

FSCV Protocol. FSCV in awake and behaving rats and analyte identification and quantification have been extensively described previously (38). Briefly, a micromanipulator containing a glass-insulated carbon fiber (~75 μm; Goodfellow USA) (recording) electrode was inserted into the NAc guide cannula. The recording electrode was then lowered into NAc and locked into place. An FSCV head stage (University of Washington Electronics and Materials Engineering Shop) was used to tether the rat, apply voltage changes, and measure resultant current changes. The electrode was held at −0.4 V and ramped in a triangular fashion (−0.4 to +1.3 to −0.4 V, 400 $V \cdot s^{-1}$; "scan").

While recording, scans were applied at 10 Hz. To verify that the recording location supported phasic dopamine release, electrical stimulation was delivered to the VTA (24 pulses, 60 Hz, 120 μA). If this electrical stimulation failed to evoke dopamine release, the recording electrode was advanced 0.16 mm and the process was repeated. Once a stable release site was confirmed (Figs. S5 and S6), the experimental session began. After the recording session, electrodes were removed; rats were disconnected from the head stage and returned to their home cage.

FSCV Data Analysis. Electrochemical data were recorded during the entire session. Individual trials were background-subtracted, and dopamine concentration surrounding the opening of the solenoid (intraoral infusion sessions) or the CS onset (conditioning sessions) was extracted from voltammetric data using principal component analysis (39). For intraoral infusion experiments, we calculated the average dopamine concentration during the 4-s baseline immediately before infusion (−4 to 0 s) and compared this concentration with the average dopamine concentration evoked by infusion of NaCl (0.1–4 s; Fig. 1). For conditioning experiments, we calculated the average dopamine concentration during the 2-s baseline period immediately before CS onset (−2 to 0 s) and compared this concentration with the average dopamine concentration evoked during the first 2 s of the CS (0.1–2 s) and the first 2 s of the subsequent intraoral infusion (6–8 s in Figs. 2 and 4 and 8–10 s in Fig. 3).

Statistical Analysis. For intraoral infusions, dopamine concentration evoked during the epoch of interest was compared using a two-way [epoch (baseline, infusion) × treatment (Replete, Deplete, Re-Replete, Deplete + Amiloride)] ANOVA. For conditioning experiments, dopamine concentration was compared using a two-way ANOVA with main effects of epoch (baseline, cue, infusion) and treatment (Replete, Deplete; Fig. 2), 1-d training (Trained Replete, Trained Deplete; Fig. 3), training history (Paired, Unpaired; Fig. 4), or testing (Paired-Replete, Paired-Deplete; Fig. 4) conditions. One-way ANOVAs and Tukey's honest significant difference post hoc tests were used where appropriate. NaCl intake and measures of approach behavior were compared separately using a two-tailed unpaired *t* test, Welch's corrected *t* test, the Mann–Whitney *U* test, or Wilcoxon's matched pairs test (two groups) or one-way ANOVA (more than two comparisons). Statistical analyses were performed using GraphPad 5.0 (Prism, Inc.), MATLAB (MathWorks), or SPSS Version 20.0 (IBM).

ACKNOWLEDGMENTS. We thank Dr. John H. R. Maunsell for helpful comments and Pawel Bujakowski for assistance with video scoring. This work was supported by NIH Grants R01 DA025634 (to M.F.R.), K01 DA033380 (to J.E.M.), R01 DA038168 (to G.D.S.), and T32 MH093315 (to J.A.M.) and by the University of Illinois at Chicago Dean's Scholar Fellowship (to J.J.C.).

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