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Unanticipated stressful and rewarding experiences engage the same prefrontal cortex and ventral tegmental area neuronal populations

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Overlapping representation of stress and reward

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Abstract

Brain networks that mediate motivated behavior in the context of aversive and rewarding experiences involve the prefrontal cortex (PFC) and ventral tegmental area (VTA). Neurons in both regions are activated by stress and reward, and by learned cues that predict aversive or appetitive outcomes. Recent studies have proposed that separate neuronal populations and circuits in these regions encode learned aversive versus appetitive contexts. But how about the actual experience? Do the same or different PFC and VTA neurons encode unanticipated aversive and appetitive experiences? To address this, we recorded unit activity and local field potentials (LFP) in the dorsomedial PFC (dmPFC) and VTA of male rats as they were exposed, in the same recording session, to reward (sucrose) or stress (tail pinch) spaced one hour apart. As expected, experience-specific neuronal responses were observed. About 15-25% of single units in each region responded by excitation or inhibition to either stress or reward, and only stress increased LFP theta oscillation power in both regions and coherence between regions. But the largest number of responses (29% dmPFC and 30% VTA units) involved dual-valence neurons that responded to both stress and reward exposure. Moreover, the temporal profile of neuronal population activity in dmPFC and VTA as assessed by principal component analysis were similar during both types of experiences. These results reveal that aversive and rewarding experiences engage overlapping neuronal populations in the dmPFC and the VTA. These populations may provide a locus of vulnerability for stress related disorders, which are often associated with anhedonia.

Keywords: Stress, reward, ensemble activity, local field potential, dopamine, depression, anxiety, theta oscillations

Significant Statement

Animals must recognize unexpected harmful and rewarding events in order to survive. How the brain represents these competing experiences is not fully understood. Two interconnected brain regions implicated in encoding both rewarding and stressful events are the dmPFC and the VTA. In either region, separate neurons and associated circuitry are assumed to respond to events with positive or negative valence. We find, however, that a significant subpopulation of neurons in dmPFC and VTA encode both rewarding and aversive experiences. These dual-valence neurons may provide a computational advantage for flexible planning of behavior when organisms face unexpected rewarding and harmful experiences.

Introduction

Acute aversive and rewarding events are motivationally salient experiences that may change brain function and behavior (Ferenczi et al., 2016; Hermans et al., 2014; Ulrich-Lai and Herman, 2009; Ye et al., 2016). These experiences can produce opposite behavioral outcomes including promoting avoidance after an aversive experience and approach behavior after a rewarding experience (Bissonette et al., 2014; Gentry et al., 2019), which suggest that they engage different neurophysiological responses in the brain. At the same time, however, they promote similar behaviors such as increased vigilance and motivation to act. The neurophysiological response to both aversive and rewarding stimuli is altered in psychiatric disorders (Kalivas and Volkow, 2005; Lederbogen et al., 2011; Stanton et al., 2018) suggesting common neurobiological substrates. In particular, stress related disorders such as major depressive disorder are commonly associated with anhedonia and impaired ability to enjoy experiences that were once rewarding (Stanton et al., 2018).

Two principal brain regions that have been implicated in processing aversive and rewarding experiences are the prefrontal cortex (PFC) and the ventral tegmental area (VTA) (Abercrombie et al., 1989; Kobayashi et al., 2006; Taber and Fibiger, 1997; Thierry et al., 1976). Acute stressors and rewarding events increase the release of dopamine, noradrenaline and the expression of early genes in the PFC (i.e. c-fos) (Butts et al., 2011; Smith et al., 1997; Thierry et al., 1976; Weinberg et al., 2010). Stress and expectation of a rewarding outcome also increase the activity of PFC neurons (Horst and Laubach, 2013; Jackson and Moghaddam, 2006). In the VTA, dopamine neurons are famously activated by reward (Schultz, 1998; Steinberg et al., 2013) and while VTA dopamine neurons are both activated and inhibited in aversive contexts (Anstrom and Woodward, 2005; Bromberg-Martin et al., 2010; Holly and Miczek, 2016), stress robustly increases the release of dopamine in all VTA terminal regions including ventral striatum, amygdala, and PFC (Abercrombie et al., 1989; Inglis and Moghaddam, 1999). But while it is well accepted that both stress and reward activate these regions, it remains an open question whether the same neural populations within each region represent events with opposing valence.

Recent recording studies have compared the neuronal response to aversive and rewarding contexts and show that specific PFC and VTA (dopamine and non-dopamine) circuits and neuronal populations can be activated and/or inhibited differently by either context (Caracheo et al., 2018; Cohen et al., 2012; de Jong et al., 2019; Kim et al., 2010; Vander Weele et al., 2018). Yet most of these studies involve Pavlovian or operant conditioning paradigms where a trained animal responds to an expected outcome. While the results of these studies are important for understanding how expectation of an impending appetitive

versus aversive event are encoded after learning, they do not address how these regions respond to the actual experience of an unexpected appetitive versus aversive event.

The present study was designed to compare neuronal responses to acute, unanticipated rewarding and aversive experiences in the dorsomedial PFC (dmPFC) and the VTA. Neuronal activity spanning individual neurons, neuronal populations and local field potentials (LFP) were recorded simultaneously in both areas of the brain. Critically, animals were exposed to both rewarding and aversive experiences in the same recording sessions so that we could reliably compare their effects on the same neurons across time. Food (sucrose) exposure (15 min) and tail pinch (15 min), spaced one hour apart, were used as rewarding and aversive experiences, respectively. We find that while, in both regions, some populations of neurons respond only to one experience, a significant proportion of neurons respond to both types of experiences.

Material and Methods

Animals

Adult male Long Evans rats weighing 325–360 g (n= 10) were housed in pairs on a 12 h light/dark cycle (lights on at 19:00 h). All experiments were performed during the dark active phase of the cycle. All procedures were conducted in accordance with the University of Pittsburgh's Institutional Animal Care, the University of Mississippi Animal Review Board and Use Committee, and the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

Surgery and electrophysiology procedure

Chronic microelectrode arrays were implanted under isoflurane anesthesia in the dmPFC (prelimbic) (AP= +3.0, L= 0.7, V= -4, from Bregma) and the ventral tegmental area (VTA) (AP= -5.3, L= 0.5-1.1, V= -8.3, from Bregma) of rats (Del Arco et al., 2017; Park and Moghaddam, 2017) (See Figure 1A and B). Microelectrode arrays consisting of eight polymide-insulated Tungsten wires (50 µm) made in-house were implanted in the VTA. Microelectrode arrays consisting of eight or sixteen Teflon-insulated stainless-steel wires (50 µm) (NB Labs, Tx) were implanted in the dmPFC. Electrode arrays were secured onto the skull with dental cement using six screws as anchors. A silver wire was connected to one of the screws used as a ground.

Single units were recorded by a unity-gain field-effect transistor head stage and lightweight cabling, which passed through a commutator to allow freedom of movement in the test chamber (Plexon, Dallas, TX). Recorded neuronal activity was amplified at 1000× gain and digitized at 40 kHz by the recorder software (Plexon, Dallas, TX). Single-unit activity was digitally high-pass filtered at 300 Hz, and LFPs were low-pass filtered at 125 Hz (Plexon, Dallas, TX). Single units were isolated in Offline Sorter (Plexon, Dallas, TX) using a combination of manual and semiautomatic sorting techniques (Homayoun and Moghaddam, 2007).

Tail pinch and food reward exposure

Animals were allowed to recover from surgery for at least 1 week. They were then acclimated to the recording cable in the testing cage for 2-3 days before the recording started. During this time animals were individually housed and mildly food-restricted (15 g of standard chow per day). Habituation included exposing animals to sugar pellets in the testing cage at least one day before recording started.

Recording sessions lasted 165 min (Figure 1). After 30 min of baseline recording, animals were exposed to food (sucrose) for 15 min and, 60 min later, to tail pinch for 15 min. To control for the order of exposure to these salient events, 4 animals received tail pinch before food in a second recording session. Tail pinch was performed using a foam-covered cloth pin attached 2 cm from the base of the tail. The foam was used to avoid excessive pressure on the tail. Previous studies have shown that 15-min tail pinch increases corticosterone concentrations and impairs executive functions (Butts et al., 2013, 2011). Food exposure involved giving ad lib access to sugar pellets (dustless sugar pellets, 45 mg; Bio-Serv) placed in a Petri dish in the test cage. All animals consumed sugar pellets during food exposure (45 ±8 pellets per session, averaged across 8 recording sessions).

Histology

After completion of experiments, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and perfused with saline and 4% buffered formalin. Fixed brain sections were stained with cresyl violet, and electrode-tip placements were verified using a light microscope. Only data with correct placements within the prelimbic region of the dmPFC and the VTA were included in electrophysiological analyses (Figure 1B).

Electrophysiological data and statistical analysis

Electrophysiological data were analyzed with custom-written scripts, executed in Matlab (MathWorks), along with the Chronux toolbox (http://chronux.org/). Units were classified as activated or inhibited by sucrose exposure or tail pinch in 60 s bins if their average absolute activity was $Z > 2$ or $Z < -2$, respectively. The average across time bins was computed to compare the number of units activated or inhibited by both events during the event time (15 min; 15 bins) and post-event time (15 min beginning after the end of the event; 15 bins). Units were selected as responsive when either 5 consecutive bins or at least 7 non-consecutive bins were significant during the 15 min event period (sucrose exposure or tail pinch). Significant bins were detected by using Student paired *t* tests to compare the average basal firing rate to 60 s bins during sucrose exposure and tail pinch. The response of each unit was calculated by the normalized (z-score) average firing rate of significant bins during the event. Putative dopamine (DA) and non-dopamine (non-DA) units were identified using the firing rate (<12 Hz for DA) and waveform duration (>1.2 ms for DA) as criteria (Kim et al., 2010; Park and Moghaddam, 2017). The dopamine neuron waveform patterns were consistent with the ontogenetically identified dopamine neurons in the VTA of the same strain of rats (Lohani et al., 2019).

153 Unit-pairs in the dmPFC and the VTA were detected by correlating the firing rate of units
154 recorded during the same sessions (Kim et al., 2012; Narayanan and Laubach, 2009).
155 Specifically, a Pearson's correlation of the normalized firing rate (1 min bins) for each pair of
156 units was calculated in the time period of 35 min (35 bins) centered on the event (10 min
157 baseline + 15 min stimulus + 10 min post stimulus). In the VTA, correlations were calculated
158 in within the group of putative DA and non-DA units. The Pearson correlation coefficient
159 served to detect significant unit-pairs. A p value <0.01 was considered significant.

160 The principal component analysis (PCA) was performed to find common sources of variance
161 in the temporal patterns of firing rate over the population of units (Narayanan and Laubach,
162 2009). A different matrix was built for each event containing the normalized (z-score) firing
163 rate of each unit (rows) and 60s-time bins (columns). The *pca* function form matlab was
164 used to obtain coefficients and scores. Coefficients represent the principal components
165 (PCs) and the scores represent the projection of the PCs for every unit. The variance
166 explained by each PC was also obtained by this approach. The scores of every unit were
167 represented in a 2-d space comprising the top two PCs (PC1 and PC2) to visually identify
168 potential clusters for food reward exposure and tail pinch population's activity (see Figures
169 2H and 3H). Also, the PCs that explained the maximal variance (PC1 and PC2) were
170 represented to visualize the temporal profile of the population activity (see Figures 2E-G and
171 3E-G).

172 Local field potentials (LFPs) power spectral densities were quantified using the chronux
173 routine *mtspecgramc*. The raw LFP data was split in 10s windows inside which Fourier
174 transform computation was performed using a sliding time window of 4s, with 2s steps. A
175 multitaper approach was used because it improves spectrogram estimates when dealing
176 with non-infinite time series data (Mitra and Pesaran, 1999). Windows with clipping artifacts
177 or LFP values higher and lower than 3xSD of the mean of the total signal were excluded.
178 Spectral data were normalized (z-score) against the average of the baseline period, and
179 each animal's data were averaged together to yield group mean spectral data. The
180 magnitude squared coherence between time series recorded from dmPFC and VTA was
181 calculated in the same moving window using the chronux routine *cohgramc*. Each animal's
182 normalized spectral power and coherence during each event was used for statistical
183 comparisons.

184 Chi square tests were used to test whether reward (sucrose exposure) or stress (tail pinch)
185 differentially change the proportion of units and unit-pairs responding to these events.
186 Student paired *t* test was used to compare the basal firing rate of neurons. One-way and
187 two-way ANOVAs were used to compare the LFP power and coherence values during the

two events. The statistical analysis and results are depicted in Table 1. Units and LFP data recorded in two sessions were pooled together for the analysis. This was done because the order of events in the second recording session did not change significantly the proportion of unresponsive neurons or those that responded to both events and only one event (see Results).

Results

dmPFC and VTA neuronal response to reward and stress

Neuronal Population

Both food (sucrose) and tail pinch bidirectionally modulated the neuronal activity of PFC and VTA. Figures 2 and 3 show the neuronal response to both experiences in the dmPFC and the VTA, respectively. In both Figures, the top graphs are heat plots that represent the changes in the firing rate (z-scores) during sucrose exposure and tail pinch (A) or tail pinch and sucrose exposure (B), performed in the same recording session. The middle and bottom graphs represent the significant proportion of units that were activated and inhibited during both events (C-D) and the PCA analyses (E-H).

In the dmPFC (104 units, n=10), the basal firing rate of neurons was 6.47 ±0.59 Hz before sucrose exposure and 6.41 ±0.53 Hz before tail pinch (as the average of the 10 min before each event). These values were not significantly different ($t_{(103)}$=0.27, p= 0.784, paired t test). Both events activated more units than inhibited units (Figure 2) but there were no significant differences between sucrose exposure and tail pinch in the average proportion of units activated or inhibited during the event time ($\chi^2_{(2)}$ =0.11, p> 0.1, average across time bins 15-30 min) or during the post-event time ($\chi^2_{(2)}$ =0.22, p> 0.1, average across time bins 30-45 min).

In the VTA (61 units, n=8), we classified units as putative dopamine (DA, n= 38; 62%) and non-dopamine (non-DA, n= 23; 38%) subtypes (see Methods). The basal firing rate of VTA neurons before sucrose was 10.16 ±1.74 Hz (DA= 4.20 ±0.32 Hz; non-DA= 20.02 ±3.82 Hz) and before tail pinch was 9.45 ±1.60 Hz (DA= 3.89 ±0.31 Hz; non-DA= 18.63 ±3.50 Hz) (as the average of the 10 min before each event). There were no significant differences between these values ($t_{(22)}$=1.36, p= 0.186, paired t test). The basal firing rate of DA units was slightly lower before tail pinch compared to sucrose ($t_{(37)}$ = 2.08, p= 0.044, paired *t* test). Both events activated more units than inhibited units (Figure 3), but similar to dmPFC, there were no significant differences between sucrose exposure and tail pinch in the average proportion of units activated or inhibited during the event time ($\chi^2_{(2)}$ =1.93, p> 0.1, average across time bins 15-30 min) or during the post-event time ($\chi^2_{(2)}$ =1.02, p> 0.1, average across time bins 30-45 min).

Because similar proportion of units activated, inhibited, or unresponsive were observed in the dmPFC and the VTA when the order of tail pinch and reward exposure was changed (Figure 2B and 3B), units recorded in both sessions were pooled together for the above

analyses. Specifically, in the dmPFC (Figure 2B, 36 units, n= 4), during tail pinch, 29% of units (vs 29%) were activated and 5% (vs 8%) inhibited ($\chi^2_{(2)}$ =0.08, p> 0.1, average across time bins 15-30 min); during reward exposure 36% of units (vs 30%) were activated and 10% (vs 6%) inhibited ($\chi^2_{(2)}$ =0.23, p> 0.1, average across time bin 15-30 min). Similar results were found in the VTA (Figure 3B, 13 units, n= 4). During tail pinch, 31% of units (vs 44%) were activated and 5% (vs 6%) inhibited ($\chi^2_{(2)}$ =0.70, p> 0.1, average across time bins 15-30 min); during reward exposure, 19% of units (vs 27%) were activated and 10% (vs 2%) inhibited ($\chi^2_{(2)}$ =0.33, p> 0.1, average across time bins 15-30 min).

Next, a principal component analysis (PCA) was performed to identify common sources of variance in the temporal pattern of firing rate during both events (Narayanan and Laubach, 2009). The temporal pattern of the population activity as represented by the top two PCs (PC1 and PC2) was similar between sucrose exposure and tail pinch in both areas of the brain (2E-G, 3E-G). The variance explained by the top five PCs is shown in Figures 2F and 3F. The projection of each neuron in the 2-d PCs space (PC1 and PC2) is consistent with the same pattern of population activity during both sucrose exposure and tail pinch since all units can be included in one cluster (Figures 2H and 3H).

Single units

Figures 4A and 4B show the response of every unit to food (sucrose) exposure compared to the response to tail pinch in the dmPFC and VTA, respectively. These graphs show that units in both areas of the brain are activated and inhibited by both events as well as activated or inhibited by only one of them. Figures 5C and 5D show the percentage of dmPFC and VTA units that responded to both sucrose exposure and tail pinch or only one of these events (paired t-test, at alpha= 0.05; see Methods). As shown, a high number of units responded to both events in the dmPFC (30 [29%]) and the VTA (18 [30%]). In the dmPFC, the number of units that responded specifically to sucrose or tail pinch was the same (20 [20%]). In the VTA, there were more units that responded specifically to tail pinch (17 [28%]) compared to sucrose exposure (9 [15%]) with similar responses from putative DA and non-DA units (5E). Of note, the vast majority of VTA units responding to tail pinch is consistent with previous studies showing that VTA DA and non-DA cells increase their activity in response to aversive stimuli (Brischoux et al., 2009; Morales and Margolis, 2017; Thierry et al., 1976). The percentage of units that did not respond to any of the events was similar in the dmPFC (33 [32%]) and the VTA (17 [29%]). There were no significant differences in the proportion of units that responded to sucrose exposure and tail pinch or only to one of the events, in the dmPFC ($\chi^2_{(3)}$ =4.88, p> 0.1) and the VTA ($\chi^2_{(3)}$ =3.50, p> 0.1). Similarly, there

were no differences in the proportion of DA and non-DA units in the VTA that responded to both events and to only one of the events ($\chi^2_{(3)}$ =1.85, p> 0.1) (Figure 5F).

Similar responses to both salient events were observed in the dmPFC when the order of tail pinch and food exposure was changed (36 units, n= 4) ($\chi^2_{(3)}$ =1.26, p> 0.10). There were similar proportions of unresponsive neurons (38% vs 35%), neurons that responded to both events (22% vs 17%), only to food (11% vs 20%) or only to tail pinch (27% vs 27%). The same results were found in the VTA (13 units, n= 4) ($\chi^2_{(3)}$ =6.90, p> 0.05). Units recorded in both sessions were pooled together for the above analyses.

Unit-pairs

To evaluate whether food (sucrose) exposure and tail pinch change the functional interaction (i.e. coordinated activity) between neurons in the dmPFC and the VTA, we analyzed the correlation between the firing rate of pairs of units recorded in the same sessions (Table 2). Previous studies show that brief appetitive and aversive stimuli during conditioning learning can modulate the functional connectivity between neurons in the VTA (Kim et al., 2012). This study shows a significant proportion of unit pairs during both events in the dmPFC and the VTA. In the dmPFC there were 144 and 166 significant pairs during sucrose exposure and tail pinch, respectively, out of 413 possible pairs. In the VTA, there were 68 and 82 significant pairs during sucrose exposure and tail pinch, respectively, out of 221 possible pairs. As shown in Table 2, there were no significant differences in the proportion of significant unit-pairs that overlapped during both events or emerged specifically during one of the events (food exposure or tail pinch), in the dmPFC ($\chi^2_{(2)}$ =4.44, p> 0.1) and the VTA ($\chi^2_{(2)}$ =2.44, p> 0.1). These results suggest that both events produced similar coordinated activity among units in both areas of the brain and are consistent with the neuronal population activity results shown above (i.e. PCA).

LFP

To examine if LFPs mediate neural interactions within- and between- dmPFC and VTA, we analyzed LFP oscillations at different frequencies (from 1 to 40 Hz; bin size 2.5 Hz) and compared the effects of food (sucrose) exposure and tail pinch. A two-way ANOVA (frequency x event) showed that food exposure and tail pinch produced different effects on LFP oscillations in the dmPFC ($F_{(30,336)}$= 1.67, p=0.017, frequency x event interaction) and the VTA ($F_{(30,336)}$= 2.10, p=0.001, frequency x event interaction). Specifically, as shown in Figure 5, tail pinch, but not food exposure, increased the power of low theta oscillations (2-5 Hz) in the dmPFC ($F_{(2,21)}$= 7.59, p=0.003, one-way ANOVA) and the VTA ($F_{(2,21)}$= 4.44, p=0.024, one-way ANOVA) (as the average 2-5 Hz for 20 min after the beginning of the

295 events). Furthermore, theta oscillations were significantly synchronized between the dmPFC
296 and the VTA during tail pinch ($F_{(2,21)}$= 4.39, p=0.025, one-way ANOVA) compared to food
297 exposure and baseline (as the average 2-5 Hz for 20 min after the beginning of the events).
298 Overall, these results show that tail pinch, but not food exposure, increases theta oscillations
299 and coherence between the dmPFC and the VTA.

300

Discussion

In the same recording session, we compared the effects of unanticipated aversive and rewarding experiences on dmPFC and VTA single units as well as LFP oscillations. We find experience-specific changes in LFP oscillations and in the activity of neuronal subpopulations. There were, however, no global changes in the population activity or its temporal profile that characterized each type of experience. In fact, the largest proportions of neurons in either region responded to both experiences. These results suggest that while separate networks may encode aversive and rewarding experiences, there is a considerable population of dual-valence dmPFC and VTA neurons that encode both experiences. This overlap may be significant in the context of organizing behaviors that are similarly affected by stressful and rewarding events, and in psychiatric disorders where both negative and positive valence systems are affected.

Same neurons in the dmPFC and VTA respond to unanticipated aversive and rewarding experiences

The PFC and the VTA are components of negative and positive valence systems. Both regions are sensitive to acute stressors (Holly and Miczek, 2016; Jackson and Moghaddam, 2006); and both are implicated in stress and anxiety related brain disorders (Arnsten, 2015; Cha et al., 2014; Chaudhury et al., 2013; Holmes and Wellman, 2009; Tye et al., 2012) while also processing reward related events (Cohen et al., 2012; Horst and Laubach, 2013; Kobayashi et al., 2006; Schultz, 1998). Little is known, however, about the relative response of the same PFC or VTA neurons to stressful versus rewarding experiences. To address this void, we compared the effect of exposure to a reward (sucrose) in mildly food restricted animals with an aversive experience (tail pinch) on dmPFC and VTA single units during the same recording session. Our results that stress and reward can have a mixed inhibitory and excitatory effects on unit activity, especially on dmPFC cells, is in general agreement with previous literature (Anstrom and Woodward, 2005; Brischoux et al., 2009; Bromberg-Martin et al., 2010; Jackson and Moghaddam, 2006; Kobayashi et al., 2006). Critically, however, the proportion of neurons activated/inhibited, and the temporal profile of these changes were not different between the rewarding and aversive experiences. Moreover, the global activity of the neuronal population during and after either experience was similar as evaluated by PCA. These results demonstrate that there is overlap in the neuronal representation of aversive and rewarding experiences in either region that may be masked if the relationship was assessed by only measuring global changes in the population activity such as those measured by fiber photometry.

About 30% of neurons in the dmPFC and the VTA encoded the experience of animals receiving food reward or the tail pinch stressor. This substantial proportion of neurons in the dmPFC and the VTA that encode opposing valence experiences (dual-valence neurons) could function as mixed selectivity neurons. Mixed selective neurons have been implicated in processing different sensory or motor variables and can facilitate contextual flexibility during cognitive and motor behavior (Kobak et al., 2016; Ma et al., 2016; Rigotti et al., 2013). Mixed selective neurons are prevalent in the PFC (Grunfeld and Likhtik, 2018; Rigotti et al., 2013). While VTA neurons are often assumed to be specialized, recent studies have suggested that clusters of dopamine neurons respond to a whole array of variables in addition to reward (Engelhard et al., 2019). Dual-valence neurons in the dmPFC and the VTA could fine-tune adequate behavioral outcomes depending on the emotional context (i.e. degree of averseness) (Berridge, 2019; Matsumoto et al., 2016; Park and Moghaddam, 2017). Alternatively, dual-valence neurons may facilitate learning by adapting their response to only one type of experience (aversive or rewarding) after repeated exposure (Li et al., 2017). Future studies are needed to establish which common input onto these neurons drives this activity. Possible regions include bed nucleus of the stria terminals, hypothalamus and amygdala (Burgos-robles et al., 2017; Ch'ng et al., 2018; Morales and Margolis, 2017).

Experience-specific response of dmPFC and VTA neurons

Subpopulations of neurons in the dmPFC and the VTA responded selectively to tail pinch or sucrose exposure. These neurons may represent the first building blocks for conditioned learning (Gore et al., 2015) and may have specific molecular features and input/output projections (Li et al., 2017). This aspect of our results is consistent with recent studies that have identified specific populations in the PFC (Rozeske et al., 2018; Vander Weele et al., 2018; Warden et al., 2012; Ye et al., 2016) and the VTA (de Jong et al., 2019; Kim et al., 2016; Morales and Margolis, 2017; Ye et al., 2016) that respond to rewarding or aversive events. Thus, VTA neurons that receive inputs from the lateral tegmentum and project to the nucleus accumbens respond to rewards while VTA cells that receive inputs from the lateral habenula and project to the PFC respond primarily to aversive stimuli (Lammel et al., 2012). Similarly, PFC neurons that receive inputs from the VTA and project to the periaqueductal grey selectively respond to aversive events and also process avoidance behavior (Vander Weele et al., 2018). Our results expand on these studies by showing that individual neurons as well as neuronal populations respond to prolonged aversive and rewarding experiences in the dmPFC and the VTA.

Aversive experience uniquely engages dmPFC and VTA networks

Tail pinch, but not exposure to reward, increased the power of theta oscillations in both the dmPFC and the VTA. Furthermore, tail pinch increased the synchronization of VTA-dmPFC theta oscillations (2-5 Hz) which suggest that the response to acute stress requires a stronger functional connectivity between the dmPFC and the VTA (Fries, 2005). Theta oscillations in the PFC and the VTA, and their potential role in appetitive and aversive processing (Amarante et al., 2017; Kim et al., 2012; Park and Moghaddam, 2017) as well as memory (Benchenane et al., 2011; Fujisawa and Buzsáki, 2011), is still a matter of debate. Recent studies show that theta oscillations in the PFC and limbic-connected areas (i.e. amygdala, hippocampus) contribute to conditioned fear learning and avoidance behavior. Thus, 4-Hz theta oscillations in the PFC-amygdala circuit predicts the expression of fear behavior (i.e. freezing) (Karalis et al., 2016). Furthermore, an increase in 8-Hz (and to a lesser extend 4-Hz) theta oscillations promotes avoidance behavior in the elevated plus maze (Padilla-Coreano et al., 2019). Importantly, using optogenetics, this last study also shows a causal role of PFC theta oscillations to induce avoidance behavior through the activation of ventral hippocampus-PFC inputs. In line with these studies, our results suggest that theta oscillations in the VTA-dmPFC circuit contribute to process unanticipated stressful experiences and generate innate avoidance responses.

VTA modulates information coding and valence processing in the PFC (Ellwood et al., 2017; Lohani et al., 2019; Mininni et al., 2018; Weele et al., 2019). It is possible that the increased theta oscillations in the dmPFC during tail pinch are produced by a direct modulation from the VTA because selective optogenetic stimulation of dopamine cells in the VTA can induce PFC oscillations and modulate the activity of PFC neuronal ensembles at different time scales (Lohani et al., 2019). Furthermore, VTA dopamine inputs in the PFC can regulate the occurrence of prefrontal 4-Hz theta oscillations (Parker et al., 2014) and amplify the response of prefrontal neurons that encode aversive stimuli (Vander Weele et al., 2018). Based on this evidence, we suggest that VTA promotes VTA-dmPFC communication through theta oscillations in response to tail pinch. Importantly, the degree of VTA-dmPFC theta connectivity might depend on contextual information (Park and Moghaddam, 2017) and involve other areas of the brain such as the hippocampus (Fujisawa and Buzsaki, 2011).

Unique features of the present data

Multiple studies have identified specialized populations of PFC and VTA neurons that respond to aversive and rewarding events (e.g., de Jong et al., 2019; Kobayashi et al., 2006; Warden et al., 2012). These studies have involved Pavlovian or instrumental conditioning paradigms and therefore provide data on how previously learned aversive or rewarding outcomes, or cues that predict those outcomes, are encoded by these neurons. Most of the

previous studies use either fiber photometry to assess population activity or assess neuronal activity in different sessions. They, therefore, do not measure the activity of the same neurons to both stress and reward.

Our study focused on the unanticipated experience of stress and reward. We were able to distinguish separate populations of dual-valence versus experience-specific neurons unrelated to conditioning paradigms. Notwithstanding the importance of encoding learned associations, animals must recognize unexpected aversive and appetitive events in order to survive. The relatively large proportion of neurons that responded to both experiences shown here may be critical for behavioral flexibility and future learning needed to successfully adapt to dangerous or positive elements in the environment.

Another novel aspect of the data is the selective engagement of the VTA-dmPFC networks by the stressful experience. The change in theta oscillation in the PFC, in coordination with other regions, has been implicated in state anxiety and learned fear (Padilla-Coreano 2019). The present data suggests that engagement of this network reflects an innate (not learned) response to an aversive event.

Caveats

PFC and VTA contain heterogeneous groups of cells. The PFC includes pyramidal cells and multiple types of GABA interneurons (DeFelipe and Fariñas, 1992; Somogyi et al., 1998). The VTA includes dopamine-containing and GABA-containing cells, both of which package other neurotransmitters including glutamate (Carr and Sesack, 2000; Nair-Roberts et al., 2008). We did not methodically distinguish between neuron types and indiscriminately recorded from all spontaneously active neurons. In the dmPFC, the low average firing rate of recorded neurons, and the general inefficiency of our style of electrodes to record from small interneurons, suggest that the majority, if not all, of the recorded neurons were pyramidal cells. In the VTA, we classified neurons based on firing characteristics as putative dopamine and non-dopamine. This characterization is consistent with optogenetically tagged dopamine neurons observed in previous studies (Cohen et al., 2012; Lohani et al., 2019). While we do not claim that this characterization is perfectly accurate, the finding that both DA and non-DA neurons can be activated and inhibited by both aversive and rewarding events is consistent with previous work (Cohen et al., 2012). Furthermore, regardless of the type of cells in either dmPFC or VTA that we recorded, our primary conclusion that same cells respond to both appetitive and aversive experiences holds.

Clinical implications

The gist of our finding is that a subpopulation of dmPFC and VTA neurons encodes both unanticipated aversive and rewarding experiences. These dual-valence neurons may be critical for vulnerability to develop disorders that are manifested, or exacerbated, by stress. These conditions, including mood and anxiety disorders, PTSD, addiction, and schizophrenia, involve symptoms with concomitant malfunction of negative and positive valence systems (Daviu et al., 2019; Kalivas and Volkow, 2005; Meyer-Lindenberg, 2010; Stanton et al., 2018). Animal models relevant to these disorders also suggest an alteration in communication between the PFC and other areas of the brain including the VTA (Bruchim-Samuel et al., 2016; Cha et al., 2014; Park and Moghaddam, 2017). Future work will be critical in identifying the brain circuitry that mediates the dual-valence response of dmPFC and VTA neurons.

References

Abercrombie E, Keefe K, DiFrischia D, Zigmond M (1989) Differential Effect of Stress on In Vivo Dopamine Release in Striatum, Nucleus Accumbens, and Medial Frontal Cortex. J Neurochem 52:1655–1658.

Adhikari A, Topiwala MA, Gordon JA (2010) Synchronized Activity between the Ventral Hippocampus and the Medial Prefrontal Cortex during Anxiety. Neuron 65:257–269.

Amarante LM, Caetano MS, Laubach M (2017) Medial frontal theta is entrained to rewarded actions. J Neurosci 37:1965–17.

Anstrom KK, Woodward DJ (2005) Restraint increases dopaminergic burst firing in awake rats. Neuropsychopharmacology 30:1832–1840.

Arnsten AFT (2015) Stress weakens prefrontal networks: molecular insults to higher cognition. Nat Neurosci 18:1376–1385.

Benchenane K, Tiesinga PH, Battaglia FP (2011) Oscillations in the prefrontal cortex: A gateway to memory and attention. Curr Opin Neurobiol 21:475–485.

Berridge KC (2019) Affective valence in the brain: modules or modes? Nat Rev Neurosci 20:225–234.

Bissonette GB, Gentry RN, Padmala S, Pessoa L, Roesch MR (2014) Impact of appetitive and aversive outcomes on brain responses: Linking the animal and human literatures. Front Syst Neurosci 8:1–15.

Brischoux F, Chakraborty S, Brierley DI, Ungless MA (2009) Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. Proc Natl Acad Sci U S A 106:4894–9.

Bromberg-Martin ES, Matsumoto M, Hikosaka O (2010) Dopamine in Motivational Control: Rewarding, Aversive, and Alerting. Neuron 68:815–834.

Bruchim-Samuel M, Lax E, Gazit T, Friedman A, Ahdoot H, Bairachnaya M, Pinhasov A, Yadid G (2016) Electrical stimulation of the vmPFC serves as a remote control to affect VTA activity and improve depressive-like behavior. Exp Neurol 283:255–263.

Burgos-robles A, Kimchi EY, Izadmehr EM, Porzenheim MJ, Ramos-guasp WA, Nieh EH, Felix-ortiz AC, Namburi P, Leppla CA, Presbrey KN, Anandalingam KK, Pagan-rivera PA, Anahtar M, Beyeler A, Tye KM (2017) Amygdala inputs to prefrontal cortex guide behavior amid conflicting cues of reward and punishment. Nat Neurosci 20:824–835.

Butts KA, Floresco SB, Phillips AG (2013) Acute stress impairs set-shifting but not reversal learning. Behav Brain Res 252:222–229.

Butts KA, Weinberg J, Young AH, Phillips AG (2011) Glucocorticoid receptors in the prefrontal cortex regulate stress-evoked dopamine efflux and aspects of executive function. Proc Natl Acad Sci U S A 108:18459–64.

Caracheo BF, Grewal JJS, Seamans JK (2018) Persistent Valence Representations by Ensembles of Anterior Cingulate Cortex Neurons. Front Syst Neurosci 12:1–11.

Carr DB, Sesack SR (2000) Projections from the rat prefrontal cortex to the ventral tegmental area: Target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. J Neurosci 20:3864–3873.

Ch'ng S, Fu J, Brown RM, McDougall SJ, Lawrence AJ (2018) The intersection of stress and

reward: BNST modulation of aversive and appetitive states. Prog Neuro-Psychopharmacology Biol Psychiatry 87:108–125.

Cha J, Carlson JM, DeDora DJ, Greenberg T, Proudfit GH, Mujica-Parodi LR (2014) Hyper-Reactive Human Ventral Tegmental Area and Aberrant Mesocorticolimbic Connectivity in Overgeneralization of Fear in Generalized Anxiety Disorder. J Neurosci 34:5855–5860.

Chaudhury D et al. (2013) Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. Nature 493:532–6.

Cohen JY, Haesler S, Vong L, Lowell BB, Uchida N (2012) Neuron-type-specific signals for reward and punishment in the ventral tegmental area. Nature 482:85–88.

Daviu N, Bruchas MR, Moghaddam B, Sandi C, Beyeler A (2019) Neurobiological links between stress and anxiety. Neurobiol Stress 11:100191.

de Jong JW, Afjei SA, Pollak Dorocic I, Peck JR, Liu C, Kim CK, Tian L, Deisseroth K, Lammel S (2019) A Neural Circuit Mechanism for Encoding Aversive Stimuli in the Mesolimbic Dopamine System. Neuron 101:133-151.e7.

DeFelipe J, Fariñas I (1992) The pyramidal neuron of the cerebral cortex: Morphological and chemical characteristics of the synaptic inputs. Prog Neurobiol 39:563–607.

Del Arco A, Park J, Wood J, Kim Y, Moghaddam B (2017) Adaptive encoding of outcome prediction by prefrontal cortex ensembles supports behavioral flexibility. J Neurosci 37:8363–8373.

Ellwood IT, Patel T, Wadia V, Lee AT, Liptak AT, Bender KJ, Sohal VS (2017) Tonic or Phasic Stimulation of Dopaminergic Projections to Prefrontal Cortex Causes Mice to Maintain or Deviate from Previously Learned Behavioral Strategies. J Neurosci 37:8315–8329.

Engelhard B, Finkelstein J, Cox J, Fleming W, Jang HJ, Ornelas S, Koay SA, Thiberge SY, Daw ND, Tank DW, Witten IB (2019) Specialized coding of sensory, motor and cognitive variables in VTA dopamine neurons. Nature 570:509–513.

Ferenczi EA, Zalocusky KA, Liston C, Grosenick L, Warden MR, Amatya D, Katovich K, Mehta H, Patenaude B, Ramakrishnan C, Kalanithi P, Etkin A, Knutson B, Glover GH, Deisseroth K (2016) Prefrontal cortical regulation of brainwide circuit dynamics and reward-related behavior. Science 351:aac9698.

Fries P (2005) A mechanism for cognitive dynamics: Neuronal communication through neuronal coherence. Trends Cogn Sci 9:474–480.

Fujisawa S, Buzsáki G (2011) A 4 Hz Oscillation Adaptively Synchronizes Prefrontal, VTA, and Hippocampal Activities. Neuron 72:153–165.

Gentry RN, Schuweiler DR, Roesch MR (2019) Dopamine signals related to appetitive and aversive events in paradigms that manipulate reward and avoidability. Brain Res 1713:80–90.

Gore F, Schwartz EC, Brangers BC, Aladi S, Stujenske JM, Likhtik E, Russo MJ, Gordon JA, Salzman CD, Axel R (2015) Neural Representations of Unconditioned Stimuli in Basolateral Amygdala Mediate Innate and Learned Responses. Cell 162:134–145.

Grunfeld IS, Likhtik E (2018) Mixed selectivity encoding and action selection in the prefrontal cortex during threat assessment. Curr Opin Neurobiol 49:108–115.

536 Hermans EJ, Henckens MJAG, Joels M, Fernandez G (2014) Dynamic adaptation of large-
537 scale brain networks in response to acute stressors. Trends Neurosci 37:304–314.

538 Holly EN, Miczek KA (2016) Ventral tegmental area dopamine revisited: Effects of acute and
539 repeated stress. Psychopharmacology (Berl) 233:163–186.

540 Holmes A, Wellman CL (2009) Stress-induced prefrontal reorganization and executive
541 dysfunction in rodents. Neurosci Biobehav Rev 33:773–783.

542 Homayoun H, Moghaddam B (2007) NMDA Receptor Hypofunction Produces Opposite
543 Effects on Prefrontal Cortex Interneurons and Pyramidal Neurons. J Neurosci
544 27:11496–11500.

545 Horst NK, Laubach M (2013) Reward-related activity in the medial prefrontal cortex is driven
546 by consumption. Front Neurosci 7:1–15.

547 Inglis FM, Moghaddam B (1999) Dopaminergic innervation of the amygdala is highly
548 responsive to stress. J Neurochem 72:1088–1094.

549 Jackson ME, Moghaddam B (2006) Distinct patterns of plasticity in prefrontal cortex neurons
550 that encode slow and fast responses to stress. Eur J Neurosci 24:1702–1710.

551 Kalivas PW, Volkow ND (2005) The Neural Basis of Addiction. Am J Psychiatry 162:1403–
552 1413.

553 Karalis N, Dejean C, Chaudun F, Khoder S, Rozeske RR, Wurtz H, Bagur S, Benchenane K,
554 Sirota A, Courtin J, Herry C (2016) 4-Hz oscillations synchronize prefrontal-amygdala
555 circuits during fear behavior. Nat Neurosci 19:605–12.

556 Kim CK, Yang SJ, Pichamoorthy N, Young NP, Kauvar I, Jennings JH, Lerner TN, Berndt A,
557 Lee SY, Ramakrishnan C, Davidson TJ, Inoue M, Bito H, Deisseroth K (2016)
558 Simultaneous fast measurement of circuit dynamics at multiple sites across the
559 mammalian brain. Nat Methods 13:325–328.

560 Kim Y, Wood J, Moghaddam B (2012) Coordinated activity of ventral tegmental neurons
561 adapts to appetitive and aversive learning. PLoS One 7:e29766.

562 Kim YB, Matthews M, Moghaddam B (2010) Putative gamma-aminobutyric acid neurons in
563 the ventral tegmental area have a similar pattern of plasticity as dopamine neurons
564 during appetitive and aversive learning. Eur J Neurosci 32:1564–1572.

565 Kobak D, Brendel W, Constantinidis C, Feierstein CE, Kepecs A, Mainen ZF, Romo R, Qi X,
566 Uchida N, Machens CK (2016) Demixed principal component analysis of neural
567 population data. Elife 5:1–37.

568 Kobayashi S, Nomoto K, Watanabe M, Hikosaka O, Schultz W, Sakagami M (2006)
569 Influences of Rewarding and Aversive Outcomes on Activity in Macaque Lateral
570 Prefrontal Cortex. Neuron 51:861–870.

571 Lammel S, Lim BK, Ran C, Huang KW, Betley MJ, Tye KM, Deisseroth K, Malenka RC
572 (2012) Input-specific control of reward and aversion in the ventral tegmental area.
573 Nature 491:212–217.

574 Lederbogen F, Kirsch P, Haddad L, Streit F, Tost H, Schuch P, Wüst S, Pruessner JC,
575 Rietschel M, Deuschle M, Meyer-Lindenberg A (2011) City living and urban upbringing
576 affect neural social stress processing in humans. Nature 474:498–501.

577 Li Y, Mathis A, Grewe BF, Osterhout JA, Ahanonu B, Schnitzer MJ, Murthy VN, Dulac C

(2017) Neuronal Representation of Social Information in the Medial Amygdala of Awake Behaving Mice. Cell 171:1176-1190.e17.

Lohani S, Martig AK, Deisseroth K, Witten IB, Moghaddam B (2019) Dopamine Modulation of Prefrontal Cortex Activity Is Manifold and Operates at Multiple Temporal and Spatial Scales. Cell Rep 27:99-114.e6.

Ma L, Hyman JM, Durstewitz D, Phillips AG, Seamans JK (2016) A Quantitative Analysis of Context-Dependent Remapping of Medial Frontal Cortex Neurons and Ensembles. J Neurosci 36:8258–8272.

Matsumoto H, Tian J, Uchida N, Watabe-Uchida M (2016) Midbrain dopamine neurons signal aversion in a reward-context-dependent manner. Elife 5:1–24.

Meyer-Lindenberg A (2010) From maps to mechanisms through neuroimaging of schizophrenia. Nature 468:194–202.

Mininni CJ, Caiafa CF, Zanutto BS, Tseng KY, Lew SE (2018) Putative dopamine neurons in the ventral tegmental area enhance information coding in the prefrontal cortex. Sci Rep 8:1–11.

Mitra PP, Pesaran B (1999) Analysis of dynamic brain imaging data. Biophys J 76:691–708.

Morales M, Margolis EB (2017) Ventral tegmental area: cellular heterogeneity, connectivity and behaviour. Nat Rev Neurosci 18:73–85.

Moriya S, Yamashita A, Kawashima S, Nishi R, Yamanaka A, Kuwaki T (2018) Acute Aversive Stimuli Rapidly Increase the Activity of Ventral Tegmental Area Dopamine Neurons in Awake Mice. Neuroscience 386:16–23.

Nair-Roberts RG, Chatelain-Badie SD, Benson E, White-Cooper H, Bolam JP, Ungless MA (2008) Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. Neuroscience 152:1024–1031.

Narayanan N, Laubach M (2009) Methods for studying functional interactions among neuronal populations. Methods Mol Biol 489:135–165.

Padilla-Coreano N, Canetta S, Mikofsky RM, Alway E, Passecker J, Myroshnychenko M V., Garcia-Garcia AL, Warren R, Teboul E, Blackman DR, Morton MP, Hupalo S, Tye KM, Kellendonk C, Kupferschmidt DA, Gordon JA (2019) Hippocampal-Prefrontal Theta Transmission Regulates Avoidance Behavior. Neuron 104:601-610.e4.

Park J, Moghaddam B (2017) Risk of punishment influences discrete and coordinated encoding of reward-guided actions by prefrontal cortex and VTA neurons. Elife 6:e30056.

Parker KL, Chen K-H, Kingyon JR, Cavanagh JF, Narayanan NS (2014) D1-Dependent 4 Hz Oscillations and Ramping Activity in Rodent Medial Frontal Cortex during Interval Timing. J Neurosci 34:16774–16783.

Rigotti M, Barak O, Warden MR, Wang XJ, Daw ND, Miller EK, Fusi S (2013) The importance of mixed selectivity in complex cognitive tasks. Nature 497:585–590.

Rozeske RR, Jercog D, Karalis N, Girard D, Herry C (2018) Prefrontal-Periaqueductal Gray-Projecting Neurons Mediate Context Fear Discrimination. Neuron 97:898–910.

Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80:1–27.

620 Smith WJ, Stewart J, Pfaus JG (1997) Tail pinch induces fos immunoreactivity within several
621 regions of the male rat brain: Effects of age. Physiol Behav 61:717–723.

622 Somogyi P, Tamás G, Lujan R, Buhl EH (1998) Salient features of synaptic organisation in
623 the cerebral cortex. Brain Res Rev 26:113–135.

624 Stanton CH, Holmes AJ, Chang SWC, Joormann J (2018) From Stress to Anhedonia:
625 Molecular Processes through Functional Circuits. Trends Neurosci 42:23–42.

626 Steinberg EE, Keiflin R, Boivin JR, Witten IB, Deisseroth K, Janak PH (2013) A causal link
627 between prediction errors, dopamine neurons and learning. Nat Neurosci 16:966–973.

628 Taber MT, Fibiger HC (1997) Activation of the mesocortical dopamine system by feeding:
629 lack of a selective response to stress. Neuroscience 77:295–298.

630 Thierry AM, Tassin JP, Blanc G, Glowinski J (1976) Selective activation of the mesocortical
631 DA system by stress. Nature 263:242–244.

632 Tye KM, Mirzabekov JJ, Warden MR, Ferenczi EA, Tsai H-C, Finkelstein J, Kim S-Y,
633 Adhikari A, Thompson KR, Andalman AS, Gunaydin LA, Witten IB, Deisseroth K (2012)
634 Dopamine neurons modulate neural encoding and expression of depression-related
635 behaviour. Nature 493:537–541.

636 Ulrich-Lai YM, Herman JP (2009) Neural regulation of endocrine and autonomic stress
637 responses. Nat Rev Neurosci 10:397–409.

638 Vander Weele CM, Siciliano CA, Matthews GA, Namburi P, Izadmehr EM, Espinel IC, Nieh
639 EH, Schut EHS, Padilla-Coreano N, Burgos-Robles A, Chang CJ, Kimchi EY, Beyeler
640 A, Wichmann R, Wildes CP, Tye KM (2018) Dopamine enhances signal-to-noise ratio
641 in cortical-brainstem encoding of aversive stimuli. Nature 563:397–401.

642 Warden MR, Selimbeyoglu A, Mirzabekov JJ, Lo M, Thompson KR, Kim SY, Adhikari A, Tye
643 KM, Frank LM, Deisseroth K (2012) A prefrontal cortex-brainstem neuronal projection
644 that controls response to behavioural challenge. Nature 492:428–432.

645 Weele CMV, Siciliano CA, Tye KM (2019) Dopamine tunes prefrontal outputs to orchestrate
646 aversive processing. Brain Res 1713:16–31.

647 Weinberg MS, Johnson DC, Bhatt AP, Spencer RL (2010) Medial prefrontal cortex activity
648 can disrupt the expression of stress response habituation. Neuroscience 168:744–756.

649 Ye L, Allen WE, Thompson KR, Tian Q, Hsueh B, Ramakrishnan C, Wang A-C, Jennings
650 JH, Adhikari A, Halpern CH, Witten IB, Barth AL, Luo L, McNab JA, Deisseroth K
651 (2016) Wiring and molecular features of prefrontal ensembles representing distinct
652 experiences. Cell 165:1–13.

653

654 **Figure Legends**

655 **Figure 1**. Electrode location and experimental protocol. **A**. Schematic of electrode arrays
656 implantation for recording simultaneously in the dmPFC and the VTA. **B**. Representation of
657 the electrode's placement in the dmPFC (prelimbic) and the VTA of the rat. **C**. Protocol
658 performed during the recording sessions.

659 **Figure 2**. dmPFC population activity during rewarding and stressful events. **A-B**. Heat plots
660 represent the baseline normalized firing rate for single units. Each row is the activity of a
661 single unit in 60 s time bins aligned to the first event ─ sucrose food exposure (A) or tail
662 pinch (B) (15 min; dashed lines) ─ and sorted from lowest to highest average normalize
663 firing rate. **C-D**. Time course of single unit's activation and inhibition during sucrose food
664 exposure (C) and tail pinch (D). All units represented in A-B are included. The percentage of
665 units was categorized as activated or inhibited based on whether their averaged activity by
666 60 s time bins was significantly different from baseline activity. **E-G**. Temporal profile of the
667 population activity associated with the top two principal components (PC1 and PC2) for
668 sucrose food exposure and tail pinch. **F**. Variance explained by the top five principal
669 components for both events. **H**. Representation of single units in the 2-d space according to
670 the top two principal components.

671 **Figure 3**. VTA population activity during rewarding and stressful events. **A-B**. Heat plots
672 represent the baseline normalized firing rate for single units. Each row is the activity of a
673 single unit in 60 s time bins aligned to the first event ─sucrose food exposure (A) or tail pinch
674 (B) (15 min; dashed lines) ─ and sorted from lowest to highest average normalize firing rate.
675 **C-D**. Time course of single unit's activation and inhibition during sucrose food exposure (C)
676 and tail pinch (D). All units represented in A-B are included. The percentage of units was
677 categorized as activated or inhibited based on whether their averaged activity by 60 s time
678 bins was significantly different from baseline activity. **E-G**. Temporal profile of the population
679 activity associated with the top two principal components (PC1 and PC2) for sucrose food
680 exposure and tail pinch. **F**. Variance explained by the top five principal components for both
681 events. **H**. Representation of single units in the 2-d space according to the top two principal
682 components.

683 **Figure 4**. dmPFC and VTA single units respond to rewarding and stressful events. **A-B**.
684 Representation of single units according to their response to sucrose food exposure and tail
685 pinch in the dmPFC (A) and the VTA (B). The response is the averaged normalized (z-score)
686 firing rate (FR) during the event (15 min). **C-D**. Proportion (percentage) of single units that
687 respond to sucrose food exposure and/or tail pinch in the dmPFC (C) and the VTA (D). **E**.
688 Electrophysiological characterization of VTA units in putative DA and non-DA according to

their basal firing rate and wave form duration. Each point represents one recorded unit. **F**. Proportion of putative DA and non-DA units that respond to sucrose food exposure and tail pinch (overlapped on D bar graph).

Figure 5. dmPFC and VTA LFP oscillations change during sucrose food exposure and tail pinch. **A-B**. Baseline normalized LFP power spectrum during sucrose food exposure and tail pinch in the dmPFC (A) and the VTA (B). **C**. Normalized power-spectrum plots comparing both events in the dmPFC and the VTA. **D**. Normalized dmPFC-VTA coherence plots comparing both events, sucrose food exposure and tail pinch. * $p<0.05$ one-way ANOVA.

698 **Table 1.** Statistical results according to brain area, data analyzed, and test used.

Neuronal Population	dmPFC	Basal firing rate (food vs stress)	paired *t* test	$t_{103} = 0.27$, p= 0.784
		Units activated/inhibited (food vs stress)	Chi square	$\chi^2_2 = 0.11$, p>0.1 (event time) $\chi^2_2 = 0.22$, p>0.1 (post-event time)
	VTA	Basal firing rate (food vs stress)	paired *t* test	$t_{37} = 2.08$, p= 0.044 (DA) $t_{23} = 1.36$, p= 0.186 (non-DA)

699

		Units activated/inhibited (food vs stress)	Chi square	χ^2_2= 1.93, p>0.1 (event time) χ^2_2= 1.02, p>0.1 (post-event time)
Single Units	dmPFC	Units responses	Chi square	χ^2_3= 4.88, p>0.1
	VTA	Units responses DA vs non-DA	Chi square Chi square	χ^2_3= 3.50, p>0.1 χ^2_3= 1.85, p>0.1
Unit Pairs	dmPFC	Units pairs	Chi square	χ^2_2= 4.44, p>0.1
	VTA	Units pairs	Chi square	χ^2_2= 2.44, p>0.1
LFP	dmPFC	LFP power (food vs stress)	Two-way ANOVA frequency effect event effect freq x event	 $F_{(15,336)}$= 6.77, p<0.001 $F_{(2,336)}$= 0.33, p>0.1 $F_{(30,336)}$= 1.67, p=0.017
			One-way ANOVA	$F_{(2,21)}$= 7.59, p=0.003
	VTA	LFP power (food vs stress)	Two-way ANOVA frequency effect event effect freq x event	$F_{(15,336)}$= 6.38, p<0.001 $F_{(2,336)}$= 11.54, p<0.001 $F_{(30,336)}$= 2.10, p=0.001
			One-way ANOVA	$F_{(2,21)}$= 4.44, p=0.024
	dmPFC-VTA	LFP coherence	One-way ANOVA	$F_{(2,21)}$= 4.39, p=0.025

704
705
706
707 **Table 2.** Proportion of significant unit-pairs in the PFC and the VTA. In
708 parenthesis, unit-pairs for dopamine (DA) and non-dopamine (Non-DA)
709 units.
710

	PFC	**VTA**	(DA / Non-DA)
Food consumption	0.35	0.31	(0.21 / 0.05)
Tail pinch	0.40	0.37	(0.22 / 0.09)
Food and Tail pinch	0.17	0.16	(0.10 / 0.04)
Only Food	0.18	0.15	(0.12 / 0.01)
Only Tail pinch	0.23	0.21	(0.13 / 0.01)

711

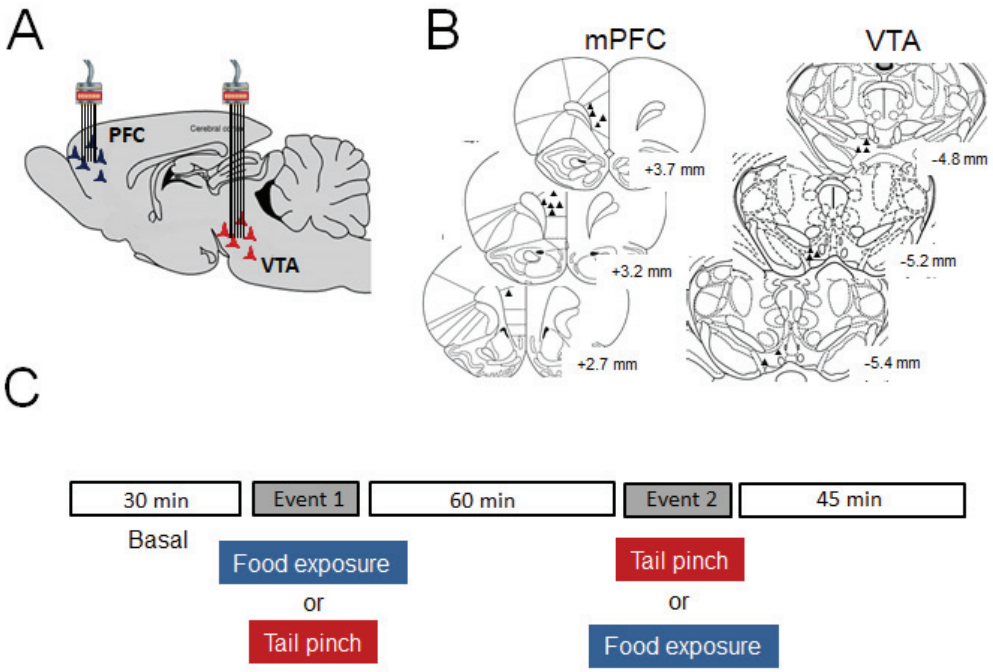

FIGURE 1

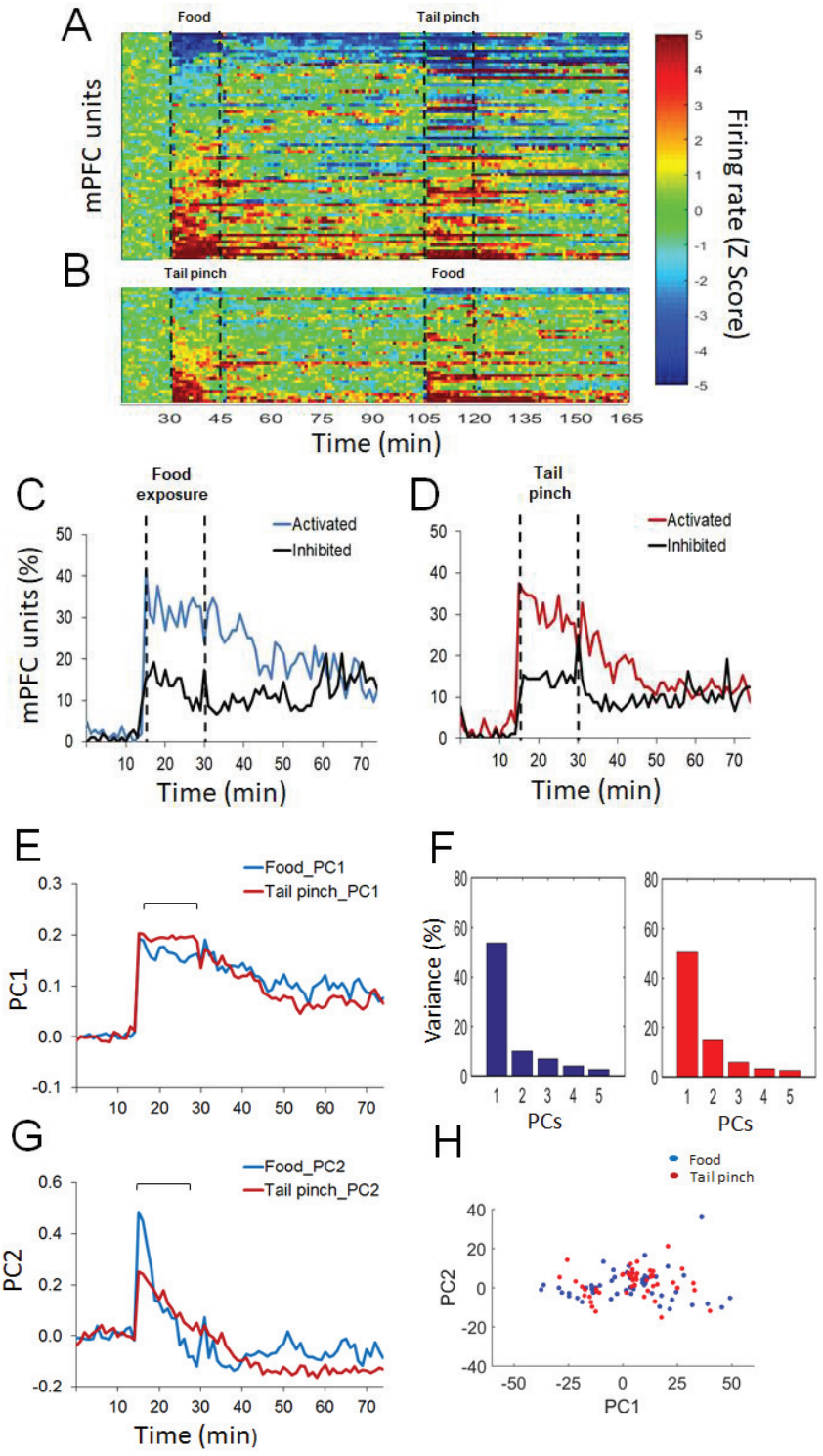

FIGURE 2

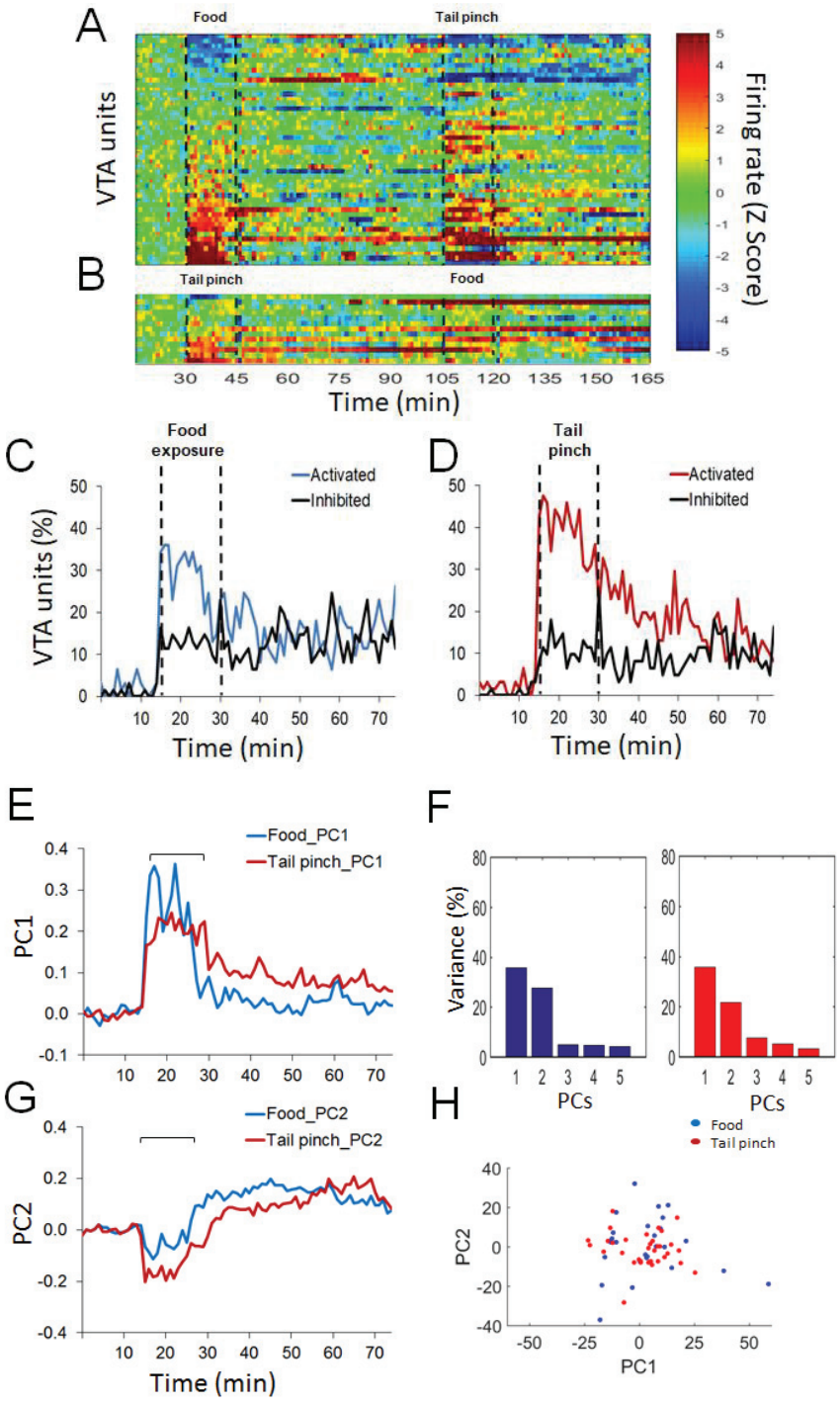

FIGURE 3

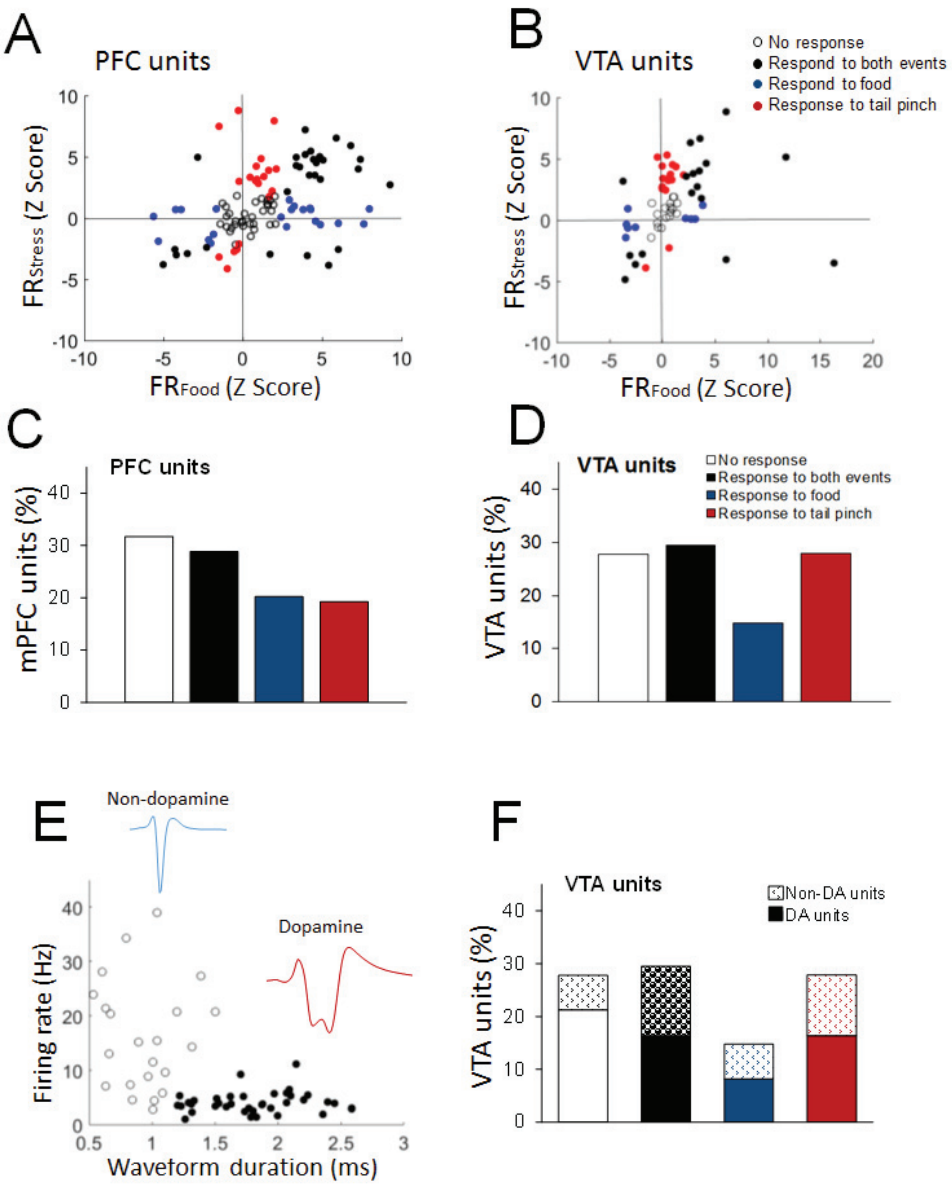

FIGURE 4

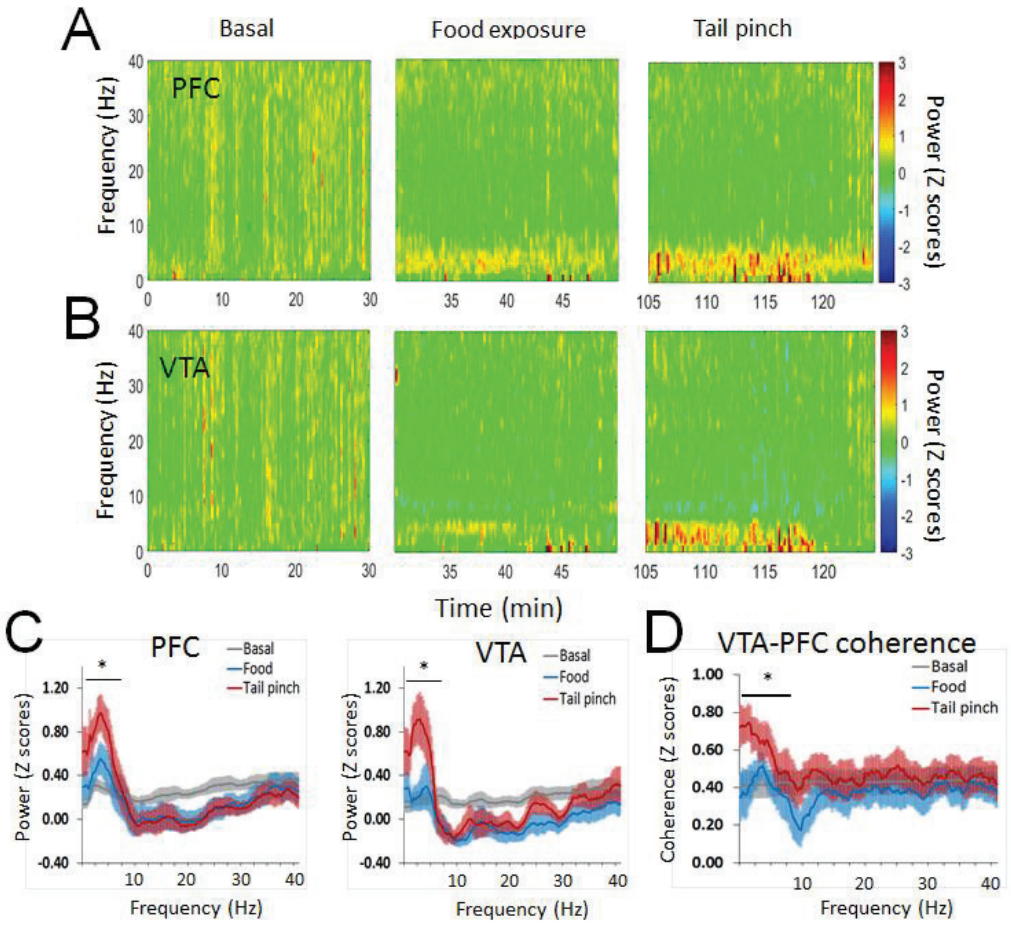

FIGURE 5