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Putative γ -aminobutyric acid neurons in the ventral tegmental area have a similar pattern of plasticity as dopamine neurons during appetitive and aversive learning

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Abstract

Dopamine influences affective, motor and cognitive processing, and multiple forms of learning and memory. This multifaceted functionality, which operates across long temporal windows, is broader than the narrow and temporally constrained role often ascribed to dopamine neurons as reward prediction error detectors. Given the modulatory nature of dopamine neurotransmission, that dopamine release is activated by both aversive and appetitive stimuli, and that dopamine receptors are often localized extrasynaptically, a role for dopamine in transmitting precise error signals has been questioned. Here we recorded from ventral tegmental area (VTA) neurons, while exposing rats to novel stimuli that were predictive of an appetitive or aversive outcome in the same behavioral session. The VTA contains dopamine and γ -aminobutyric acid (GABA) neurons that project to striatal and cortical regions and are strongly implicated in learning and affective processing. The response of VTA neurons, regardless of whether they had putative dopamine or GABA waveforms, transformed flexibly as animals learned to associate novel stimuli from different sensory modalities to appetitive or aversive outcomes. Learning the appetitive association led to larger excitatory VTA responses, whereas acquiring the aversive association led to a biphasic response of brief excitation followed by sustained inhibition. These responses shifted rapidly as outcome contingencies changed. These data suggest that VTA neurons interface sensory information with representational memory of aversive and appetitive events. This pattern of plasticity was not selective for putative dopamine neurons and generalized to other cells, suggesting that the temporally precise information transfer from the VTA may be mediated by faster acting GABA neurons.

Keywords

addiction; freely moving rat; memory; reward; schizophrenia

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Supporting Information Additional supporting information may be found in the online version of this article:

Fig. S1. Example of VTA recording data and recording site.

Fig. S2. The difference in responses to CS_{av} and CS₊.

Fig. S3. Changes in the proportion of responsive neurons to either CS during sessions 1–16.

Fig. S4. Examples of neurons recorded on the same channel during several sessions across the contingency reversal.

Fig. S5. Effects of sugar pellet delivery and shock on VTA neurons.

Fig. S6. VTA neural response profiles to CSs and USs.

Fig. S7. Comparison of the neural responses of different firing rate VTA neuron groups.

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Introduction

Dopamine is implicated in nearly all aspects of higher brain function including goal-directed movement, affective processing, multiple forms of learning and memory, and executive functions (Beninger, 1983; Goldman-Rakic, 1996; Frank *et al.*, 2004; Wise, 2004; Gibbs *et al.*, 2007). Our understanding of the role of dopamine, however, is hindered by several dichotomies in the literature. Dopamine neurons show rapid stereotypical excitatory responses to sensory stimuli (Ljungberg *et al.*, 1992; Horvitz *et al.*, 1997; Dommett *et al.*, 2005) or learned responses to reward-related cues (Mirenowicz & Schultz, 1996; Fiorillo *et al.*, 2003). However, dopamine is a slow-acting neuromodulator (Rice & Cragg, 2008) with its post-synaptic receptors mostly distal from the synapse (Smiley *et al.*, 1994; Paspalas & Goldman-Rakic, 2004; Pinto & Sesack, 2008). Its functional role in target areas is therefore more consistent with a role in the modulation of precise excitatory or inhibitory post-synaptic potentials mediated by fast-acting neurotransmitters such as glutamate or γ -aminobutyric acid (GABA) (Seamans & Yang, 2004). Thus, although dopamine neurons fire rapidly in response to reward-related cues, the phasic dopamine signal probably encodes reinforcement information in combination with a more temporally and spatially precise signal. Although there are many possible sources for such a signal, the ventral tegmental area (VTA) contains intermingled dopaminergic and projection GABA-ergic neurons (Carr & Sesack, 2000), which receive overlapping input from a number of regions. This raises the interesting possibility that non-dopaminergic neurons in the VTA may likewise participate in the encoding of information commonly ascribed to the dopamine system.

To address these issues, we recorded from multiple classes of VTA neurons during a modified classical conditioning task in which two novel conditioned stimuli (CSs) predicted different unconditioned stimuli (USs). One CS was followed by an appetitive outcome and the other by an aversive outcome. This allowed us to sample the response of the same population of neurons to both CSs during the same recording sessions. We found that VTA neurons, regardless of dopamine-like or GABA-like waveform shapes, developed similar patterns of outcome-specific responses as associative learning progressed. This suggests that dopamine and GABA neurons in the VTA participate similarly in encoding novel sensory stimuli and the learning of associating those stimuli with appetitive or aversive outcomes. Given the neuromodulatory nature of dopamine signaling (Rice & Cragg, 2008), we suggest that a concomitant response of VTA GABA neurons to salient events might provide a mechanism through which temporally precise information is transferred out of the VTA to the same regions targeted by dopamine neurons.

Materials and methods

Subjects

Adult male Sprague–Dawley rats weighing 300–360 g were used ($n = 7$). Rats were housed individually on a 12 h light/dark cycle (lights on at 19:00 h). All experiments were performed during the dark phase when the animals are most active. All procedures were in accordance with the University of Pittsburgh's Institutional Animal Care and Use Committee.

Electrophysiological experiments

Seven rats were chronically implanted with microelectrode arrays (NB Laboratories, Denison, TX, USA) into the VTA [target coordinates for the center of array at AP, -5.3 ; ML, 0.5 – 1.1 ; DV, 7.7 – 8.3 mm (Paxinos & Watson, 1998)]. Electrodes were implanted under isoflurane anesthesia as described previously (Jackson *et al.*, 2004; Homayoun &

Moghaddam, 2006). The electrode arrays consisted of eight 50- μ m-diameter Teflon-insulated, stainless steel wires arranged in a 2×4 pattern (0.25×0.7 mm) with 300–700 k Ω impedance. Recordings were made during daily behavioral sessions. Extracellular single-unit recordings were performed on up to 16 channels simultaneously. The signal passed through a unity-gain JFET headstage amplifier before being analog filtered (0.3–8 kHz band pass) and amplified $1000 \times$ (Plexon, Dallas, TX, USA). The amplified signal from each electrode was digitized (40 kHz sampling rate) and each waveform segment (1.2–3 ms) that crossed an experimenter-defined threshold was saved for offline sorting. For an example filtered voltage trace, see Supporting Information Fig. S1A.

Behavioral task

At a minimum of 1 week after surgery, rats were placed on a food-restricted diet of 15 g of rat chow per day and given 2 days of handling and habituation to the experimental chambers (Coulbourn Instruments, Allentown, PA, USA). Fortified sugar pellets (10 dustless precision pellets 45 mg; Bioserv, Frenchtown, NJ, USA) were placed by the investigator in the food trough of each chamber and were consumed by the animals during habituation. After habituation, animals began the first session of a double conditioning task. During sessions 1–8, two different CSs were randomly associated with appetitive or aversive USs. The CSs consisted of a 10 s duration tone or a flashing house light. The USs, which were presented immediately after the termination of either CS, were a sugar pellet (appetitive US) delivered to the food trough or a mild electric shock (aversive US) (180 ms, 0.2 mA) delivered through a stainless steel grid floor (Coulbourn Instruments). Rats underwent 60 trials (30 trials of each contingency) delivered pseudo-randomly with a 20 s intertrial interval (Fig. 1A). After animals learned the initial appetitive CS (CS_{ap})–appetitive US and aversive CS (CS_{av})–aversive US associations for eight sessions, the appetitive and aversive CS–US contingencies were reversed across sessions 9–16 for six of the seven rats. The experimental procedures and behavioral recordings were performed with GRAPHIC STATE software (Coulbourn Instruments). During the behavioral task, rats were connected to a lightweight headstage cable attached to a commutator (NB Laboratories) that allowed rats to move freely in the experimental chamber.

Classification of units

Units recorded on the same electrodes in different sessions were counted as distinct units despite the fact that some of the recorded units had a similar spike shape and firing rate across sessions. Although some units were sampled more than once, this ensured that different units were not incorrectly assumed to be the same. The population activity and percentage of responding units were measured across sessions. Spike sorting was performed with OFF-LINE SORTER software (Plexon) using a combination of automatic and manual methods as described previously (Jackson *et al.*, 2004; Jackson & Moghaddam, 2006). After careful isolation, each unit was classified by its average spike waveform ($n = 784$). Initially, natural clusters of spike waveforms (1.2 ms in duration) were identified by *K*-means cluster analysis with correlation method ($K = 10$). Ultimately, all but 14 units (which were excluded because their spike waveforms were difficult to classify) were clustered in three groups based on width and after-response hyperpolarization of the waveform. Neurons with a long-duration triphasic waveform were designated as Type I ($n = 529$). Neurons with a short-duration waveform were designated as Type II ($n = 113$). A third group ($n = 128$) had medium-duration action potentials without a triphasic shape and was classified as Type III. It should be emphasized that, even though we used common single-unit recording techniques to classify cell types in freely moving animals, potential misclassification errors are associated with this approach (Margolis *et al.*, 2006; Brischoux *et al.*, 2009). Our results should be interpreted with this caveat in mind.

Histology

Animals were anesthetized with chloral hydrate and intracardially perfused with saline, followed by 10% buffered formalin. Fixed brains were cut in 60–100 μm slices and sections were stained with cresyl violet to locate the microdialysis probe and electrode placements. Under a light microscope, the tips of recording electrodes and stimulation electrodes were confirmed to be in the VTA (Fig. S1B).

Data analysis

Behavior—We used the number of nose pokes into the food trough as an index of the conditioned behavior (Wan & Peoples, 2008). For each recording session, the number of food trough nose pokes was tabulated during each CSap and CSav presentation interval: CSap (10 s) $\times$ 30 trials, CSav (10 s) $\times$ 30 trials. This measure was normalized by dividing the number of nose pokes during each CS by the number of nose pokes during the second half of each 20 s intertrial interval (10 s) $\times$ 60 trials (R ; if no conditioning expected $R = 0.5$). Instead of using only the 30 intertrial intervals preceding each specific CS, we pooled the nose pokes from all 60 intertrial intervals for this measure. Importantly, there was no significant difference in the number of nose pokes preceding the CSap and CSav (t -test, $P > 0.05$, 17.66 ± 1.39 and 18.44 ± 1.46 , respectively).

Neural activity—To identify neural responses to the stimuli, we compared the neural activity before and after each stimulus. For a baseline period, we used the 5 s interval (–5.5 to –0.5 s) before each CS onset. Baseline values were compared with neural activity in the 0.5 s after stimulus onset. For neural population analysis, each neuron's Peri-Stimulus Time Histogram (bin size 50 ms) was normalized with firing rate (NEUROEXPLORER; Nex Technologies, Littleton, MA, USA). The normalized values were then averaged for all of the neurons in the population. The population response was quantified as the summation of activity from zero to 0.5 s. In addition to between-session analyses of neural activity, within-session analyses were conducted by binning the 30 presentations of each CS (60 total trials) into five bins of six consecutive trials. The responses of each neuron to a stimulus were normalized in these bins. In addition, a sliding receiver operating characteristic (ROC) analysis was used to examine the time-course of the neuronal response to the CSs (Wallis & Miller, 2003). The area under the ROC curve was utilized as an index of neural selectivity between the CSap, CSav and baseline periods. A value of 0.5 was indicative of no difference in neural activity between two conditions. ROC values were calculated with a sliding 100 ms window in 20 ms steps.

All statistics were performed with MATLAB software (Mathworks, Natick, MA, USA). Paired t -tests were used to compare individual neural responses to each stimulus ($\alpha = 0.01$) and to test behavioral biases to CSap and CSav ($\alpha = 0.05$). The relationship between the magnitude of the population response to each CS and the number of sessions ($n = 8$) was assessed using Pearson's correlation analysis. The relationship between the proportion of neurons responsive to each CS and the number of sessions ($n = 8$) was also assessed using Pearson's correlations. Chi-square tests ($\alpha = 0.05$) were used to compare the proportion of responding neurons between groups. The comparisons between the population responses of each group were analyzed with two-way repeated-measures anova.

Results

Behavior

The R_{ap} increased during sessions 1–8, indicating that Pavlovian approach behavior increased during CSap as learning progressed ($r = 0.93$, $P < 0.01$). Conversely, the R_{av} was < 0.5 in sessions 2, 4, 6 and 8 (t -test, $P < 0.05$), indicating that the rats learned the

association between both the CS and US. Following the CS–US reversal, the R_{ap} and R_{av} shifted according to the new contingencies. Interestingly, responding to the new CSav was not depressed below 0.5 although, during sessions 9–16, responding was decreased during the presentation of the new CSav and increased during the presentation of the new CSap ($r = -0.92$, $P < 0.01$ and $r = 0.77$, $P < 0.05$, respectively, Fig. 1B). We performed an additional control experiment that contained a CS+ (appetitive) and a CS– (no association). Doing this allowed us to compare our previous CSav responses with CS– to make sure that previously measured responses to CSav reflected a learned aversive association rather than simply no association (Fig. S2). We observed that responses to the CSav were reduced (below $R = 0.5$) but the responses to the CS–, which had no associated contingency, were not depressed.

Ventral tegmental area neural activity to appetitive and aversive conditioned stimuli

A total of 784 units were recorded during 102 sessions in seven rats (average 7.7 units per recording session). VTA neurons had similar excitatory responses to either CS during sessions 1–2 at which point both stimuli were novel and animals did not show a biased behavioral response (Figs 1B and 2A–C) and population activities to the CSap and CSav did not differ significantly ($P > 0.05$, repeated-measures two-way ANOVA, Fig. 2B). Likewise, the proportion of the neurons responding with excitation to the CSs did not differ as 16.5% (14/85) responded to the novel CSap and 17.6% (15/85) responded to the novel CSav in sessions 1–2 ($P > 0.05$, Chi square test, Fig. 2C).

In subsequent sessions, as associative learning progressed, the responses to the CSap became more excitatory, whereas responses to the CSav became primarily inhibitory.

The change in response to the CSap or CSav was reflected in both the magnitude of population activity (Fig. 2B, left) and the proportion of the responding neurons (Fig. 2C, shaded area). As learning progressed from session 1 to 8, the population responses to the CSap became larger ($r = 0.918$, $P < 0.01$), whereas the population responses to the CSav became smaller ($r = -0.960$, $P < 0.01$). On session 7–8, 43.8% (46/105) of neurons exhibited excitatory responses to the CSap, whereas 12.4% (13/105) of neurons decreased their activity to the CSav ($P < 0.01$, Chi square test). There was a strong positive correlation between session number and the number of neurons with excitatory responses to the CSap ($r = 0.809$, $P < 0.05$) and a strong negative correlation between session number and the number of neurons exhibiting excitatory responses to the CSav ($r = -0.883$, $P < 0.01$). We also observed a positive correlation between session number and the proportion of neurons inhibited during the CSav ($r = 0.818$, $P < 0.05$). Those units responding with inhibition to the CSav were the same neurons exhibiting excitation to the CSap.

When contingencies were reversed in session 9, the neural response evoked by the CSs shifted correspondingly, such that the response to the previous CSap, which then predicted the aversive outcome, changed to inhibition and responses to the previous CSav, which then predicted an appetitive outcome, became excitatory. After the reversal, the population responses evoked by the new CSap (previous CSav) were predominantly excitatory and the population responses evoked by the new CSav (previously CSap) became predominantly inhibitory. Again, the magnitude of the population response to both the CSap and CSav was strongly correlated with the session number ($r = 0.925$ and $r = -0.973$, respectively, $P < 0.01$ in all cases, Fig. 2B, right). In comparing the proportion of responsive neurons in sessions 9–16, more neurons were depressed by the new CSav and excited by the new CSap ($r = -0.932$ and $r = 0.872$, respectively, $P < 0.01$ in all cases, Fig. 2C). We also found similar responses when using a 1 s window (Fig. S3). An example of a single-unit response is depicted in Fig. S4.

Examination of the trial-by-trial neural response during the first session of reversed contingencies (session 9) showed that VTA neurons began exhibiting activated and inhibited responses to the new contingencies during this session (Fig. 2D and E). The response to the new CSap was increased ($r = 0.988$, $P < 0.01$) and the response to the new CSav was decreased ($r = -0.988$, $P < 0.01$) during the course of the session ($n = 53$).

In addition, the neural responses to sugar pellet delivery and foot shock were assessed across session (Fig. S5). Consistent with primate studies, putative dopamine neurons displayed excitatory responses to sugar pellet delivery and inhibited responses to the foot shock. Note that the neural response to the foot shock was measured not during the shock delivery time but immediately after the offset of shock as the electrical shock produces artifacts that interfere with data collection. The profiles of responses to the CSs and USs are depicted in Fig. S6.

Comparisons of neural responses between ventral tegmental area neuron subgroups

Several types of neurons are localized in the VTA, including dopamine-containing neurons and GABA-containing projections and interneurons (Carr & Sesack, 2000; Yamaguchi *et al.*, 2007; Nair-Roberts *et al.*, 2008). The responses of these subpopulations may vary according to neurotransmitter content. Although several research groups have suggested using various pharmacological ligands to uncover neural identity, this methodology is not suitable for chronic recordings where drug exposure could complicate the interpretation of neural and behavioral data. As such, we opted to employ electrophysiological criteria commonly used to identify VTA neurons, i.e. firing rate and spike waveform duration. Although these classification criteria have been called into question in recent studies in adolescent animals (Margolis *et al.*, 2006), many single-unit recording studies have utilized some combination of these criteria to distinguish dopamine from other neurons (Romo & Schultz, 1989; Roesch *et al.*, 2007; Matsumoto & Hikosaka, 2009). Figure 3A–C depicts a typical spike waveform from each group. Analysis of the response of Type I, which may be considered putative dopamine neurons ($n = 529$ from seven animals during 16 recording sessions, median $n = 34$ per session, Fig. 4A), Type II ($n = 113$ from seven animals during 16 recording sessions, median $n = 8$ per session, Fig. 3B) and Type III ($n = 128$ from seven animals during 16 recording sessions, median $n = 8$ per session, Fig. 3C) neurons revealed similar responses across cell type to the CSap and CSav (Fig. 3D–I).

All three types of neurons had similar excitatory responses to either CS during session 1–2, at which point both stimuli were novel to the animals (Fig. 3D, H and I). Neither the percentage of excitatory CS-responsive neurons ($P > 0.05$, Chi square test, Fig. 3H and I) nor population activities to either CS differed significantly ($P > 0.05$, repeated-measures two-way ANOVA, Fig. 3D). In subsequent sessions, as associative learning progressed, the responses to the CSap became more excitatory, whereas responses to the CSav became primarily inhibitory (Fig. 3E, H and I). When contingencies were reversed in session 9 (Fig. 3F), the neural response to each CS also shifted correspondingly so that the response to the original CSap, which switched to predict the aversive outcome, changed to inhibition and responses to the original CSav, which switched to predict the appetitive outcome, became excitatory (Fig. 3G–I). These changes in response to the CSap or CSav were reflected in both the magnitude of the population response and the proportion of responding neurons.

For Type I neurons, the population response magnitude increased to the CSap and decreased to the CSav as learning progressed ($r = 0.881$ and $r = -0.941$, respectively, $P < 0.01$ in all cases, Fig. 3D and E). Comparing the proportion of responsive neurons in session 1–2 and session 7–8, excitatory responses increased to the CSap and decreased to the CSav ($P < 0.05$, Chi square test in all cases, Fig. 3H and I). At the same time, the number of inhibitory CSav-responsive neurons increased from 3.9% (2/51) to 12.5% (9/72). There was a strong

positive correlation between the number of neurons showing excitatory responses to the CSap and the number of sessions ($r = 0.799$, $P < 0.05$) and a strong negative correlation between the number of sessions and number of neurons with an excitatory response to the CSav ($r = -0.902$, $P < 0.01$). As expected, there was a positive correlation between session number and the number of neurons with an inhibitory response to the CSav ($r = 0.757$, $P < 0.05$). When contingencies were reversed in session 9, the population responses shifted following the reversal with excitatory responses to the new CSap (previous CSav) and inhibitory responses to the new CSav (previous CSap) becoming increasingly larger ($r = 0.905$ and $r = -0.963$, respectively, $P < 0.01$ in all cases, Fig. 3F and G). In comparing the proportion of responsive neurons in session 9–10 with session 15–16, more neurons were depressed by the new CSav (23.8%, 15/63, $P < 0.01$, Chi square test, Fig. 3H) but excited by the new CSap (73.0%, 46/63, $P < 0.01$, Chi square test, Fig. 3I).

Similar to the response of Type I neurons, for Type II neurons, as learning progressed, the population response magnitude evoked by the CSap increased ($r = 0.633$, $P = 0.091$) and that evoked by the CSav decreased ($r = 0.799$, $P < 0.01$) from session 1 to session 8 (Fig. 3E). The percentage of excitatory CSap-responsive neurons increased from 10.0% (2/20) in sessions 1–2 to 35.3% (6/17) in sessions 7–8, whereas 17.6% (3/17) of neurons showed inhibited responses in sessions 7–8 (Fig. 3H and I). After session 9, the population responses shifted following the reversal with excitatory responses to the new CSap (previous CSav) and inhibitory responses to the new CSav (previous CSap) becoming larger ($r = 0.859$, $P < 0.01$ and $r = -0.614$, $P = 0.104$, respectively, Fig. 3G). The percentage of neurons exhibiting excitatory responses to the new CSap increased from 31.3% (5/16) in sessions 9–10 to 50% (7/14) in sessions 15–16. Likewise, 7.1% (1/14) of neurons exhibited inhibited responses to the CSav in session 15–16 (Fig. 3H and I).

For Type III neurons, there was a progressive increase in the magnitude of the excitatory population response evoked by the CSap ($r = 0.687$, $P = 0.06$) and inhibitory response evoked by the CSav ($r = 0.502$, $P = 0.206$) as learning progressed from session 1 to session 8 (Fig. 3E). Population responses shifted with the changing contingencies, showing greater excitatory responses to the new CSap and inhibitory responses to the new CSav as learning progressed from session 9 to 16 ($r = 0.814$ and $r = -0.958$, in both cases $P < 0.01$, Fig. 3G). In sessions 1–2, 14.3% (2/14) and 14.3% (2/14) of neurons displayed excitatory phasic responses to the CSap and CSav. In subsequent sessions, more neurons displayed excitatory responses to the CSap, whereas the excitatory responses to the CSav were reduced. By session 7–8, 37.5% (6/16) of neurons were activated by the CSap and 6.3% (1/16) were inhibited by the CSav. After shifting association contingencies, the proportion of excitatory CSap-responsive neurons increased from 33.3% (6/18) in sessions 9–10 to 53.8% (7/13) in sessions 15–16. Likewise, 30.8% (4/13) of neurons showed inhibited responses to the CSav in sessions 15–16 (Fig. 3H and I). Note that some statistical comparisons within Type II and Type III neurons were not tested because of limited sample size.

The population responses to both the CSap and CSav were not significantly different between VTA subgroups ($P > 0.05$ for the interaction [session $\times$ group] and group effect). There was a significant effect of time, indicating that the population was cue responsive ($P < 0.01$ for the time effect in all cases, repeated two-way ANOVA, Fig. 3D–G). The proportions of the neurons responsive to either the CSap or CSav were not significantly different ($P > 0.05$, Chi-square test in all cases, Fig. 3H and I).

As VTA dopamine neurons are traditionally thought to possess slow firing rates, whereas GABAergic VTA neurons are thought to have higher firing rates (Anstrom & Woodward, 2005), we compared the neural responses of high-firing-rate (> 12 Hz) and low-firing-rate ($<$

7 Hz) neurons. In both groups, we observed a similar pattern of neural responses as learning progressed. However, it should be noted that the magnitudes were not equivalent (Fig. S7).

We observed inhibitory responses evoked by the CSav that were more complex than previously reported. As seen in Fig. S4, the CSav evoked a brief activation before inhibition in VTA neurons. This biphasic population response pattern is clearly visible in sessions 1–8 and 9–16 (Fig. 4A). We calculated the ROC value to compare the selectivity of population responses to the CSap and CSav (Fig. 4B). Because no differences were detected between Type I, II or III neurons, all neurons were included in the ROC analysis. In sessions 1–2, selectively divergent neural activity was not evoked by either the CSap or CSav. However, in sessions 5–8 and 13–16, when the pre-reversal and post-reversal associations were best learned [as demonstrated by the behavioral index *R* (see behavioral results)], differences in neural activity evoked by the CSap and CSav caused increases in the ROC value during later stages of the CS presentation on the trial level. These data suggest a double dissociation in the pattern of neural activity evoked by the CSs. The differences in neural activity evoked by either CS emerge only as learning progresses, as the differences in the pattern of population responses are only evident in the later stages of learning. These differences in population responses to either CS occur not in the time periods immediately after CS presentation but rather in the time periods that occur after the CS has been continuously presented for several seconds.

Discussion

The present study demonstrates that both putative dopamine and GABA neurons in the VTA similarly signal appetitive and aversive information, once discrete novel sensory stimuli become associated with a predicted outcome. Excitatory responses to either stimulus were present before any association was formed, indicating that VTA neurons are activated by sensory events without knowledge of the contingencies being associated with those events. This is consistent with previous findings that dopamine neurons respond to novel auditory and visual stimuli (Ljungberg *et al.*, 1992; Horvitz *et al.*, 1997; Dommett *et al.*, 2005) and further demonstrates that non-dopaminergic neurons including GABA neurons in the VTA also respond to novel salient events.

The responses to either stimulus changed quantitatively and qualitatively over the subsequent sessions as behavioral evidence of learning emerged. Thus, although VTA neurons initially responded to novel events, with further associations these neurons came to represent stimuli predictive of appetitive and aversive outcomes. Overall, these findings are consistent with the idea that dopamine neurons are involved in both appetitive and aversive learning. The fact that a similar pattern of plasticity was observed across VTA cell types suggests that GABA neurons in the VTA may also be critical for associative learning.

When the predictive outcome of each CS was switched, VTA neurons changed their response in accordance with the shift in associations. These findings indicate that once VTA neurons learn appetitive or aversive stimulus–outcome associations, they can quickly extinguish and update these associations if contingencies change. This rapid adjustment in the neural response to changed associations is consistent with previous work (Roesch *et al.*, 2007; Pan *et al.*, 2008) but further demonstrates that such plasticity is observed with aversive as well as appetitive stimulus associations. Importantly, putative non-dopaminergic neurons in the VTA displayed a similar pattern of activity, consistent with the idea that these neurons encode information related to appetitive and aversive outcomes.

Interestingly, even though the overall VTA neural response to the CSav was inhibitory, we observed a brief excitation prior to a sustained depression in firing rate. Previous studies

have found that some dopamine neurons are activated by aversive stimuli (Brischoux *et al.*, 2009; Matsumoto & Hikosaka, 2009), whereas other studies have suggested that dopamine neurons are inhibited by aversive stimuli (Mirenowicz & Schultz, 1996). Although it is possible that stimuli predictive of an aversive outcome evoke a biphasic response from putative dopamine neurons, it is notable that previous stimulus exposure may likewise drive this response. We found that the biphasic nature of the response evoked by the CSav increased after reversal of the initial association. Dopamine neurons tend toward stimulus generalization (Mirenowicz & Schultz, 1996), and thus the biphasic nature of the response may be in part related to prior stimulus exposure.

Our finding that putative dopamine and GABA neurons in the VTA respond similarly to novel stimuli and learned associations related to these stimuli is significant in the context of how dopamine neurotransmission influences behavior. Although the rapid response of dopamine neurons has been implicated in mediating reward-related signaling, dopamine is a slow-acting modulator with its post-synaptic actions primarily dictated by volume transmission (Rice & Cragg, 2008). Dopamine receptors and transporters, especially in the prefrontal cortex, are localized extrasynaptically (Paspalas & Goldman-Rakic, 2004), and extracellular increases in dopamine as measured by voltammetry or microdialysis remain elevated for seconds to minutes (Stefani & Moghaddam, 2006). Given that VTA GABA neurons project to similar cortical and ventral striatal regions as dopamine neurons (Carr & Sesack, 2000; Fields *et al.*, 2007), our data address this theoretical discrepancy by suggesting that temporally precise information may be transferred out of the VTA via GABA neurons and then modulated by dopamine. In addition to GABAergic VTA neurons, VTA glutamate neurons are another possible rapid-signaling candidate (Yamaguchi *et al.*, 2007).

The change in the magnitude and type of response in VTA neurons during learning indicates that the same sensory event recruits different mechanisms of afferent drive on these neurons as the animal learns that an event predicts an aversive or appetitive outcome. Both dopamine and GABA VTA neurons receive a wide range of direct and indirect inputs from lower structures such as the superior colliculus (Comoli *et al.*, 2003) and higher structures such as the prefrontal cortex (Carr *et al.*, 1999). The response to either novel stimulus is likely to be modulated by bottom-up sensory drive by regions such as the superior colliculus (Dommett *et al.*, 2005). After learning, the contingency-specific inhibitory and excitatory responses of these neurons, and flexibility of these responses to changing contingencies, suggests a more complex regulation. For example, regions such as the habenula, rostromedial tegmental nucleus, hippocampus and prefrontal cortex, which are critical for learning and affective processing, and which regulate the firing of VTA dopamine neurons (Grace *et al.*, 2007; Matsumoto & Hikosaka, 2007; Jhou *et al.*, 2009), may increase their influence after contingencies are assigned to a stimulus.

Overall, our findings suggest that multiple subpopulations of VTA neurons play a role in associative learning by initially detecting novel stimuli in the environment without familiarity with the contingencies associated with these stimuli. As the contingencies are learned, an increasing positive or negative value is assigned to that stimulus. This response shifts with the reversal of outcome valence, suggesting that the response of VTA neurons to sensory stimuli is subject to rapid modification by emotional memory. Thus, these neurons may provide a critical interface where sensory information gains access to internal representations of appetitive or aversive memories and action plans. Given the slow and modulatory nature of dopamine neurotransmission, the temporally precise information transfer related to this interface may be mediated by GABA neurons. The concomitant activation of dopamine neurons, which leads to the sustained increase of extracellular dopamine in terminal regions, would then modulate this response to subserve 'slower'

functions such as learning, working memory, sustained attention and regulation of emotional behavior (Goldman-Rakic, 1996; Kelley, 2004; Montague *et al.*, 2004).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CS	conditioned stimulus
CSap	appetitive conditioned stimulus
CSav	aversive conditioned stimulus
GABA	γ -aminobutyric acid
ROC	receiver operating characteristic
US	unconditioned stimulus
VTa	ventral tegmental area

References

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Fig. 1.

Behavioral task and performance. (A) Behavioral task. For eight consecutive daily sessions, a double conditioning paradigm was used in which two novel stimuli (tone or flashing light) were followed by the delivery of an appetitive (sugar pellet) or aversive (mild electric foot shock; 180 ms, 0.2 mA) US. Tone or light stimuli were delivered for 10 s and were randomly assigned to be paired with either sugar pellet or foot shock for the first eight sessions. For sessions 9–16 this association was reversed. The CS that preceded sugar pellet delivery is referred to as CS_{ap} and the CS predictive of shock is referred to as CS_{av}. Rats ($n = 7$) underwent 60 trials (30 trials of each contingency) delivered pseudo-randomly. (B) Behavioral performance. As animals learned the associations across multiple sessions, the R_{ap} (behavioral association index; see Materials and methods) was increased and R_{av} was depressed. After CS associations were reversed, R_{ap} and R_{av} were developed to the new association contingencies. Error bars represent SEM.

Fig. 2.

The changing response of VTA neurons to the CSs. (A) Representative examples of phasic responses in one animal to the same CS in session 1 vs. session 8. Raster plots represent neural activity, with each row corresponding to a single trial. All 30 trials are shown in each plot with the corresponding peristimulus time histogram. Blue ticks represent the time that the animal nose pokes into the food trough. (B) The population response of VTA neurons to the CSap and CSav during sessions 1–16. There was a progressive increase in the magnitude of excitatory responses to the CSap across sessions 1–8. The magnitude of the population response was strongly correlated with the session number ($r = 0.918$, $P < 0.01$). The initial excitatory response to the CSav changed to a delayed inhibitory response as task learning progressed. The population response of neurons to the CSav decreased as learning progressed and the magnitude of the population response was inversely correlated with the session number ($r = -0.960$, $P < 0.01$). Again, the population responses shifted in response to contingency reversal with increasingly excitatory responses to the new CSap (right lower panel) and inhibitory responses to the new CSav (right upper panel) as learning progressed ($r = 0.925$ and $r = -0.973$, respectively, $P < 0.01$ in all cases). Shadows represent SEM. (C) Changes in the proportion of responsive neurons to either CS during sessions 1–16. In session 1–2, a small proportion of neurons displayed excitatory phasic responses to the CSap (16.5%; 14/85) and CSav (17.6%; 15/85). In subsequent sessions, a larger proportion of neurons displayed excitatory phasic responses to the CSap and inhibitory responses to the CSav ($r = 0.809$ and $r = 0.818$, respectively, $P < 0.05$ in all cases). On session 7–8, 43.8% (46/105) of VTA neurons were activated by the CSap and 12.4% (13/105) were inhibited by the CSav. The percentage of excitatory or inhibitory response to each CS shifted as contingencies changed. After the association contingencies were shifted, more neurons showed excited responses to the new CSap and inhibited responses to the new CSav as learning progressed ($r = 0.872$ and $r = -0.932$, respectively, $P < 0.01$ in all cases). (D) Trial-by-trial responses of an individual neuron during session 9 to a CS that previously predicted foot shock (on sessions 1–8) but now predicts sugar pellet delivery (left) and to a CS that previously predicted sugar pellet delivery and now predicts foot shock (right). The neuron began exhibiting activation and inhibition to the new CSap and CSav, respectively, in the later trials of this session. Color indicates the firing rates per second. (E) The normalized response of VTA neurons recorded in session 9 reflects learning of the new CS contingencies ($n = 53$ in session 9). Thirty trials of CS type were divided into five bins of six consecutive trials. The responses of each neuron to a stimulus were normalized in these bins. The response to the new CSap was increased ($r = 0.988$, $P < 0.01$) and the response to the new CSav was decreased ($r = -0.988$, $P < 0.01$) during the course of the session. Significant correlations were not found for either CSap or CSav in session 8 ($r = 0.579$ and $r = 0.146$, respectively, $P > 0.05$ in all cases, $n = 54$). Circles and triangles represent neural activity and behavior separately. Error bars represent SEM.

Fig. 3.

Comparison of the neural responses of VTA subpopulations. (A–C) Representative waveforms for neurons classified as Type I ($n = 529$ from seven animals during 16 recording sessions, median $n = 34$ per session), Type II ($n = 113$, median $n = 8$ per session) and Type III ($n = 128$, median $n = 8$ per session). Scale: 0.2 ms and 0.05 mv. Subtyped population responses to CSap and CSav of VTA neurons during sessions 1–2 (D), 7–8 (E), 9–10(F) and 15–16 (G). There was a significant effect of time with no interaction [group $\times$ time] or group effects ($P < 0.01$ for time, $P > 0.05$ for group and interaction [group $\times$ time] effect, repeated two-way ANOVA). (H and I) Changes in the proportion of subtyped VTA CSap- and CSav-responsive neurons. More Type I neurons were activated to CSap and depressed to CSav on session 7–8 compared with sessions 1–2 ($P < 0.05$ for both cases, Chi-square test). Because of the limited sample sizes of Type II and Type III neurons, these comparisons were not executed for these groups (although we observed qualitatively similar patterns of responding between these and Type I neurons).

Fig. 4.

Time-course of the response to the CSap and CSav. (A) Type I neurons responsive to the CSav exhibited a biphasic pattern, first exhibiting excitation followed by inhibition. Histograms represent sliding 100 ms time bins with each time bin advanced by 20 ms from the beginning of the previous time bin. This analysis demonstrates a biphasic response to the CSav during sessions 1–8 (left, $n = 248$) and sessions 9–16 (right, $n = 281$), with an early period of excitation followed by a more sustained period of inhibition. (B) Time-course of the signal change in the neural response to the CSap and CSav. ROC analyses revealed that the signal change in response to the presentation of either CS developed as learning progressed. In sessions 1–2, VTA neurons did not significantly alter their responding to the presentation of either CS (filled circle). In sessions 5–8, VTA neurons developed significantly different responses (activation or inhibition) to the CSap and CSav (filled circle). By sessions 13–16 (after contingency reversal), VTA neurons exhibited strong differential responding to the presentation of the CSap or CSav in a non-monotonic fashion (filled circle). Examination of the signal change from baseline to the presentation of either the CSap (empty circle) or CSav (triangle) indicates that the early peak in differential responding is best explained by the increase in activity to the CSap (empty circle), which then declines rapidly. Differential responding to the CSav was maintained for a longer period of time, best explaining the second peak in VTA neuron differential activity (second peak of filled circles).