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Learn. Mem. 2011 18: 260-271
Access the most recent version at doi:10.1101/lm.1895211

Research

Ventral tegmental area and substantia nigra neural correlates of spatial learning

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The ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) may provide modulatory signals that, respectively, influence hippocampal (HPC)- and striatal-dependent memory. Electrophysiological studies investigating neural correlates of learning and memory of dopamine (DA) neurons during classical conditioning tasks have found DA neural activity in VTA and SNc to be tightly coupled with reinforcement expectations. Also, VTA integrity and DA in HPC have been found to regulate the encoding of HPC-dependent memories. Therefore, to determine the nature of the neural code HPC may receive from midbrain DA regions, the present study investigated VTA and SNc neural activity as navigating rats engaged in new spatial learning and experienced changes in expected goal locations. VTA and SNc cells were differentially engaged during training to a series of three novel goal locations. During task acquisition, the peak firing rates of VTA neurons decreased at the time of reward and shifted to time points before reward retrieval, whereas the peak firing rates of SNc neurons remained elevated at the time of reward during training to all three goal locations. Both VTA and SNc egocentric coding was strongest during training to the first goal location, which coincided with the time subjects learned the behavioral rules specific to the task. These data imply that VTA and SNc play complementary yet distinct roles in spatial learning to optimize adaptive behavior.

Dopamine (DA) is a neuromodulator that is known to regulate several forms of learning and memory. The DA system has been studied extensively in terms of its involvement in stimulus–response learning, reinforcement learning, working memory, and spatial memory. DA-producing neurons are found in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) (Ungerstedt 1971), and VTA and SNc, respectively, project to limbic and frontostriatal structures that are necessary for different mnemonic systems. These widespread innervations suggest that DA plays a general role in learning and memory. Importantly, DA is crucially implicated in reinforcement and motivation (for review, see Fields et al. 2007). Therefore, DA may regulate the effects of reinforcement and motivation on multiple memory systems (Mizumori et al. 2004, 2009; Fields et al. 2007; Yin et al. 2008).

A mnemonic role of DA has traditionally been studied in the context of tasks that require intact DA-striatal circuitry. As examples, DA antagonism in the nucleus accumbens (NAc) impairs acquisition of appetitive Pavlovian and operant tasks (Dalley et al. 2005; Hernandez et al. 2005) and DA levels in NAc are correlated with learning cue–stimulus relationships (Stuber et al. 2008). Functional dissociations, however, can be found between medial (mdSTR) and lateral dorsal striatum (ldSTR) such that mdSTR is implicated in action–outcome learning and ldSTR in stimulus–response learning, respectively (Featherstone and McDonald 2004; Yin et al. 2005, 2006). Thus, DA projections from SNc may regulate action–outcome and stimulus–response learning (for review, see Yin et al. 2008). DA also exerts profound effects on HPC-dependent learning and memory such as spatial learning, novelty detection, and context processing. Intrahippocampal injections of DA agonists and antagonists improve and impair HPC-dependent behaviors, respectively (Packard and White 1991; Gasbarri et al. 1996; Bernabeu et al. 1997; O'Carroll et al. 2006; Bethus et al. 2010). The majority of DA innervations to HPC arise from the VTA (Gasbarri et al. 1994), and VTA inactivation disrupts HPC-dependent learning, memory, and place fields (Martig et al. 2009; Rossato et al. 2009; Martig and Mizumori 2011).

DA appears to regulate memory systems via physiological mechanisms that influence neural plasticity. With regard to HPC-dependent memory processing in particular, D1 receptor agonism facilitates induction of LTP in HPC (Huang and Kandel 1995; Li et al. 2003; Nai et al. 2010) and D2 receptor antagonism impairs spatial memory and LTP in dentate gyrus (Saab et al. 2009) indicating that D1 and D2 receptors may work together to promote optimal plasticity in HPC subregions. Single unit recording studies also support the view that DA regulates memory related neural plasticity mechanisms in HPC. The primary behavioral correlate of principal cells in HPC is location-specific firing (O'Keefe and Dostrovsky 1971) and these "place cells" exhibit properties consistent with the view that HPC evaluates the extent to which a context changes (Mizumori 2008). DA influences these properties, as DA agonists increase place field specificity, and DA antagonists and VTA inactivation decrease place field specificity (Kentros et al. 2004; Martig and Mizumori 2010). Further, DA regulates place field stability in context-dependent ways (Gill and Mizumori 2006; Tran et al. 2008).

VTA and HPC are proposed to function as a loop whereby novel information detected by HPC regulates DA neuron firing in VTA. Novelty induced DA activity then promotes neural plasticity mechanisms in HPC to enhance behaviors such as spatial learning and context processing (Mizumori et al. 2004, 2009; Lisman and Grace 2005). Phasic DA neural activity in VTA and SNc is tightly coupled with reinforcement expectations (Schultz et al. 1997; Pan et al. 2005) and is thought to broadcast a teaching signal to efferent structures to reinforce learning and ongoing behaviors (Schultz and Dickinson 2000). Little is known about DA cell representations during performance of a

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Article is online at http://www.learnmem.org/cgi/doi/10.1101/lm.1895211.

HPC-dependent task. Puryear et al. (2010) recorded VTA neurons from navigating rodents that were performing a well-learned context-dependent spatial working memory task. Under these conditions, VTA activity was coupled to both reinforcement expectations and egocentric variables, such as the velocity and acceleration of movement by the animal. Interestingly, VTA reward-related phasic activity was also context-dependent and thus not reflective of only sensory or motor aspects of reward consumption. Contrary to the expectations of the prediction error hypothesis of DA function (for review, see Schultz 1998), reward-related DA cell firing was observed even though the rats were well trained. It was suggested that continued reward responding by DA cells reflected the inherent greater uncertainty of working memory tasks.

Recently DA has been found to be selectively involved during acquisition of HPC-dependent tasks (e.g., Rossato et al. 2009; Bethus et al. 2010). Thus, the present study recorded VTA neurons during the acquisition of a nonworking memory task in which rats were rewarded for finding food at a single maze location. Recording continued as rats were trained (in sequence) to find food at two additional (novel) reward locations on the elevated plus maze. In terms of the behavior, we predict that rats will initially perform poorly when they are required to switch to new goal locations. As rats experience more goal location switches, performance should improve relative to initial learning at previous goal locations because they will more quickly implement spatial search strategies to locate the reward. We hypothesize that when rats receive reward, VTA and SNc activity will reflect choice accuracy, showing more robust neural responses during early training that gradually decrease as rats learn where the reward is located. Due to differential connectivity patterns, VTA and SNc are implicated in HPC- and STR-dependent memories, respectively. Thus, as rats experience more goal location changes, we expect activity at the time of reward to decrease in VTA, reflecting HPC influences on behavioral expectations for spatial changes in the goal location. SNc activity at the time of reward might not reflect expectations for spatial changes of reward. Rather, we expect SNc activity to be robust during all three goal location sessions, reflecting mdSTR involvement in learning action–outcome relationships. DA is also heavily implicated in movement (Campanella et al. 1987) and action selection (Wickens et al. 2007). Therefore, both VTA and SNc egocentric coding, in this case, velocity tuning, may be strongest when rats are learning movement strategies necessary to navigate the task. Consistent with this prediction, we found VTA and SNc egocentric coding to be strongest during initial acquisition phases of the task. We found that VTA and SNc cells are differentially engaged in terms of reward activity as a function of changes in the goal location. Consistent with our hypothesis, VTA responses at the time of reward decreased as rats experienced more goal location changes, whereas SNc activity remained robust.

Results

Behavior

VTA and SNc neural activity was recorded as rats were tested on a plus maze task during which they were habituated (HAB) and sequentially trained to retrieve reward from one of three goal locations (Goal 1, Goal 2, and Goal 3) (Fig. 1). Errors were marked when all four limbs were placed on an erroneous arm and trial times were recorded with a stopwatch. To characterize differences in performance across individual goal locations, the goal conditions (Goal 1, Goal 2, and Goal 3) were subdivided based on a median split of the average number of errors per trial, resulting in training and asymptotic performance conditions. A total of

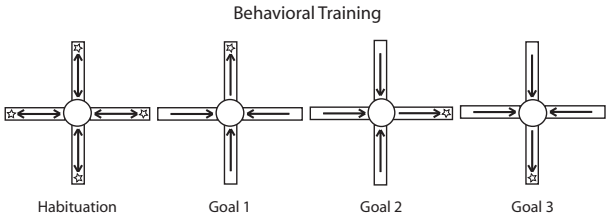

Figure 1. Schematic of the plus maze task. During the habituation phase, all four food cups are replenished immediately after rats consume the chocolate milk reward. After rats traverse the maze reliably and consume reward consistently, plus maze training begins. Rats are sequentially trained to a total of three goal locations (Goal 1, Goal 2, and Goal 3). Rats receive 12 trials per day (session). A trail begins when they are placed on a randomly chosen start arm; they are allowed to explore all arms until they find the goal location. The goal location remains constant until a criterion of nine or more correct trials is met for each of two consecutive sessions. Upon meeting the criterion, rats are trained to a new goal, until the same criterion is achieved. Finally, rats are trained to the third goal location. Visual cues are displayed in fixed positions throughout training to the three goal locations.

59 sessions were analyzed. There were significant main effects of condition in terms of the average errors per trial (see Fig. 2) ($F_{(5,58)} = 12.34$, $P < 0.01$). Significant differences existed between performance conditions ($F_{(1,58)} = 51.47$, $P < 0.01$) with rats making more errors during training (0.74 ± 0.06) than during asymptotic performance (0.07 ± 0.06). There were no significant interaction effects between goal conditions (Goal 1, Goal 2, and Goal 3) and performance conditions (training vs. asymptotic performance) ($F_{(2,58)} = 0.847$, ns) and no significant differences between goal conditions ($F_{(2,58)} = 0.624$, ns), indicating that choice accuracy did not differ between Goal 1 (0.06 ± 0.09), Goal 2 (0.08 ± 0.10), and Goal 3 (0.07 ± 0.15): rats made more errors during training in all three goal conditions.

There was also a significant main effect of condition on the average time per arm entry ($F_{(5,58)} = 3.07$, $P < 0.05$) with significant differences found between goal conditions ($F_{(2,58)} = 4.00$, $P < 0.05$). Post hoc analysis revealed that arm entry times at Goal 1 (19.92 ± 3.06 sec) were greater than arm entry times at Goal 3 (6.87 ± 4.3 sec), $P = 0.05$. There was neither a significant interaction ($F_{(2,58)} = 2.53$, ns) nor significant difference between performance conditions ($F_{(1,58)} = 2.62$, ns). Independent *t*-tests were used to compare arm entry times between training and asymptotic performance during separate goal conditions. There was a significant difference between performance conditions at Goal 1 ($t_{(23)} = 2.34$, $P < 0.05$) with rats taking longer during training (29.07 ± 4.04 sec) than during asymptotic performance (10.77 ± 4.23 sec). Arm entry times did not differ between performance conditions at Goal 2 ($t_{(19)} = 0.24$, ns) and Goal 3 ($t_{(11)} = 1.80$, ns).

To summarize, rats made more errors during training than during asymptotic performance regardless of goal condition. Behavior differed in terms of latency to reward across goal conditions such that rats were slower during training than during asymptotic performance, but only at the first learned goal location (Fig. 2).

Units

Baseline properties of VTA and SNc neurons

The average firing rates ranged from 0.02 to 43.61 Hz, with a large number of cells exhibiting firing rates below 15 Hz (Fig. 3). Recording of putative DA and non-DA neurons was restricted

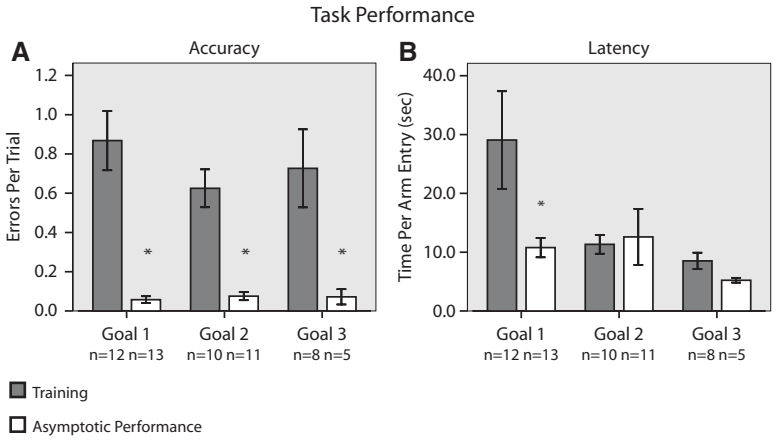

Figure 2. Task performance. (*A*) Plus maze performance presented as the average number of errors per trial across goal locations during training and asymptotic performance sessions. Rats made similar numbers of errors, and more errors, during training sessions at all goal locations. Asymptotic performance was characterized by few, if any, errors. (*B*) Plus maze performance across goal locations presented as the average time per arm entry during training and asymptotic sessions. During training sessions to the first goal location, rats took longer to complete trials compared to the asymptotic performance sessions.

using criteria that excluded cells based on average firing rates and reward-related activity. Reward-related activity was assessed using peri-event time histograms (PETHs) constructed around an experimenter defined time window of 4 sec surrounding reward receipt. A cell was considered to have a significant excitatory reward response if it exhibited elevated average firing rates within 150 msec of reward receipt that exceeded average firing rates by 2 standard deviations (SD); see Materials and Methods for details. The distribution of firing rates of cells that exhibited significant reward activity ranged from 0.02 to 22.92 Hz (Fig. 3). After a firing rate criterion of >1 Hz and <15 Hz was imposed, a total of 169 VTA neurons and 77 SNc neurons were analyzed. Average spike duration (latency difference between the maximum and minimum points of the analog voltage signal) were broad (338.9 ± 12.9 μsec) and there were no differences between cells recorded in VTA (341.0 ± 13.5 μsec) and SNc (334.4 ± 11.6 μsec), $t_{(244)} = 0.37$, ns. Average firing rates in VTA (4.34 ± 0.23 Hz) were higher than in SNc (3.09 ± 0.13 Hz), $t_{(244)} = 3.45$, $P < 0.01$. When compared across habituation and goal conditions average firing rates in VTA or SNc did not differ, $F_{(3,165)} = 1.34$, ns; $F_{(3,73)} = 1.80$, ns, for VTA and SNc, respectively. There were also no differences when goal conditions were subdivided according to performance conditions, $F_{(5,115)} = 0.16$, ns; $F_{(5,64)} = 0.50$, ns, for VTA and SNc, respectively. The percentage of spikes that fired in bursts (see Materials and Methods for bursting criteria) was also significantly greater in VTA ($60.98 \pm 1.93\%$) than in SNc ($31.56 \pm 1.48\%$), $t_{(244)} = 9.69$, $P < 0.01$. When compared across habituation and goal conditions burst activity in VTA or SNc did not differ, $F_{(3,165)} = 1.13$, ns; $F_{(3,73)} = 2.70$, ns, for VTA and SNc, respectively; there were no differences when goal conditions were subdivided according to performance conditions, $F_{(5,115)} = 1.02$, ns; $F_{(5,64)} = 0.63$, ns, for VTA and SNc, respectively (Fig. 4). To summarize, overall average firing rates and burst activity were higher in VTA than in SNc. When analyzed separately, however, firing rates and burst activity in VTA and SNc did not differentially respond to the different goal or performance conditions.

Reward–related firing properties in VTA and SNc neurons

VTA and SNc reward activity differed in that a higher *proportion of cells* in SNc (0.55) had significant reward responses than in VTA (0.38), $\chi^2(1, N = 246) = 5.57$, $P < 0.05$. The proportion of cells exhibiting significant reward responses was compared separately across habituation and goal conditions in VTA and SNc. In VTA there was a significant effect of condition, $\chi^2(3, N = 169) = 10.45$, $P < 0.05$. Post hoc analysis adjusted for multiple comparisons ($\alpha = 0.008$) revealed significant differences between Habituation (0.47) and Goal 3 (0.15), $\chi^2(1, N = 87) = 9.66$, $P < 0.005$, and Goal 1 (0.44) and Goal 3 $\chi^2(1, N = 73) = 7.19$, $P < 0.008$. Differences between Goal 2 (0.42) and Goal 3 approached significance, $\chi^2(1, N = 77) = 6.68$, $P = 0.009$. Thus, in VTA, relative to Habituation and Goal 1 there was a significant decrease in the proportion of cells that exhibited significant reward responses during testing at Goal 3. In SNc there was not a significant effect of condition, $\chi^2(3, N = 77) = 1.34$, $P = 0.72$, indicating the proportion of cells with significant reward responses did not change across conditions (Fig. 5).

Next, the *strength of the reward response* was evaluated by comparing the normalized peak firing rates between structures. Overall the peak firing rates in SNc ($z = 2.00 \pm 0.16$) were significantly higher than those in VTA ($z = 1.54 \pm 0.12$), $t_{(244)} = 2.25$, $P < 0.05$. The peak firing rates were further examined across goal and performance conditions for VTA and SNc cells. There was a significant main effect of condition on VTA peak firing rates, $F_{(5,115)} = 3.25$, $P < 0.01$. There was a difference across goal conditions, $F_{(2,115)} = 12.89$, $P < 0.01$, such that the peak firing rates at

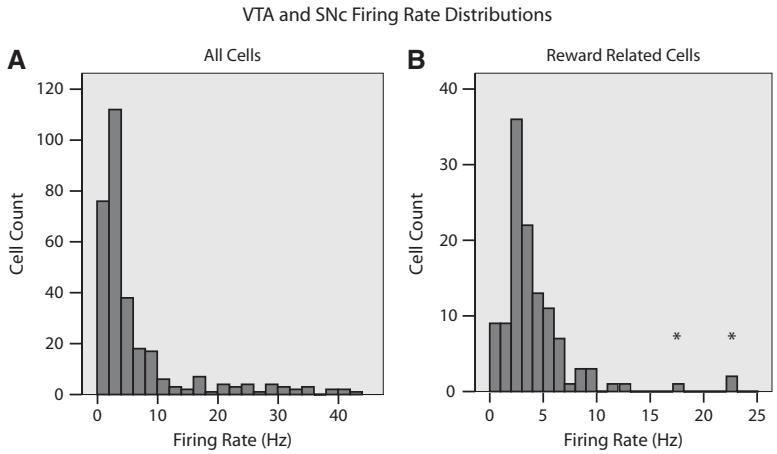

Figure 3. Firing rate distributions. (*A*) Distribution of average firing rates for all cells (i.e., VTA combined with SNc). (*B*) Distribution of average firing rates for all cells with significant reward-related responses. Cells with firing rates over 2 standard deviations outside the normal distribution are indicated with asterisks.

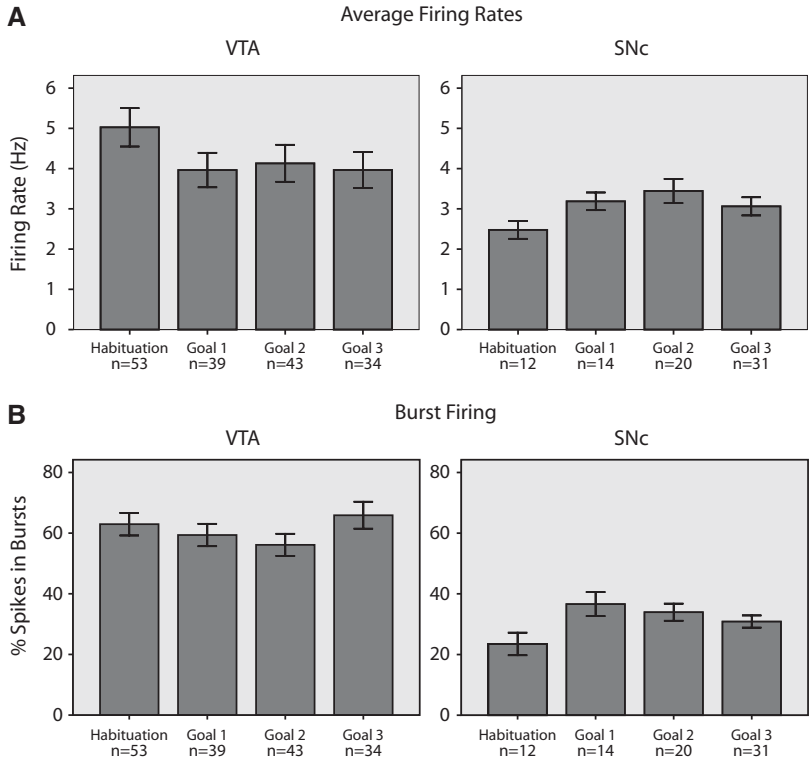

Figure 4. Average firing rates and burst firing. (*A*) Average firing rates across habituation and goal locations for VTA and SNc cells. There were no differences as a function of behavioral condition in average firing rates for cells recorded in VTA or SNc. (*B*) Percent of spikes fired in bursts across habituation and goal locations for VTA and SNc cells. No differences as a function of behavioral condition were observed.

the time of reward were significantly greater during Goal 1 ($z = 1.81 \pm 0.22$) and Goal 2 ($z = 1.52 \pm 0.20$) than the peak firing rates during Goal 3 ($z = 0.64 \pm 0.23$), $P < 0.05$ for both comparisons. There were no interaction effects, $F_{(2,115)} = 0.82$, ns, nor were there differences based on performance condition $F_{(1,115)} = 0.28$, ns, indicating that the strength of the reward response did not change when rats were training or at asymptotic levels of performance. There were no main effects of conditions on the peak firing rates in SNc, $F_{(5,64)} = 0.10$, ns, indicating that there were no changes in the strength of reward responses across goal or performance conditions (Fig. 5).

Finally, the *temporal specificity of the reward response* was evaluated by investigating the time of peak activity within the 4-sec epoch surrounding the time of reward acquisition. Negative and positive values represent the peak firing rates that occur before and after the reward, respectively. Overall PETH window peak times for SNc cells (-472.40 ± 96.12 msec) were not different from those in VTA (-405.77 ± 80.68 msec), $t_{(244)} = 0.49$, ns. Peak times of VTA and SNc cell firing were further examined across goal and performance conditions. There was a significant main effect of condition on peak times in VTA, $F_{(5,115)} = 2.53$, $P < 0.05$. There was a difference across goal conditions, $F_{(2,115)} = 5.638$, $P < 0.01$ such that the peak time of activity occurred significantly earlier for Goal 3 (-851.40 ± 175.61 msec) than for Goal 2 (-166.79 ± 155.45 msec) and Goal 1 (129.17 ± 167.35 msec), $P < 0.05$ for both comparisons. There were no interaction effects, $F_{(2,115)} = 0.96$, ns, nor were there differences based on performance condition, $F_{(1,115)} = 0.33$, ns, indicating that the specificity of the reward response did not change based on whether rats were training or at asymptotic levels of performance. There were no main effects of condition on peak times in SNc, $F_{(5,64)} = 0.31$, ns, indicating that there were no changes in the specificity of reward responses according to goal or performance conditions (Fig. 5).

To summarize, the proportion of SNc neurons with significant reward responses was greater than that of VTA neurons. The number of SNc cells with excitatory reward responses did not change across goal conditions, whereas the number of VTA cells with excitatory reward responses decreased during testing at Goal 3. The overall strength of the reward response in terms of the peak firing rates at the time of reward was greater for SNc cells than VTA cells. There were also differences in the peak firing rates between SNc and VTA in terms of goal conditions. Consistent with the pattern of changes in the proportion of cells with reward responses, the SNc peak firing rates did not change as a function of testing phase, whereas the peak firing rates of VTA cells decreased substantially when rats were tested at Goal 3. There were no changes in the strength of the reward response based on performance condition for either structure. Finally, VTA reward responses were more specific to reward retrieval when rats were tested at Goal 1 and Goal 2 than when tested at Goal 3. There were no differences in peak times as a function of training condition in SNc and there were no differences based on performance condition in either structure. Thus VTA, and not SNc, neurons appear to change their reward responsiveness as a function of experience. Example histograms for reward-related firing patterns can be found in Figure 6.

Correlations between velocity and firing rates in VTA and SNc

Pearson's correlation coefficients were calculated to assess the relationship between unit activity and an animal's movement. A total of 139 cells were analyzed from VTA and 77 cells from SNc. Collapsed across conditions, velocity correlations with the firing rate of cells in VTA ($R = 0.29 \pm 0.02$) were stronger than those of SNc cells ($R = 0.20 \pm 0.02$), $t_{(214)} = 2.41$, $P < 0.05$. Velocity correlations were compared across habituation and goal conditions separately for VTA and SNc neurons. Velocity correlations did not differ across conditions, $F_{(3,138)} = 0.96$, ns; $F_{(3,73)} = 0.89$, ns, for VTA and SNc, respectively. When goal conditions were subdivided according to performance conditions (i.e., training vs. asymptotic levels), there were significant main effects of condition on velocity correlations of VTA and SNc cells, $F_{(5,88)} = 2.4$, $P < 0.05$; $F_{(5,64)} = 3.18$, $P < 0.05$, for VTA and SNc, respectively. In both regions there was a significant effect of performance condition $F_{(1,88)} = 5.79$, $P < 0.05$; $F_{(1,64)} = 8.69$, $P < 0.05$, for VTA and SNc, respectively, but there were no significant goal condition, $F_{(2,88)} = 0.49$, ns; $F_{(2,64)} = 0.72$, ns, for VTA and SNc, respectively, or interaction effects, $F_{(2,88)} = 2.47$, ns; $F_{(2,64)} = 0.08$, ns, for VTA and SNc, respectively. Thus, the overall level of training was the most reliable predictor of how an animal's movement velocity relates to cell firing rates.

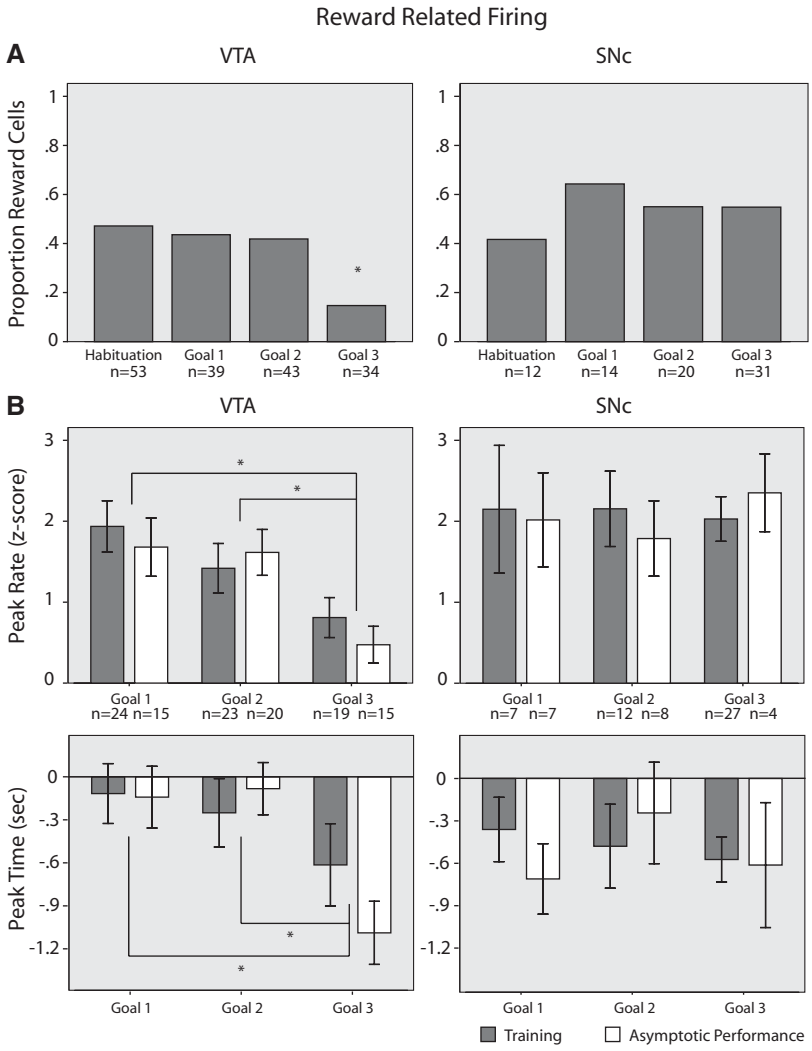

Figure 5. Reward-related activity. (*A*) Proportion of cells with significant reward responses across habituation and training to different goal locations. There was a significant decrease in the proportion of cells with significant reward correlates during testing at the third goal condition relative to habituation and the first goal condition for only VTA cells. (*B*) Normalized peak reward firing rates and peak times of activity in VTA and SNc across goal conditions during training and asymptote. There was a significant decrease in peak reward firing rates and peak times of activity during testing at the third goal condition for VTA, but not SNc cells.

Independent *t*-tests were used to compare velocity correlations between training and asymptotic performance at all three goal conditions. For VTA cells there were significant differences in velocity correlations between training ($R = 0.44 \pm 0.07$) and asymptotic performance ($R = 0.11 \pm 0.04$) at Goal 1, $t_{(28)} = 2.68$, $P < 0.05$, such that stronger correlations between the rat's velocity and the firing rate of the cell were evident during training. There were no significant differences between performance conditions at Goal 2, $t_{(35)} = 0.09$, ns, or Goal 3, $t_{(20)} = 1.3$, ns. Similar to VTA responses, SNc cells showed significant differences in velocity correlations between training ($R = 0.40 \pm 0.06$) and asymptotic performance ($R = 0.08 \pm 0.04$) at Goal 1, $t_{(12)} = 4.27$, $P < 0.01$. Also, there were no significant differences between performance conditions at Goal 2, $t_{(18)} = 0.95$, ns, or Goal 3, $t_{(29)} = 0.59$, ns, in SNc (Fig. 7).

To summarize, velocity correlations with the firing rate were stronger in VTA than in SNc. When analyzed separately, the same patterns of changes in correlations between the rat's velocity and the firing rate of the cell were evident in both VTA and SNc such that correlations were strongest during training to the first goal location (Fig. 7).

Relationships between reward responses and training duration

A Pearson's correlation analysis was implemented to investigate the relationship between reward responses and time spent performing the task. In VTA, there was a significant, weak correlation between peak reward activity and number of rewarded arm entries, $R = 0.06$, $N = 169$, $P < 0.01$. There was not a significant relationship between peak time and rewarded arm entries, $R = 0.0009$, $N = 169$, ns. In SNc, there was not a significant relationship between peak reward activity and the number of rewarded arm entries, $R = 0.004$, $N = 77$, ns, nor was there a significant relationship between peak time and the number of rewarded arm entries, $R = 0.005$, $N = 77$, ns.

Discussion

The present study investigated VTA and SNc neural responses as unrestrained rats acquired both motor skills and mnemonic strategies needed to solve a naturalistic foraging task. It appears that VTA and SNc population activity are differentially influenced when animals are engaged in a HPC-dependent task. The number and extent of VTA, but not SNc, reward responses declined after animals learned a spatial task. Both VTA and SNc movement-correlated firing was strongest during the initial stages of learning. These findings generally support current theories about SNc and VTA roles in regulating action selection and multiple memory systems, respectively. Moreover, the observed differential responding by VTA and SNc neurons is consistent with their different patterns of afferent and efferent connections with HPC.

Differential reward-related responses by VTA and SNc neurons

There were proportionally greater numbers of SNc neurons that exhibited significant reward activity relative to VTA. Also, the normalized peak firing rates at the time of reward were greater in SNc than in VTA. Given that a primary correlate for DA neurons is reward activity, the finding that more SNc neurons had significant reward correlates is consistent with the differential distribution of DA neurons between VTA and SNc (Margolis et al. 2006a).

We hypothesized that reward activity of VTA and SNc cells would be more robust when rats initially learned about a new goal location (i.e., during the training phase) relative to

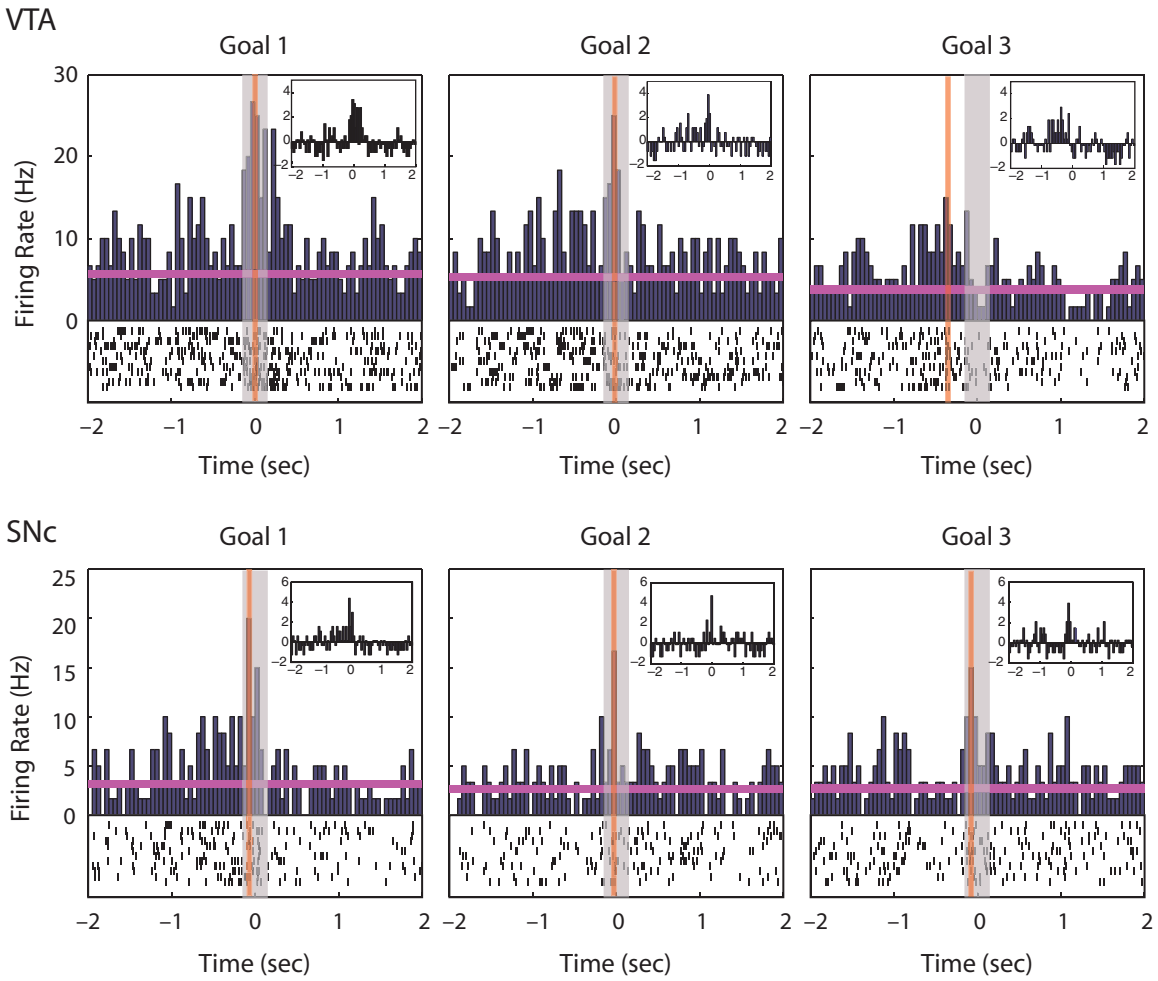

Figure 6. Individual examples of reward-related activity. Peri-event time histograms (PETHs) constructed for six representative cells recorded in VTA or SNc during testing at all three goal conditions. Time 0 is the time when the rat licked the food cup. Individual spikes are represented by tick marks in the raster plots below the PETH. The area highlighted in gray represents the experimenter defined reward interval. The area highlighted in orange represents peak times of activity. The purple line represents the average firing rate during the 4-sec time window. Insets are *Z*-score representations of firing rate histograms with time on the *x*-axis and *Z*-scores on the *y*-axis.

asymptotic performance for that same goal location. Our results are inconsistent with this hypothesis. Reward activity in VTA and SNc did not differ depending on whether the rats were being trained initially or performing at asymptotic levels. Numerous electrophysiological studies in primates have found that DA neurons no longer respond to the reward after the animal is trained to predict reward delivery (for review, see Schultz 1998). This apparent discrepancy may be accounted for by considering differences between studies in terms of when the unit recordings were collected relative to the extent of behavioral training. Primate recordings occur after the subject has been overtrained to predict reward whereas recordings in this study were collected during initial learning. Pan and colleagues (2005) recorded from DA neurons as rats were being trained on an appetitive classical conditioning task and found that the reward response does not subside until well after the development of conditioned responding. Our results could be considered consistent with these findings as testing after the goal location was learned lasted for only about 2 d. Therefore, our rats may never have received sufficiently extensive training to a particular goal location to result in reduced reward responding, even during the asymptotic performance phase.

The proportion of SNc neurons exhibiting significant reward responses remained constant across all testing phases for all three goal locations, whereas the proportion of VTA neurons exhibiting reward responses decreased during testing at the third goal location. Moreover peak firing to rewards declined for VTA, and not SNc, cells by the time of training to the third goal location. Also, the latency to the peak response occurred earlier for VTA, and not SNc, reward-related activity only during testing at the third goal location. These results are partially consistent with our prediction that reward activity would decrease as the rats experienced more goal location changes in that reward activity was weaker and less specific to the reward time by the third goal location. Further, correlation analyses investigating the relationships between the numbers of rewarded arm entries and the peak firing rates and times revealed either weak significant correlations or no correlations at all. Therefore, it appears that decreases in reward activity at the third goal location may be more readily explained in terms of experience with the task procedure of changing spatial locations. Conversely, SNc reward-related activity remained stable throughout training, perhaps reflecting the repeatedly changing behavioral requirements for reward retrieval across the different goal conditions.

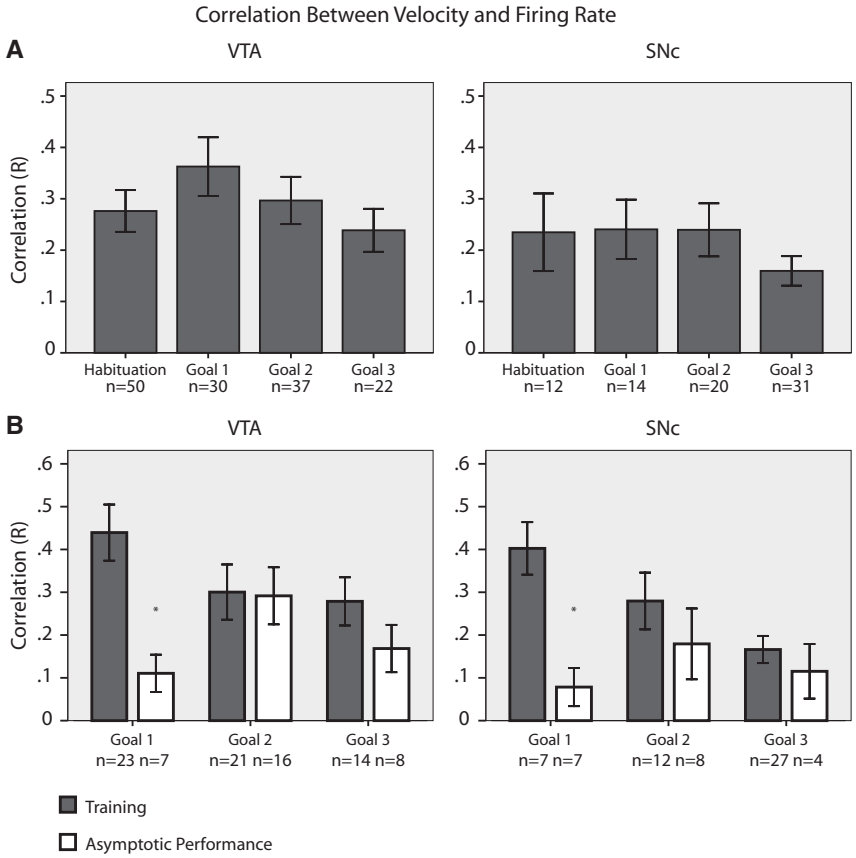

Figure 7. Velocity and firing rate correlations. (*A*) Average correlations between velocity and firing rate across habituation and goal locations for VTA and SNc cells. There were no differences in velocity-rate correlations. (*B*) Average velocity correlations with firing rate across goal locations during training and asymptotic performance. The velocity correlations of VTA and SNc neurons were significantly higher during training than asymptotic performance during testing at the first goal location. No such distinction was found when training to Goals 2 and 3.

The overall pattern of reward-related responses by VTA neurons suggests that VTA may enhance learning relationships between the reward and spatial aspects of the environment. In contrast, SNc reward-related activity may enhance learning of contingencies required for successful behavioral performance. These results are consistent with current theories suggesting that VTA and HPC operate as a circuit (Lisman and Otmakhova 2001; Mizumori et al. 2004) to mediate successful encoding and/or persistence of HPC-dependent memories (Lisman and Otmakhova 2001; Lisman and Grace 2005; Rossato et al. 2009; Bethus et al. 2010), whereas SNc and mdSTR interactions mediate a separate although parallel circuit to facilitate action–outcome learning (for review, see Yin et al. 2008).

Velocity and firing rate correlations of VTA and SNc cells

VTA and SNc neurons exhibited similar changes in velocity correlations across training conditions. In terms of behavior, rats took longer to complete trials during training to the first goal location. After this initial phase, arm entry times decreased and remained stable throughout the experiment indicating that rats learned how to effectively navigate the maze after training to the first goal location. Changes in VTA and SNc egocentric codes paralleled behavior in that the strongest firing rate correlations with velocity were found during training to the first goal location when the animals were taking longer to complete individual trials. Thus, the stronger egocentric coding by VTA and SNc neurons may relate to the development and subsequent utilization of goal-directed search strategies.

VTA–HPC interactions during learning and memory

Current theories propose that VTA and HPC reciprocally interact such that novel, context relevant information detected by HPC enhances VTA DA release, which in turn enables encoding of new information into long-term memory (Mizumori et al. 2004; Lisman and Grace 2005; Bethus et al. 2010). VTA input to HPC may be direct (Gasbarri et al. 1994), whereas HPC output to VTA is likely indirect, arriving via an accumbens to ventral pallidal to pedunculopontine nucleus route (Yang and Mogenson 1987; Floresco et al. 2001). Supporting this view, VTA has been shown to regulate HPC neural activity during spatial learning (Martig and Mizumori 2011) and encoding of HPC-dependent memories (Rossato et al. 2009). The pedunculopontine nucleus has been shown to regulate DA neuron firing (Kelland et al. 1993; Floresco et al. 2003; Zweifel et al. 2009) and HPC regulates VTA DA neuron firing (Floresco et al. 2001). Also, HPC has been shown to regulate DA responses to novelty (Legault and Wise 2001) and analogous to hippocampal place fields, phasic reward responses of putative DA neurons in VTA are sensitive to changes in the visuo-spatial context (Puryear et al. 2010).

The present study allowed us to investigate VTA neural responses during acquisition of a HPC-dependent task as navigating rats engaged in new spatial learning or as trained rats experienced changes in the expected spatial location of reward. Training to the first two goal locations presents new rules to the rat. During training to the first goal location, rats learned the general rules for solving the plus maze task (e.g., food is found at the end of a maze arm) and they were exposed to new environmental cues. Then, the reward location was changed for the first time to a different (i.e., the second) goal as the cues and general rules remained constant. By the time rats were trained to go to the third goal location, rats had experienced the procedure of changing goal locations. Thus, the rules and procedural contingencies are learned by the time rats experience the change to the third goal location. However, the rats still had to learn a new reward location during testing to Goal 3. That is, rats learned that reward was located at the end of a maze arm that did not previously contain reward. Under similar conditions HPC neurons continue to reorganize and discriminate reward locations (Smith and Mizumori 2006). Therefore, HPC itself was likely engaged during learning the third goal location, presumably to signal a change in context. It is possible then that VTA–HPC interactions are recruited at the time of reward when rats are learning the first two reward locations: VTA reward activity may facilitate encoding of spatial properties of the reward location by HPC neurons. However, this is not to say that reward-related DA activity was not present during later changes in the goal location. Unfortunately, elevated VTA activity to

specific behavioral acts or cues that preceded the reward such as those found during classical conditioning (for review, see Schultz 1998) was not identifiable when rats were performing the plus maze task. However, predictive firing can be observed in VTA neurons when rats are performing a well-learned spatial working memory task (Puryear et al. 2010). It would be interesting to determine whether or not the predictive firing of VTA neurons is regulated by changes in context during a well-learned spatial task.

DA–STR interactions during learning and memory

Currently there are several theories of how the DA and STR systems interact to support learning. STR is divided into at least three separate functional domains consisting of NAc, mdSTR, and ldSTR (for reviews, see Ikemoto 2007; Yin et al. 2008). Current models propose that DA from VTA and medial to lateral compartments of SNc, respectively, facilitate stimulus–outcome, action–outcome, and stimulus–response learning (Yin et al. 2008). It is thought that DA facilitates LTP in STR to enhance learning (Reynolds and Wickens 2002). Specifically, *phasic* DA activity enhances D1 receptor activation (Goto and Grace 2005) and leads to strengthening of dSTR synapses (West and Grace 2002) that are activated by corticolimbic input carrying information related to behavioral responses or specific stimuli (Wickens et al. 2007). The patterns of SNc reward responses observed in the current study support the view that medial SNc–mdSTR interactions facilitate action–outcome learning as SNc reward responses remained robust with changes in action–outcome contingencies experienced with switching the goal location. In addition, although SNc-mdSTR interactions are not traditionally implicated in HPC-dependent spatial tasks (Da Cunha et al. 2003), Darvas and Palmiter (2010) found that DA deficient mice with viral rescue mediated restoration of DA signaling in mdSTR was sufficient to restore spatial learning deficits. In fact, viral rescue of DA signaling to both medial and lateral compartments of STR was sufficient to restore most behavioral deficits. These results imply that models based on STR compartmentalization may not fully explain DA influences on STR-dependent learning and memory.

An alternative model proposes that DA regulates learning and memory through different modes of firing, such that differing levels of phasic and tonic activity, respectively, regulate goal-directed behaviors and response selection (Grace 1991; Goto and Grace 2005; Zweifel et al. 2009). Indeed, selective disruption of phasic DA activity impairs several forms of cue-dependent learning while leaving motor learning intact (Zweifel et al. 2009). The current study did not directly test a role for tonic vs. phasic activity in regulating learning. Nevertheless, consistent with the goal oriented demands of the task, phasic levels of activity by VTA and SNc cells were evident throughout training. Initially, rodents often employ spatial strategies to solve navigation tasks and gradually switch to using a response-based strategy (Packard and McGaugh 1996). Thus, it is possible that with extended training in the current behavioral paradigm elevated tonic levels of DA neuron activity would be observed with concurrent reductions in phasic activity and elevations in average firing rates that may reflect the use of egocentric strategies to solve the task.

VTA cell firing rates and burst firing are higher than those of SNc cells

VTA neurons, overall, had higher average firing rates and emitted more spikes in bursts than SNc neurons. These findings are consistent with previous work investigating electrophysiological differences between mesolimbic and nigrostriatal DA neurons. Mesoaccumbens and mesocortical DA neurons have been shown to fire at higher rates and emit more spikes in bursts than nigrostriatal DA neurons (Chiodo et al. 1984; Clark and Chiodo 1988; Grenhoff et al. 1988; Zhang et al. 2008). These differences are thought to be due to varying distributions of autoreceptors or autoreceptor sensitivity such that mesolimbic DA neurons are less responsive to regulatory feedback systems than nigrostriatal DA neurons (Chiodo et al. 1984; White and Wang 1984). When analyzed separately according to behavioral conditions there were no differences in patterns of activity between the structures indicating that these measures may not reflect neural mechanisms underlying differences in behavior found across conditions in this paradigm.

Conclusions

The current study revealed distinguishing behavioral correlates in VTA and SNc neural activity in navigating rodents as they learned a HPC-dependent task. Dynamic changes in neural activity that correspond to learning a spatial rule were found to be specific to VTA. Further, VTA and SNc egocentric coding likely reflected learning of navigational and goal-directed strategies. Together these data imply that VTA and SNc play complementary yet distinct roles during spatial learning. Patterns of activity within these two structures differed in a way that complements current theories of parallel processing by HPC and STR memory systems during learning (e.g., Mizumori et al. 2004; Yeshenko et al. 2004). Notably, these findings provide new electrophysiological insight into VTA-specific DA contributions to HPC-dependent navigation.

Materials and Methods

Subjects

Six male Long-Evans rats between the ages of 4 and 8 months old were individually housed in a temperature controlled environment with a 12 h light/dark cycle. All subjects were given ad libitum food and water and handled for 5 min for at least five days before behavioral testing began. During all phases of behavioral testing rats were maintained at 85% of their maximum free feeding body weight. All animal care was conducted according to guidelines established by the University of Washington's Institute for Animal Care and Use Committee.

Apparatus

All rats were trained on a modified eight arm radial maze in which four arms were arranged to form a plus configuration. The maze was elevated 79 cm above the floor and consisted of four black Plexiglas runways (58 × 5.5 cm) that radiated out from a circular center platform (19.5 cm in diameter). The maze area was enclosed with a black curtain hung on a circular track. During plus maze training several extramaze visual cues were fixed to the curtain. For two of the six rats, intramaze visual cues fixed to the ends of the maze arms were available in addition to the extramaze cues.

Behavioral training

Rats were acclimated to the maze and trained to retrieve chocolate milk reward from a food cup located at the end of a single elevated arm in a maze environment devoid of visual cues before recording electrodes were implanted and unit recording began. Plus maze training began when stable, isolated units were found, thus rats were not included in the study if stable unit activity was not evident ($n = 2$). During unit recording, rats were first trained to forage for reward located at the ends of all four arms of the plus maze (habituation). During habituation visual cues were not displayed and chocolate milk was continuously replaced such that reward was always available at the ends of the maze arms. After rats traversed the maze reliably and consumed reward consistently, plus maze training began. Visual cues were available during all phases of plus maze training and remained in the same

configuration for the duration of training. Rats were sequentially trained to retrieve reward from one of three goal locations (Goal 1, Goal 2, and Goal 3) (Fig. 1). Training to individual goal locations (goal conditions) consisted of 12 trials per day until rats met a behavioral criterion of nine or more correct trials for two consecutive days. A trial began when the rat was removed from an intertrial interval platform located adjacent to the maze and placed on a pseudorandomly chosen start arm. The trial was terminated after the rat located and consumed reward. Rats were placed on the intertrial interval platform between trials for about 1 min at which time the maze arms were cleaned with unscented baby wipes. After behavioral criterion was met for each of the goal conditions, rats were subject to reward manipulations in which they were given 36 trials per day split into three blocks. This portion of training consisted of changing the type of reward received (strawberry, mint, or white chocolate milk) and omitting rewards during the second block of 12 trials. However, due to insufficient unit sampling across conditions, this phase of training is not presented. After reward manipulations were conducted rats were trained to a new goal location and reward manipulations were conducted after rats reached criterion at the new goal.

Surgical procedures

After rats consumed reward consistently from a single food cup they were given ad libitum access to food and water and prepared for surgery. Rats were anesthetized with an isoflurane/oxygen mixture (5% and 2%–4% isoflurane for induction and maintaining anesthesia, respectively) and given subcutaneous injections of an analgesic (Ketoprofen at 5 mg/kg), and an antibiotic (Baytril at 5 mg/kg). Four rats were implanted bilaterally with custom-built microdrives targeting VTA (−5.3 mm AP relative to Bregma, ±0.08 ML, and 7.0-mm ventral to dura), and two rats were implanted unilaterally with Neuralynx hyperdrives targeting VTA. Given the proximity of SNc to VTA, some electrodes tips were misplaced and located in SNc. Microdrives consisted of two recording tetrodes per drive, or two tetrodes per hemisphere. Hyperdrives consisted of 12 recording tetrodes per hemisphere. Recording tetrodes were constructed out of four 25-μm lacquer coated tungsten wires twisted together. The tips of each of the four wires were gold plated to reach impedances of 300–1000 kΩ. A ground wire was attached to the skull and a reference electrode (114-μm stainless steel) was implanted near corpus callosum in rats with microdrive implants. For rats with hyperdrive implants, reference channels were chosen from tetrode channels without discernable unit activity. All rats were allowed to recover for at least 7 d before recording and training began.

Data Analysis

Position and unit activity collection

An infrared light emitting diode was mounted to the recording headstage and used to monitor each rat's position on the maze as they were trained on the plus maze. Position data were sampled at 15 Hz in two rats and 30 Hz in four rats at a 2.8-cm/pixel resolution. Position data were viewed offline and event markers were inserted to delineate errors and the start and end of each trial. In addition, event markers that indicated when the rat first licked the reward at the goal location were inserted online via lick detectors (Neuralynx, Inc.) connected to the food cups. Analog waveform traces were digitized, amplified 500–7000 times, filtered between 600 Hz and 6 kHz, and then passed through a discriminator that triggered a 2-msec sampling period at 16 Hz whenever an impulse from any of the four tetrode channels passed a user-defined threshold. Single units were considered to be well isolated and suitable for recording if the waveform amplitude exceeded background noise levels by at least 3 times. All position and unit data were acquired by Cheetah data acquisition software (Neuralynx). Single unit activity was isolated from other units and background activity using Mclust sorting software (A. Redish) and Plexon sorting software (Plexon Inc.). Chris Higginson provided additional template matching software for use with Mclust.

Behavioral analysis

The experimenter recorded performance on the plus maze. The average number of errors per trial across training sessions was computed to assess plus maze accuracy and the average amount of time per arm entry was computed to assess movement differences across training sessions. Because some rats were trained with intramaze cues and some without, an outlier analysis was conducted on the average number of errors per trial, as well as time per arm entry between training sessions to assess if there were any differences between rats. No outliers were found in terms of the average number of errors per trial. Three sessions from two rats, one trained with intramaze cues, one without, were outliers in terms of the average time per arm entry. Therefore, consistent with previous work showing distal cues to be more effective for spatial navigation than proximal cues (Hudon et al. 2003; Allen et al. 2004), we did not find reliable differences in behavior among these rats. Thus, behavioral data from all rats were combined for analysis. To separately characterize differences in performance within individual goal locations, goal conditions (Goal 1, Goal 2, and Goal 3) were subdivided based on a median split of average number of errors per trial into training and asymptotic performance conditions. Training conditions consisted of sessions with more than 0.23 errors per trial and asymptotic performance conditions were sessions with less than 0.23 errors per trial. All statistical analysis was conducted using SPSS Statistical Software. Two-way ANOVAs were conducted separately on the average number of errors per trial and time per arm entry with goal and performance conditions as between subject factors. Bonferonni-adjusted post hoc tests were conducted when significant ($P < 0.05$) group differences were detected. Descriptive statistics are presented as the mean ± standard error.

Unit analysis

Selection criteria. Traditionally, in vivo studies have identified putative DA neurons based on electrophysiological characteristics such as long action potential duration, low average firing rates, and intermittent burst firing properties (Guyenet and Aghajanian 1978). Neurons in SNc exhibiting standard DA neuron electrophysiological criteria have been confirmed in vivo and in vitro using histological and pharmacological techniques (Grace and Bunney 1980, 1983a,b). Due to the disruptive nature of DA agonists and antagonists on behavior, neuron classification for the current study was determined using electrophysiological characteristics without pharmacological confirmation. In VTA, only 50%–60% of the neurons are DA producing and there are large populations of GABAergic neurons (Margolis et al. 2006a, b; Fields et al. 2007) and glutamatergic neurons (Lavin et al. 2005; Chuhma et al. 2009). Further, in vivo recordings combined with juxtacellular labeling and neurochemical identification via immunoflourescence for tyrosine hydroxylase suggest neurons in VTA exhibiting standard DA neuron electrophysiological criteria are not always DA neurons (Brischoux et al. 2009). However, VTA neurons that do not exhibit standard electrophysiological DA neuron criteria in terms of firing rates (>10 Hz) can be reliably identified as non-DA neurons and are thought to be GABA containing cells (Steffensen et al. 1998). During data collection, we recorded from cells that appeared to have low overall firing rates. Cells with high firing rates were recorded if a low firing rate cell was present on the same tetrode. In addition, an attempt was made to record from the same cells across training phases. After histological verification of recording locations, all cells determined to be in VTA or SNc were subject to the following analysis.

A primary behavioral correlate of principal cells in VTA and SNc is reward-related firing. Reward-related firing was assessed using peri-event time histograms (PETHs) constructed out of 50 msec bins 2 sec before and after the rat first licked the food cup. A cell was identified as exhibiting significant excitatory reward

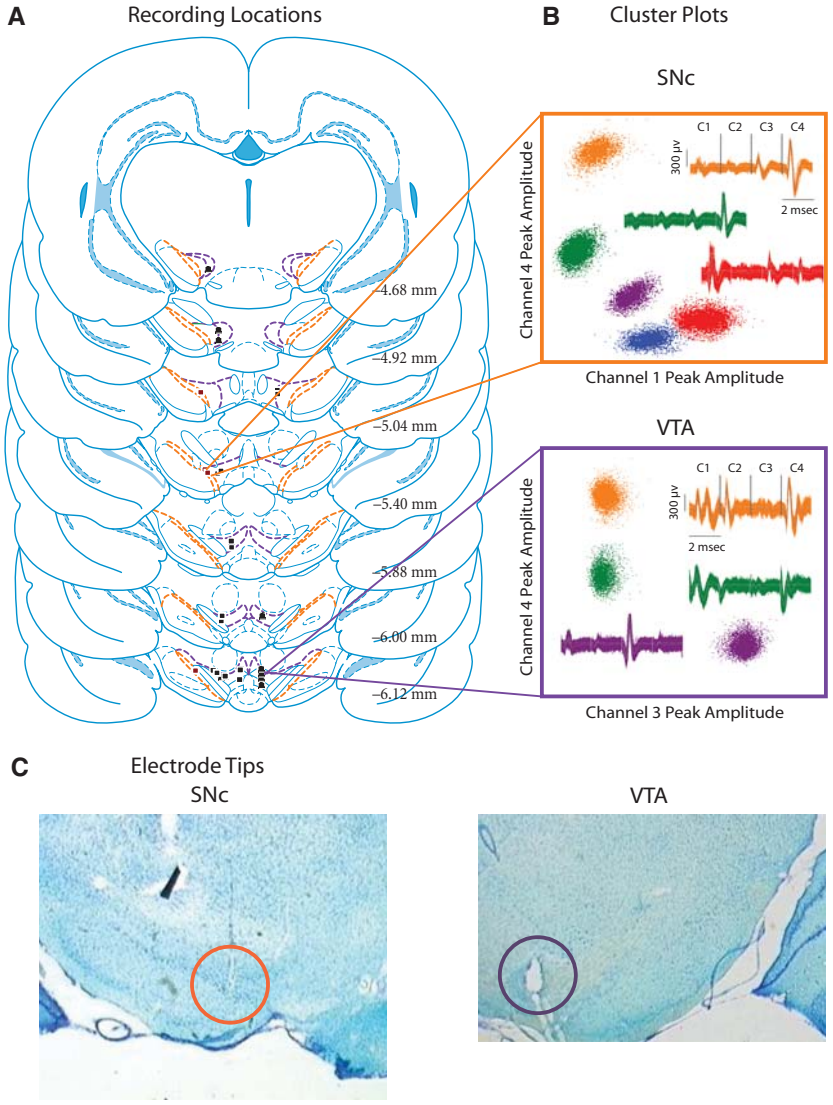

Figure 8. Histology and recording examples. (*A*) Reconstructed VTA and SNc recording locations. VTA is outlined in purple and SNc is outlined in orange. Recording locations in VTA and SNc are marked by black and red squares, respectively. Reconstructed from Paxinos and Watson (2009). (*B*) Cluster plots and waveform examples from individual cells isolated on tetrodes placed in SNc and VTA. Waveforms from selected units (clusters of dots) are illustrated in the corresponding color. (C) Representative examples of recording electrode lesion locations in SNc and VTA.

activity if the average firing rate in a bin within 150 msec of reward receipt exceeded the average firing rate of the 4 sec PETH window by 2 SD above the mean. Three reward cells had firing rates over 15 Hz and were determined to be outliers with average firing rates that were all at least 2 SD above the mean. Therefore, we limited analysis to DAergic and glutamatergic cells in VTA and SNc by excluding all cells with firing rates >15 Hz. In addition, cells with very low average firing rates (<1 Hz) were excluded from analysis to limit artifacts in normalization due to low background activity. In sum, this restricted analysis does not allow for identification of putative DA neurons. Rather, these criteria allowed us to assess the activity of putative principal (i.e., DAergic or glutamatergic) neurons.

Reward–related firing properties. PETH analysis as described previously was used to assess neural activity surrounding the reward event. The proportion of cells displaying significant reward activity (Z-score ≥ 2 within 150 msec of reward) across habituation and goal phases was assessed using a Pearson's χ^2 test. Neural activity at the time of reward was determined by calculating the normalized peak firing rate within 150 msec of reward receipt. In addition, the time point at which the bin with the highest firing rate occurred within the 4-sec period surrounding reward receipt was used as a measure of temporal specificity of reward-related firing. Separate two-way ANOVAs were used to determine if there were significant differences in reward activity and specificity with goal and performance conditions as between subject factors. Bonferonni-adjusted post hoc tests were conducted when significant ($P < 0.05$) group differences were detected. Descriptive statistics are presented as the mean ± standard error. In addition, relationships between reward-based neural activity and time were assessed with linear correlations between reward activity and specificity and the number of rewarded arm entries.

Velocity and firing rate analysis. To assess relationships between unit activity and an animal's movement on the maze, Pearson's correlation coefficients were calculated for average firing rates and movement velocity (14 cm/sec bin size, excluding intertrial interval times and error entries). The upper velocity range was limited to the lowest maximum velocity of a given session (280 cm/sec) to eliminate erroneous correlations due to inadequate sampling at very high velocities. In addition, position data were inadvertently collected at 15 Hz for two animals. These sessions were not included in the movement analysis as position sampling at this resolution was determined to be inadequate for this purpose. One-way ANOVAs were used to determine if there were significant differences in velocity/firing rate R values between habituation and testing phases. Two-way ANOVAs were used to determine if there were significant differences in R values with performance and testing phase conditions as between subject factors. Bonferonni-adjusted post hoc tests were conducted when significant ($P < 0.05$) main effects were detected. Descriptive statistics are presented as the mean ± standard error.

Baseline firing properties. One-way ANOVAs were used to determine if there were significant differences in average firing rates (excluding intertrial interval times and error entries) between habituation and testing phases. In addition, evaluation of burst firing properties was conducted using previously established criteria of ≤80 msec to signal burst onset and >160 msec to signal burst offset (Grace and Bunney 1980, 1983a,b; Pan et al. 2005; Zweifel et al. 2009; Puryear et al. 2010). One-way ANOVAs were used to determine if there were significant differences in the percentage of spikes that occur in bursts between habituation and testing phases. Two-way ANOVAs were used to determine if there were significant differences in average firing rates and the percentage of spikes that occur in bursts with performance and testing phase conditions as between subject factors.

Because two rats were trained with intramaze cues, an outlier analysis was conducted to assess differences in average firing rates, reward activity, and reward specificity between testing phases and performance conditions. Valid statistical analysis could not be conducted between groups as there were a total of 26 cells recorded with intramaze cues and 220 cells recorded with extramaze cues; therefore homogeneity assumptions were violated. Outlier analysis indicated that a total of two cells in VTA had average firing rates of 2 SD above the mean. Outliers were not detected in any other measure of neural activity. Therefore, as with the behavioral analysis, data from intramaze cue and extramaze sessions were combined. Statistical differences were found between brain regions, therefore unit activity from VTA and SNc were analyzed separately.

Histology

Once tetrodes were lowered past the region of interest (DV 8.0 mm) or rats completed behavioral training, the final position of the tetrode was marked by passing a 25 μA current through the electrode for 25 sec to create a lesion while the rats were under isoflurane anesthesia. Rats were immediately transcardially perfused with 9% saline followed by a 10% formalin/saline solution. Brains were extracted and placed in a 30% sucrose formalin solution. Following sucrose absorption, brains were cut using a cryostat into 40 μm coronal sections and stained with cresyl violet. Lesions were compared with depth records to determine final recording locations. See Figure 8 for an illustration of recording electrode positions in VTA and SNc.

Acknowledgments

The authors thank Yong Sang Jo, Alix Norton, and Marsha Penner for helpful discussions and Graham Jones for behavioral testing of animals and data processing.

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Received June 4, 2010; accepted in revised form January 24, 2011.