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Rapid Communication

Consolidation of object recognition memory requires simultaneous activation of dopamine D_1/D_5 receptors in the amygdala and medial prefrontal cortex but not in the hippocampus

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ABSTRACT

The mesocorticolimbic dopaminergic system includes the ventral tegmental area (VTA) and its projections to the amygdala (AMY), the hippocampus (HIP) and the medial prefrontal cortex (mPFC), among others. Object recognition (OR) long-term memory (LTM) processing requires dopaminergic activity but, although some of the brain regions mentioned above are necessary for OR LTM consolidation, their possible dopamine-mediated interplay remains to be analyzed. Using adult male Wistar rats, we found that posttraining microinjection of the dopamine D_1/D_5 receptor antagonist SCH23390 in mPFC or AMY, but not in HIP, impaired OR LTM. The dopamine D_2 receptor agonist quinpirole had no effect on retention. VTA inactivation also hindered OR LTM, and even though this effect was unaffected by co-infusion of the dopamine D_1/D_5 receptor agonist SKF38393 in HIP, mPFC or AMY alone, it was reversed by simultaneous activation of D_1/D_5 receptors in the last two regions. Our results demonstrate that the mesocorticolimbic dopaminergic system is indeed essential for OR LTM consolidation and suggest that the role played by some of its components during this process is much more complex than previously thought.

The mesocorticolimbic dopaminergic pathway originates in the ventral tegmental area (VTA) and controls motivated behavior and movement initiation strategies as well as attention, reward processing, fear, and novelty detection (Cole & Robbins, 1989; Beninger & Miller, 1998; Nader & LeDoux, 1999; Granon et al., 2000; Schultz, 2002). This pathway includes several brain areas essential for learning and memory, such as the amygdala (AMY), the hippocampus (HIP) and the medial prefrontal cortex (mPFC). Indeed, it has been hypothesized that a functional loop including VTA-hippocampus dopamine connections regulates the influx of information into long-term memory (LTM; Lisman & Grace, 2005). We previously demonstrated that, in order to persist, fear memories require activation of this pathway (Rossato, Bevilaqua, Izquierdo, Medina, & Cammarota, 2009). Here, we investigated whether VTA function is also needed for consolidation of object recognition (OR) LTM. Since different brain regions are necessary for discriminating objects (Bussey, Muir, & Aggleton, 1999; Hotte, Naudon, & Jay, 2005; Tinsley et al., 2011; Zhu, McCabe, Aggleton, & Brown, 1997), and AMY, mPFC and HIP seem to serve distinct functions during OR memory processing, including, respectively, arousal, judgment of recency and familiarity (Akirav & Maroun, 2006; Barker, Bird, Alexander, & Warburton, 2007; Barker & Warburton, 2008; Roozendaal, Castello, Vedana, Barsegyan, & McGaugh, 2008; Balderas et al., 2008; Nelson, Cooper, Thur, Marsden, & Cassaday, 2011; Werenicz et al., 2012; Cross, Brown, Aggleton, & Warburton, 2012; Albasser, Amin, Lin, Iordanova, & Aggleton, 2012; Fortress, Fan, Orr, Zhao, & Frick, 2013), there are good reasons to expect that dopaminergic influence on OR LTM may differ in these brain areas. Therefore, we also examined the possible dopamine-mediated interplay between these regions. To address these questions, male Wistar rats (3-month-old) implanted with 22-gauge guides aimed to the VTA [stereotaxic coordinates AP −4.8/LL ±1.0/DV −9.0], CA_1 region of the dorsal HIP [stereotaxic coordinates AP −4.2/LL ±3.0/DV −3.0], the basolateral nucleus of AMY [stereotaxic coordinates AP −2.4/LL ±5.1/DV −8.1], and/or the pre-limbic sub-division of the mPFC [stereotaxic coordinates AP +3.2/LL ±0.8/DV −4.0] (Paxinos & Watson, 1986)] were trained in the novel object recognition task

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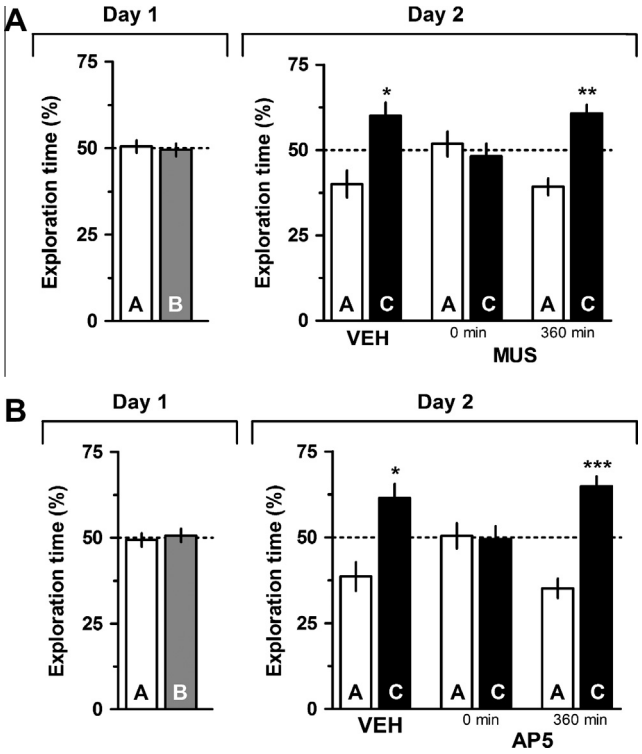

Fig. 1. Reversible inactivation of the VTA hinders OR LTM consolidation. *A*: On day 1, rats were exposed to two different objects (A and B) for 5 min and immediately or 360 min after that received bilateral infusions (0.5 μl/side) of vehicle (VEH; 0.1% DMSO in saline) or muscimol (MUS; 0.1 μg/side) in VTA. On day 2, animals were exposed to a familiar (A) and a novel object (C) for five extra minutes to evaluate OR LTM. *B*: Animals were treated exactly as in A, except that immediately or 360 min after training they received bilateral infusions of VEH or AP5 (1.0 μg/side) in VTA. Data (mean ± SEM) are presented as percentage of total exploration time. $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ in one-sample Student's *t*-test with theoretical mean = 50; $n = 8–10$ per group.

(NOR), a novelty-preference learning paradigm based on the rats' natural preference to explore novel objects involving a 5-min exposure to two novel stimuli objects in an open field arena (Dix & Aggleton, 1999; Ennaceur & Delacour, 1988; Ennaceur, Neave, & Aggleton 1997), which is susceptible to dopaminergic modulation (de Lima et al., 2011). Before that, the animals were habituated to the training arena by allowing them to freely explore it during 20 min per day for 4 days in the absence of any other behaviorally relevant stimulus. The stimuli objects were made of metal, glass or glazed ceramic, and their role (familiar or novel) and relative position were counterbalanced and randomly permuted for each experimental animal. Exploration was defined as sniffing or touching the stimuli objects with the muzzle and/or forepaws. Sitting on or turning around the objects was not considered exploratory behavior. Approximately 4% of the animals did not accumulate at least 20 s of object exploration during the training session and, consequently, were excluded from the experiments.

To analyze the involvement of the VTA in OR LTM consolidation, rats trained in the NOR task received bilateral intra-VTA infusions of the $GABA_A$ receptor agonist muscimol (MUS; 0.1 μg/side), the NMDA receptor antagonist AP5 (1 μg/side) or vehicle (VEH; 0.1% DMSO in saline) immediately or 360 min after training. Retention was assessed 24 h later. During the test session, the animals were exposed to one of the stimuli objects presented in the training session together with a novel one. Rats that received VEH explored the novel object longer than the familiar one ($p < 0.05$; $t(8)=2.483$, in one-sample Student's *t*-test with theoretical mean = 50). In contrast, animals that received MUS or AP5 immediately but not 360 min after training spent the same amount of time exploring the novel and the familiar objects (Fig. 1A and B, respectively; $p < 0.01$; $t(7) = 4.255$ for MUS and $p < 0.001$; $t(7)=5.177$ for AP5, in one-sample Student's t-test with theoretical mean = 50). Neither MUS nor AP5 affected the animals' performance in the plus-maze or the open field tasks when given in VTA 24 h before the respective behavioral session (Table 1). The VTA sends projections to several brain regions known to participate in OR memory processing, including AMY (Roozendaal et al., 2008), mPFC (Mitchell & Laiacona, 1998), and HIP (Fortress et al., 2013). Therefore, we investigated whether dopamine receptors regulate OR LTM consolidation in any of these regions. When given in AMY (Fig. 2A; $p < 0.01$, $t(7) = 3.497$ for VEH; $p < 0.001$, $t(7) = 7.798$ for SCH) or mPFC (Fig. 2B; $p < 0.05$, $t(6)=2.689$ for VEH; $p < 0.01$, $t(7)=3.160$ for SCH) immediately but not 360 min after training, the dopamine D_1/D_5 receptor antagonist SCH23390 (1.5 μg/side) impaired OR LTM. SCH23390 did not affect retention when injected in HIP at any post-training time analyzed (Fig. 2C; $p < 0.001$,

Table 1
Intra-VTA infusions of muscimol and AP5 have no effect on locomotor and exploratory activities or anxiety. VEH, MUS (0.1 μg/side) or AP5 (1 μg/side) were infused in VTA 24 h before Open Field or Plus Maze behavioral sessions. Data are expressed as mean (±SEM) of the total number of entries and the percentage of time spent in the open arms (Plus Maze) or of the number of crossings and rearings (Open Field); $n = 10$ per group). VEH = vehicle. A different set of animals was used for each behavioral test.

	VEH	AP5	MUS
Plus maze			
Total entries	20.3 ± 1.9	18.3 ± 2.9	23.3 ± 2.9
Entries in the open arms	8.2 ± 0.9	7.2 ± 1.9	8.2 ± 1.3
% Time in the open arms	25.6 ± 4.1	21.6 ± 5.1	27.6 ± 5.1
Open field			
Crossings	77.3 ± 6.8	80.3 ± 7.8	81.3 ± 9.9
Rearings	18.8 ± 2.0	21.8 ± 3.0	21.0 ± 2.0