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Dopamine Receptor Activity Participates in Hippocampal Synaptic Plasticity Associated with Novel Object Recognition

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Abstract

Physiological and behavioral evidence supports that dopamine (DA) receptor signaling influences hippocampal function. While several recent studies examined how DA influences CA1 plasticity and learning, there are fewer studies investigating the influence of DA signaling to the dentate gyrus. The dentate gyrus receives convergent cortical input through the perforant path fiber tracts and has been conceptualized to detect novelty in spatial memory tasks. To test whether DA-receptor activity influences novelty-detection, we used a novel object recognition (NOR) task where mice remember previously presented objects as an indication of learning. Although DA innervation arises from other sources and the main DA signaling may be from those sources, our molecular approaches verified that midbrain dopaminergic fibers also sparsely innervate the dentate gyrus. During the NOR task, wild-type mice spent significantly more time investigating novel objects rather than previously observed objects. Dentate granule cells in slices cut from those mice showed an increased AMPA/NMDA-receptor current ratio indicative of potentiated synaptic transmission. Post-training injection of a D1-like receptor antagonist not only effectively blocked the preference for the novel objects, but also prevented the increased AMPA/NMDA ratio. Consistent with that finding, neither NOR learning nor the increase in the AMPA/NMDA ratio were observed in DA-receptor KO mice under the same experimental conditions. The results indicate that DA-receptor signaling contributes to the successful completion of the NOR task and to the associated synaptic plasticity of the dentate gyrus that likely contributes to the learning.

Graphical Abstract

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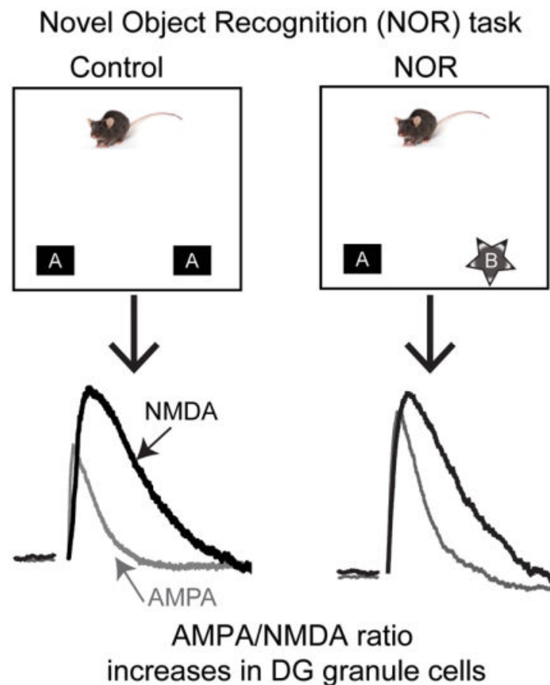
Novel object recognition (NOR) engages the hippocampus as mice remember previously presented objects as an indication of learning. In this study, Yang et al. measured the ratio of AMPA to NMDA currents. When mice were exposed to novel objects *(right),* the AMPA/NMDA ratio increased in dentate granule neurons. Accurately performing the task and the associated increase of the AMPA/NMDA ratio were dependent upon D1-like receptor activity.



Keywords

dentate gyrus; AMPA/NMDA ratio; memory; learning; CA1

INTRODUCTION

While there is increasing evidence that dopaminergic neurotransmission influences hippocampal learning and memory, many recent behavioral experiments have restricted study to the CA1 region, which is the final node of the trisynaptic loop of the hippocampus (McNamara *et al.*, 2014; Rosen *et al.*, 2015; Broussard *et al.*, 2016). Drugs of abuse, such as nicotine (Tang & Dani, 2009; Zhang *et al.*, 2010) and methylphenidate (Jenson *et al.*, 2015), have been shown to influence synaptic plasticity in the dentate gyrus (DG), the first node in the hippocampal loop. Furthermore, D1 and D5 receptors are prominently expressed in the DG (Mu *et al.*, 2011). Therefore, we set out to test the hypothesis that dopaminergic signaling participates in DG-specific synaptic plasticity associated with the novel object recognition (NOR) task.

The NOR task allows researchers to examine an animal's behavioral changes in response to a new object introduced into a familiar environment (Bevins & Besheer, 2006; Broadbent *et al.*, 2010; Antunes & Biala, 2012; Cohen & Stackman, 2015). The natural preference for

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novel objects displayed by an animal suggests that it retains a memory of the familiar objects presented previously in the environment (Ennaceur, 2010). Unlike many other behavioral assays, the NOR task does not require external motivation, reward, or punishment but only requires habituation and training (Antunes & Biala, 2012; Leger *et al.*, 2013). Thus, the NOR task has been routinely applied to evaluate object recognition memory and development of attention processes in rodents and humans (David & Witryol, 1990; Carey *et al.*, 2008; Taglialatela *et al.*, 2009).

The hippocampus is integral for spatial memory (King *et al.*, 2004) and aversive contextual memory (Broussard *et al.*, 2016). Studies specifically designed to test subregions of the hippocampus have indicated that the DG participates in spatial pattern separation (Rolls & Kesner, 2006; Kesner, 2013; Kesner & Rolls, 2015). Some studies report impaired object recognition performance caused by hippocampal damage (Clark *et al.*, 2000; Broadbent *et al.*, 2010; Cohen *et al.*, 2013), and blockade of neurogenesis within the DG was sufficient to block NOR in mice (Jessberger *et al.*, 2009). Altered intracellular zinc signaling spatially restricted to the DG also was sufficient to affect NOR (Tamano *et al.*, 2015). In addition, NOR produces *in vivo* LTP in mice (Clarke *et al.*, 2010). All of those studies served as a background that inspired us to ask whether successful completion of the NOR task engaged synaptic plasticity that required DA-receptor participation.

Evidence supports that substantial dopaminergic signaling in the hippocampus arises from outside the midbrain ventral tegmental area (VTA) and substantia nigra compacta (SNc) (Smith & Greene, 2012; Walling *et al.*, 2012). Other studies, however, also indicate a sparse direct dopaminergic projection from midbrain DA neurons to the CA1 region of the hippocampus (Gasbarri *et al.*, 1994; Gasbarri *et al.*, 1996; McNamara *et al.*, 2014; Rosen *et al.*, 2015; Broussard *et al.*, 2016). Electrophysiological evidence from freely moving mice and from *ex vivo* hippocampal slices shows that contextual learning during inhibitory avoidance training directly induces LTP expressed in CA1 pyramidal neurons, but not in DG granule cells. Furthermore, D1-like receptor activation is required for inhibitory avoidance to induce CA1 synaptic plasticity (Broussard *et al.*, 2016). Both D1 and D5 receptors are also important for controlling spike timing-dependent plasticity (STDP) of the medial perforant path synapse onto DG granule cells (Yang & Dani, 2014; Jenson *et al.*, 2015). In addition, D1 and D5 receptors are integral for hippocampal neurogenesis in the DG (Mu *et al.*, 2011). Taken together, DA signaling has broad influence over learning, memory, and synaptic plasticity in the hippocampus, including both the CA1 and the DG. However, it remains unclear whether DA significantly participates during NOR and regulates the associated synaptic plasticity in the hippocampal DG.

Here we wanted to test two hypotheses: (1) that NOR is associated with synaptic plasticity as indicated by the AMPA/NMDA-receptor ratio in the DG, and (2) that DA-receptor activity influences this plasticity and successful completion of the NOR task. First, we verified that DA projections originating in the midbrain contribute to the DG innervation (Broussard *et al.*, 2016). Then, we assessed which subregion of the hippocampus participates in synaptic plasticity associated with the later phases of the NOR task by measuring AMPA/NMDA ratios in both the DG and CA1 at 3 h after NOR testing. The next aim was to determine whether D1-like receptors are important for the NOR task using either a D1-like

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receptor antagonist, SCH 23390 (SCH, 0.05 mg/kg i.p.), or D1- or D5-receptor knockout (KO) mice.

After finding sparse GFP-labeled DA projections directly innervating the DG, we found that the NOR task is associated with an increased AMPA/NMDA ratio measured in the DG of hippocampal slices only in wild-type mice, but not in D1- or D5-KO mice. D1-like receptor inhibition not only blocked successful completion of the NOR task but also prevented the increase in the AMPA/NMDA ratio. Our findings indicate that dopaminergic signaling has a significant role in regulating NOR behavior and in the associated DG synaptic plasticity.

MATERIALS AND METHODS

Dopamine transporter fluorescent labeling

Dopamine transporters (DAT) have been shown to be located on DA fibers and terminals (Shimada *et al.*, 1991; Shimada *et al.*, 1992; Nirenberg *et al.*, 1996). To examine whether there is a direct midbrain dopaminergic projection to the hippocampus, we injected a recombinant adeno-associated virus (Grimm *et al.*, 2008; Tsai *et al.*, 2009) *AAV-EF1a-DIO-synaptophysin:GFP* containing a double-floxed inverted open reading frame encoding synaptophysin-GFP into the midbrain DA area of adult $DAT^{ires\text{-}cre/+}$ knock-in (n = 5) and $DAT^{+/+}$ (WT, n = 3) mice (Fig. 1A). We bilaterally injected AAV-EF1α-DIO synaptophysin-GFP (1 μl) into the VTA (± 3.64 A/P, ± 0.5 M/L, ± 4.0 D/V) of $DAT^{cre/+}$ or WT C57BL/6J mice (Backman *et al.*, 2006). This AAV vector facilitates the expression of GFP specifically in the synaptic terminals of neurons containing DAT, exclusively in Cre-expressing cells. Because synaptophysin is a synaptic vesicle protein, this procedure concentrated the GFP fluorophore into DA terminals of neurons from the ventral tegmental area (VTA) and the substantia nigra (SN). Control mice were littermates of the mutants. The production of the synaptophysin vector was described previously (Broussard *et al.*, 2016). Briefly, synaptophysin-GFP was excised using *NotI* and *Bsr*GI and shuttled into a custom chloramphenicol resistant shuttle plasmid that contained the MCS *Spe*I-*Not*I-*Bsr*GI-*Sca*I-*Xho*I-*Spe*I. From there, synaptophysin-GFP was excised using *Spe*I and cloned into the pAAV-flex vector. Clones were verified for flex orientation by restriction digest and transfection (Arenkiel & Ehlers, 2009; Arenkiel, 2011). After two weeks we transcardially perfused the mice, fixed the brains, and cut slices (40 μm). Sections were analyzed in 2-μm steps on a confocal microscope and images from the DG and CA1 were analyzed for green fluorescence using ImageJ (http://rsbweb.nih.gov/ij/).

Novel object recognition (NOR) task

The Institutional Animal Care and Use Committee at Baylor College of Medicine or University of Pennsylvania approved all of the protocols. Male wild-type C57BL/6J (Jackson Laboratory, Bar Harbor, Maine) or DA D1- or D5-receptor KO mice 2–3 months old had free access to food. Experiments were conducted in the dark, active cycle. A 25-cm cube with no top was covered in black contact paper on the sides and white paper on the bottom. Two 2x2 Lego brackets held the Lego objects for training and testing. Mice were habituated to the empty training box for 20 min/d for 4 d before training (Fig. 2A). Following each habituation to the box, mice were injected with small volumes of saline. In

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the training phase of this task, a mouse was placed in a box for five minutes with two identical objects stuck to the floor (e.g., two Lego blocks that look like steering wheels, the "A A" condition, Fig. 2B). The training box was cleaned with 70% ethanol between each mouse to dissipate the odors. After a 24 h interval, we tested the mice by placing them back in the same box for 5 min with the familiar object ("A", e.g., steering wheel) and a new object ("B", e.g., a Lego block with roses, the "A B" condition, Fig. 2D, top). As a control, a separate group of mice was presented the same objects from the training session ("A A", Fig. 2C, top). In separate experiments, we injected animals with a low dose of the D1-like receptor antagonist, SCH 23390, after training to test whether DA-receptor signaling contributes to the successful completion of the NOR task (Broussard *et al.*, 2016).

Simple webcams captured the interactions with the objects, and independent investigators blind to the circumstances scored the videos. For the videos for each animal, a card with the subject number was presented before the mouse was placed to act as a watermark. A mouse was scored as interacting with an object when its nose was in contact with the object or was directed toward the object within 2 cm. Moving past or resting on or over the object was not considered interaction (Bevins & Besheer, 2006). Time spent with objects during training (A A) was recorded to assess any left or right biases of the mice, and during testing a discrimination ratio was calculated (novel object interaction/total interaction with both objects). A discrimination ratio above 0.5 (i.e., 50%) indicates greater preference for the novel object.

For measurement of the AMPA/NMDA ratio associated with NOR, whole cell patch-clamp recordings were made from *ex vivo* hippocampal slices prepared from either NOR tested or littermate controls. The experimenter was blind to the behavioral condition of the mouse at the time of the electrophysiology recordings.

Hippocampal slice preparation

Hippocampal slices were prepared from mice 1.5 h after testing with the novel object. To obtain slices, all the mice were deeply anesthetized with an over-dose of ketamine and xylazine (4:1) and were transcardially perfused (Yang *et al.*, 2011; Broussard *et al.*, 2016) with ice-cold oxygenated sucrose based low-calcium, high-magnesium artificial cerebrospinal fluid (ACSF, in mM): 250 sucrose, 11 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 10 $MgSO_4$, and 0.5 $CaCl_2$. After decapitation, the brain was rapidly dissected and sliced horizontally at a thickness of 220 μm in the same solution using a vibratome (Leica VT 1000S). Horizontal hippocampal slices were kept in low-calcium, high-magnesium ACSF (in mM): 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 7 $MgSO_4$, 0.5 $CaCl_2$, and 25 glucose saturated with 95% O_2 and 5% CO_2 at 32 °C for 20 min and then at room temperature for at least 1 h until recording.

Electrophysiology

The slices were placed in a recording chamber and were continuously bathed (1–2 ml/min) in oxygenated ACSF (in mM): 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgSO_4$, 2 $CaCl_2$, 25 D-(+)-glucose, maintained at 32–34°C via an inline heater system (TC-324B, Warner Instrument Corp, Hamden, CT). Picrotoxin (100 μM, Sigma-Aldrich), a

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noncompetitive $GABA_A$ receptor antagonist, was routinely included in the ACSF. Recording electrodes (2–3 MΩ) were pulled from borosilicate glass capillaries (TW 150-4, WPI) using a Narishige PC-10 micropipette puller and were filled with a cesium-based intracellular solution (in mM): 130 cesium methanesulfonate, 10 KCl, 10 HEPES, 1 BAPTA, 3 Na_2ATP, 10 TEA-Cl, 1 GTP, 2 $MgSO_4$, pH 7.3 with CsOH. After the pipette formed a gigaseal (> 2 GΩ), a gentle suction was briefly applied to achieve a whole-cell patch-clamp recording configuration (access resistance < 10 MΩ). We recorded AMPA and NMDA receptor mediated excitatory postsynaptic currents (EPSCs) under voltage clamp conditions (+40 mV, to enable NMDA-receptor currents) from either DG granule cells or CA1 pyramidal neurons. We stimulated using a bipolar electrode (Stereotrode Tungsten, WPI, Inc., Sarasota, FL) placed 100–150 μm away from the recording electrode within the medial perforant pathway or the Schaffer collateral pathway. Input-output curves were first generated by various intensity of stimulation pulses with a duration of 0.1 ms and approximately 50–60% of maximal peak currents were used for current measurements. Both the NOR tested and control slices and recording were handled in the same way.

To obtain pure AMPA receptor-mediated EPSCs, the competitive NMDA receptor antagonist, DL-APV (100 μM, Sigma-Aldrich), was bath applied to inhibit NMDA receptor-mediated EPSCs right after a stable 10-sweep (at 0.1 Hz) baseline was recorded. Pure NMDA receptor-mediated EPSCs were digitally defined by subtraction with the aid of the Clampfit 10.4 (Molecular Devices, LLC). Electrophysiological signals were acquired using Axopatch 200B (Axon Instruments) via a 1322A digitizer (Axon Instruments). All signals were averaged from 10 EPSCs evoked by repetitive stimuli generated by a stimulus isolator (A365, WPI, Inc, Sarasota, FL) at 0.1 Hz (Broussard *et al.*, 2016). For all recordings made in the CA1 area, connections between CA3 and CA1 were cut (see didactic white “cut”, Fig. 3C) prior to bath application of 100-μM picrotoxin to prevent the spread of recurrent electrical activity into the CA1 (Kehoe *et al.*, 2014). The electrode access resistance (Ra) was monitored before and after recordings. The recordings were not used if the Ra changed by 30% or more. Investigators blind to the behavioral conditions did all the electrophysiological recordings.

Data analysis

We analyzed the data using Microsoft Excel and GraphPad Prism 6. All the data were expressed as mean ± SEM. Behavioral data were expressed as % of total exploration time for each object and analyzed by using a one-sample Student t test ($\alpha = 0.05$) by comparing the group’s means with the fixed value of 50%, which represents no change in exploration between objects. Comparisons between differences in means were assessed by paired/unpaired Student’s *t* test with the criterion significance set at $\alpha = 0.05$.

RESULTS

Evidence for sparse innervation of the dentate gyrus by midbrain dopamine neurons

The *AAV-EF1a-DIO-synaptophysin:GFP* vector facilitates the expression of GFP specifically in the synaptic terminals of neurons containing DAT, exclusively in Cre-expressing cells. Thus, when injected into the midbrain of $DAT^{ires\text{-}cre/+}$ mice, this procedure

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labeled with GFP DA terminals from neurons of the ventral tegmental area (VTA) and the substantia nigra (SN), as shown previously (Broussard *et al.*, 2016). Sparse GFP positive terminals were found throughout the DG region of $DAT^{ires\text{-}cre/+}$ mice in significantly greater proportion than WT (Fig. 1B): 1.06% ± 0.03, n = 5 for $DAT^{ires\text{-}cre/+}$; 0.08% ± 0.004, n = 3 for WT; $p = 0.001$, with the greatest representation in the hilus region (green, Fig. 1C, D, compared to Fig. 1E).

Novel object recognition (NOR) task

For behavior studies, wild-type mice were habituated for 20 min/d for 4 d to the training box (Fig. 2A). Then we trained the mice with 2 identical objects under the "A A" condition (Fig. 2B). The littermate mice were then randomly separated into 2 groups. One group was tested the next day under the "A A" condition with the same identical objects (Fig. 2C Control, top). These mice showed no significant difference for the percent of time spent exploring the two identical objects (Fig. 2C, bottom): right object, 50.5 ± 0.6%; left object, 49.5 ± 0.6%; $p = 0.4$, right vs left, n = 221. The other group of mice were tested under the "A B" condition, where one original object was replaced by a novel object (Fig. 2D NOR, top). The mice spent more time exploring the novel object (Fig. 2D, bottom): familiar object, 40.8 ± 1.2%; novel object, 59.2 ± 1.2%; with one-sample t test = t(116) = 7.6, $p < 0.0001$, novel vs familiar, n = 117. Consistent with previously published reports (Clark *et al.*, 2000; Bevins *et al.*, 2002; Mumby *et al.*, 2002; Bevins & Besheer, 2006; Fernandez *et al.*, 2008), the results indicate that wild-type mice exhibit a preference for the novel object.

NOR increased the AMPA/NMDA ratio in the dentate gyrus

Because the lateral entorhinal cortex projects directly to the DG and this circuit is critical for NOR memory (Kinnavane *et al.*, 2015), we tested whether synaptic potentiation, which increases the AMPA/NMDA-receptor current ratio (Ungless *et al.*, 2001), occurred in *ex vivo* hippocampal slices following NOR testing with the novel object. In order to determine which subregion of the hippocampus contributes to the synaptic plasticity underlying the final NOR task, both DG granule cells (Fig. 3A) and CA1 pyramidal neurons (Fig. 3C) were recorded 3 h after testing either with the novel object (test group) or with the same, familiar objects in littermates (control group). We found that after NOR testing (3 h) the AMPA/NMDA ratio increased in DG granule cells (Fig. 3B, red data): 0.56 ± 0.04 in control vs 1.08 ± 0.13 after NOR, $p = 0.003$, n = 6, 8. After NOR testing (3 h) the ratio did not change in CA1 pyramidal cells (Fig. 3D): 0.74 ± 0.11 in littermate control vs 0.69 ± 0.08 after NOR, $p = 0.78$, n = 7, 8. These findings support an association between successful NOR testing and increases in the AMPA/NMDA ratio in the DG (3 h after testing).

D1-like DA receptors required for NOR performance and synaptic plasticity

It was previously demonstrated that D1-like DA receptors facilitate synaptic plasticity at the medial perforant path to DG granule cell synapses owing to an addictive drug (Tang & Dani, 2009; Zhang *et al.*, 2010; Jenson *et al.*, 2015), and also enhance aversive contextual memory and plasticity in the CA3-CA1 synapses (Broussard *et al.*, 2016). We asked whether D1/D5 receptors may have a role in regulating successful NOR performance and the associated increase in the AMPA/NMDA ratio. First, we injected wild-type mice with a low dose of the D1-like receptor inhibitor, SCH (0.05 mg/kg, i.p.), immediately after training to examine

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DA's effect on memory of the original objects. Mice injected with SCH did not visit the novel object more (Fig 4A): 50.4 ± 3.6% with the novel object, 49.6 ± 3.6% with the familiar object; t(9) = 0.09, *p* = 0.46, n = 10. Littermates trained in NOR but receiving saline, a subset of the control group, interacted with the novel object 62 ± 3% of the time, t(10) = 4.02, p <0.05, n = 11.

Using D1- or D5-receptor KO mice, we examined the participation of these individual DA receptor subtypes. Consistent with the results from systematic administration of SCH, both D5 KO (Fig. 4B) and D1 KO (Fig. 4C) prevented novel objection recognition. The mice spent nearly equal times with both the familiar and the novel objects: D5 KO, 49.9 ± 4.2% vs 50.1 ± 4.1%, t(8) = −0.44, *p* = 0.66, n = 9 (Fig. 4B); D1 KO, 51.4 ± 3.2% vs 48.6 ± 3.2%, t(8) = −0.26, *p* = 0.60, n = 9 (Fig. 4C). These data indicate that both D1 and D5 DA receptors influence NOR performance in mice. Like the WT mice, the DA-receptor heterozygous littermates explored novel objects more commonly: D1 heterozygous, 58 ± 3%, t(9) = 3.56, p < 0.05, n = 10; and D5 heterozygous, 60 ± 2%, t(10) = 3.78, p < 0.05, n = 11.

Preventing D1-like receptor activity throughout the brain with DA-receptor KO or systemic DA-receptor inhibition, prevents normal NOR behavior. Therefore, we examined whether the NOR associated DG synaptic plasticity also required D1-like receptor activity. When wild-type mice were injected with SCH (0.05 mg/kg, i.p.), then there was no significant change in the AMPA/NMDA ratio 3 h after the NOR test compared to the control condition with both familiar objects (Fig 4D): 0.52 ± 0.02 in control vs 0.59 ± 0.08 after SCH; *p* = 0.49, n = 6, 7. Also using the D5- or D1-receptor KO mice, we measured the AMPA/NMDA ratios 3 h after the NOR test: 0.48 ± 0.04 in control vs 0.52 ± 0.05 in D5 KO; *p* = 0.53, n = 7, 6 (Fig. 4E); and 0.53 ± 0.06 in control vs 0.46 ± 0.03 in D1 KO; *p* = 0.31, n = 6, 9 (Fig. 4F). These electrophysiological data support that D1 and D5 DA receptor activity is necessary for the synaptic plasticity (as indicated by the AMPA/NMDA ratio) associated with the NOR memory task.

DISCUSSION

The dopamine transporter (DAT) is a marker for most DA neurons (Shimada *et al.*, 1991; Shimada *et al.*, 1992; Nirenberg *et al.*, 1996). Fluorescent markers associated with DAT indicated sparse dopaminergic terminals arising from the midbrain ventral tegmental area and substantia nigra innervate the dorsal DG (Fig. 1). After establishing a consistent, stable protocol for NOR (Fig. 2), the presentation of novel objects in the testing phase was associated with an increased AMPA/NMDA ratio in the DG, but not in the CA1, 3 h after NOR testing (Fig. 3). A low systemic dose of a D1-like receptor antagonist post-training decreased the exploration time of novel objects and prevented the subsequent hippocampal plasticity, an effect that was replicated in both D1 and D5 KO mice (Fig. 4). In all 3 of these cases, the DA receptors were affected throughout the brain. Taken together, these results suggest that D1-like receptor activity in the brain contributes to DG synaptic plasticity associated with the NOR task. While the synaptic plasticity was recorded specifically in the DG, the treatments with SCH or with D1- and D5-receptor KO mice acted throughout the

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brain, and it should be anticipated DA has related roles beyond the hippocampus (Kudolo *et al.*, 2010).

Anatomical evidence for DA innervation in the dentate gyrus

DA signaling to the hippocampus can arise from sources outside the midbrain VTA/SNc. There is known noradrenergic innervation to the hippocampus, and much of the DA receptor activity may arise from neurons originating in the locus coerulus (Smith & Greene, 2012; Walling *et al.*, 2012). Our results, however, also support a direct projection from midbrain VTA/SNc DA neurons to the dentate gyrus as indicated by synaptophysin-GFP staining of DAT-expressing neurons (Fig. 1). Direct DA projections were particularly prominent in the hilus. The hilus is comprised of many cell types, but the most common ones are excitatory mossy cells and HIPP interneurons (Andersen *et al.*, 2006), the latter providing feed-forward and feedback inhibition onto dentate granule cells (Zhang *et al.*, 2010). Nicotinic enhancement of LTP in the perforant path to DG circuitry was dependent upon D1-like receptor activity (Tang & Dani, 2009; Zhang *et al.*, 2010). However, D1-like receptor activation in the nucleus accumbens has also been shown to influence plasticity in the dentate (Kudolo *et al.*, 2010), and likely D1-like receptor activation in many locations contributes to hippocampal-dependent memory.

Modulatory influence of D1 and D5 receptors on NOR-related memory and synaptic plasticity in the hippocampus

In the present study, testing in the NOR task increased the AMPA/NMDA ratio in the primary excitatory cells in the DG, but not the CA1 when examined 3 h after testing. Other forms of spatial novelty have enhanced tetanus-induced plasticity in the DG (Straube *et al.*, 2003). The lack of NOR-associated plasticity in the CA1 (at > 1.5 h) seems to contrast with a previous study that measured *in vivo* fEPSP responses from the CA1 (Clarke *et al.*, 2010). In that study, NOR testing immediately increased the slope of the fEPSP, then decreased the fEPSP at 1.5 h, followed by no significant change at 3 h. In our studies, we sacrificed mice at 1.5 h, and began recording around 3 h after NOR testing. Thus, the lack of NOR-associated plasticity in the CA1 in our study very likely reflects the time window we used for the measurement. Given our time window, our results are consistent with the earlier *in vivo* study that indicated no NOR-associated CA1 plasticity at the 3h time point (Clarke *et al.*, 2010).

DA has a role in learning, memory, and synaptic plasticity via activation of D1/D5 receptors expressed in the hippocampus (Tang & Dani, 2009; Sarinana *et al.*, 2014; Jenson *et al.*, 2015; Broussard *et al.*, 2016). Systemic administration of the D1-like receptor antagonist, SCH, effectively inhibited the preference for the novel object in the NOR task in wild-type mice (Fig. 4A). This finding is consistent with a previous study showing SCH, but not the D2/D3 receptor antagonist, eticlopride, blocks novelty-induced place preference in rats (Besheer *et al.*, 1999). The systemic SCH injection given after training prevented increases in the AMPA/NMDA ratio associated with NOR 24 h later, indicating a role for D1-like receptors. (Fig. 4D). To better distinguish the roles of D1-receptor and D5-receptor subunits in novel object recognition and the associated synaptic plasticity, D1 KO and D5 KO mice were used in this study. Both D1 KO and D5 KO mice failed to show preference for the

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novel object (Fig. 4B, C). Furthermore, no significant changes in the hippocampal DG AMPA/NMDA ratios were detected following NOR testing (Fig. 4E, F). Therefore, both D1- and D5-receptor activation in the brain was required for novelty detection. Both D1 and D5 receptors can be found in the DG (Mu *et al.*, 2011; Sarinana *et al.*, 2014). We recently demonstrated that D1 and D5 KO mice had similar fEPSP responses in the DG to stimulation of the perforant path, and that spike timing-dependent protocols that produce hippocampal LTP required both D1 and D5 receptors (Yang & Dani, 2014). In previous experiments, D1 KO mice have normal basal synaptic transmission in the hippocampus, but have impaired E-LTP (Granado *et al.*, 2008).

A key limitation to our studies is that our manipulations (systemic injections, whole brain KO mice) fail to target the function of D1 or D5 receptor neurotransmission specifically in the DG. An approach that could be used to solve this problem is to use the POMC-Cre mouse line (McHugh *et al.*, 2007; Sarinana *et al.*, 2014) that selectively targets the DG. Then, DA signaling could be optogenetically (Liu *et al.*, 2014) or chemogenetically (Zhao *et al.*, 2016) manipulated at precise times to determine whether DA influences consolidation of learning pairs or influences the recognition of novel objects. Recent experiments with D1/D5 receptors selectively deleted in DG granule cells demonstrate that basal synaptic transmission is intact in the DG, but late phase LTP was not (Sarinana *et al.*, 2014).

There is ample evidence in the literature that D1-like receptors throughout the brain respond to novelty. Using *in vivo* microdialysis, previous studies reported that novel stimulation increases DA efflux in the medial prefrontal cortex (Feenstra & Botterblom, 1996), the nucleus accumbus shell (De Leonibus *et al.*, 2006), and the insular cortex (Guzman-Ramos *et al.*, 2012), suggesting broad DA influence. It also was found that rats receiving an injection (i.p.) of the specific D1-like receptor agonist, SKF38393, immediately after training dose–dependently produced a long-term enhancement of NOR memory measured at 24 and 72 h after training (de Lima *et al.*, 2011). Post-training administration of the nonselective DA receptor agonist, apomorphine, in combination with the D2 receptor antagonist, raclopride, also increased novel object exploration time, suggesting that selective activation of D1-like receptors enhances NOR memory (de Lima *et al.*, 2011). Thus, the activation of D1-like receptors (possibly at numerous locations) either by novelty-induced increases in extracellular DA levels or by systematic administration of D1-like receptor agonists can be proposed as a contributor to NOR memory consolidation. Associated with the NOR task is the plastic neuronal changes in the hippocampal DG.

In conclusion, D1-like receptor activity is necessary for successful completion of the NOR task and is necessary for the associated DG plasticity in mice. Synaptic plasticity, as indicated by an increased AMPA/NMDA ratio, is associated with NOR performance. These findings support the hypothesis that the processing of the overall NOR task engages the DG to remember the original objects in the presence of the novel objects (Antunes & Biala, 2012), and D1-like receptors participate in this process.

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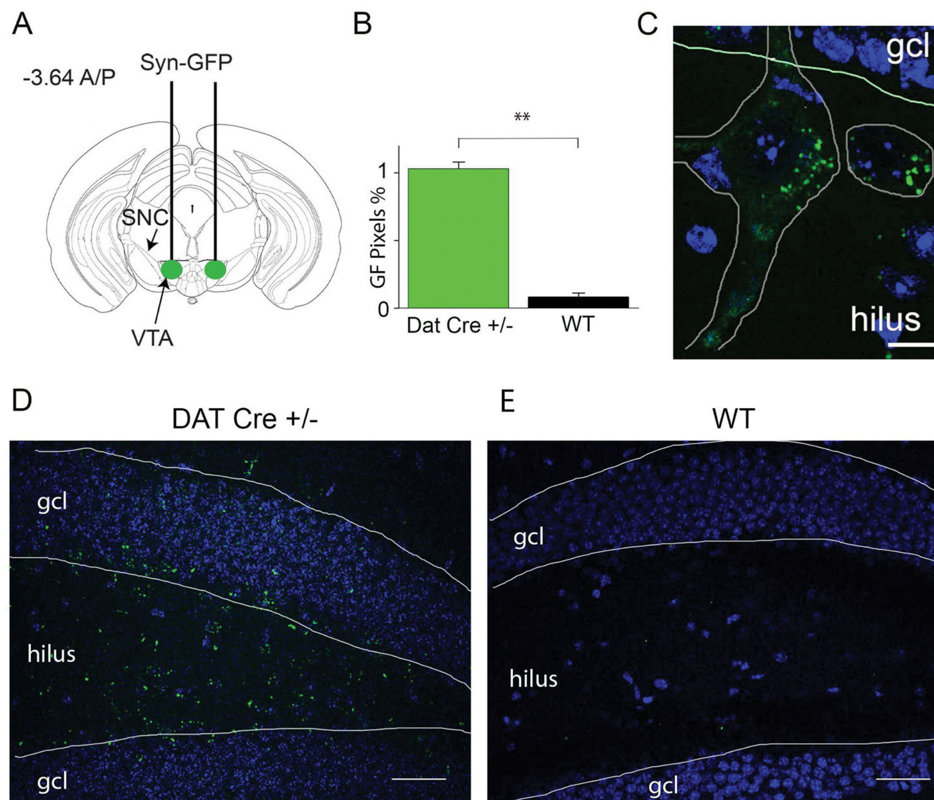

Figure 1. Midbrain DAT-containing neurons project to the DG

(A) We bilaterally injected AAV-EF1a-DIO-synaptophysin:GFP virus into the midbrain of DAT-cre (i.e., putative DA neurons) and wild-type mice as a control. The coordinates were −3.64 A/P, 0.5 M/L, and 4.0 D/V. (B) Bar graph representing the quantification of the number of green pixels in the DG from $DAT^{cre +/-}$ mice (green) compared to WT littermates (black) with $p = 0.001$. (C) A confocal image (40X) taken from the DG of a $DAT^{cre +/-}$ mouse illustrating the synaptic contact from DAT neurons on two hilar neurons outlined in gray. The larger multipolar neuron has about 12 contacts, and the smaller neuron has 6. Blue and green images are from four 2-μm sweeps that were combined at max projection. Scale bar = 10 μm. (D, E) Confocal images (20x) taken from the DG of a $DAT^{cre +/-}$ mouse (left, D) or from a wild-type (right, E) mouse injected with synaptophysin-GFP virus. The light blue is DAPI (Vector laboratories, Burlingame CA), and green fluorescence indicates the reporter from synaptophysin-GFP, which targets the putative DA axonal terminals. The white horizontal scale bars represent 50 μm. The green images were combined at max projection and blue images taken from a single confocal plane. gcl = granule cell layer.

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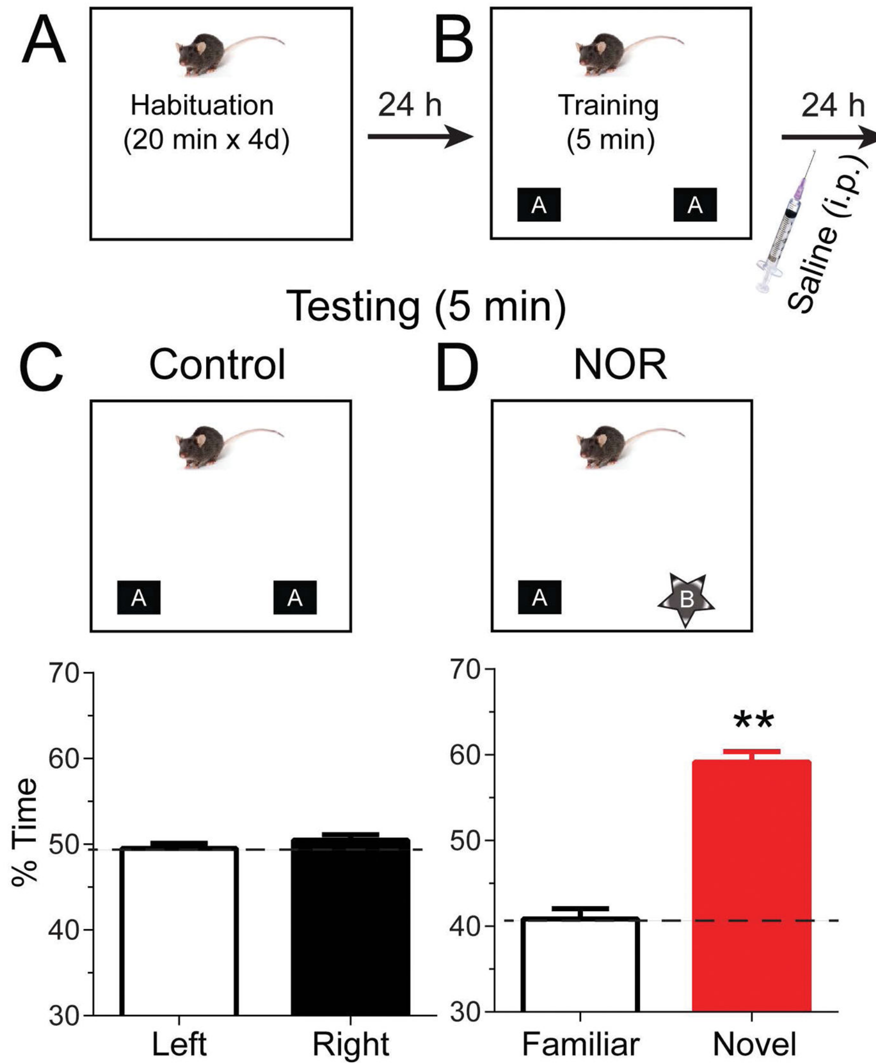

Figure 2. Novel object recognition (NOR) task in wild-type mice

The experimental protocol is illustrated. (A) Mice were placed in an open field procedural arena for 20 min daily for 4 days (Habituation). (B) 24-hours after habituation, the animals were exposed to two identical objects for 5 min in the familiar arena (Training). Immediately afterward, they were injected (i.p.) with saline. (C, top) The next day, one group of mice was placed in the same familiar field with the same familiar objects (Control). (C, bottom) The percentage of time spent exploring two identical objects was similar and not statistically different. (D, top) The second group of mice was placed in the same familiar field with one familiar object and one novel object. (D, bottom) The percentage of time spent exploring the novel object (red bar) was significantly greater than the time spent exploring the familiar object (white bar).

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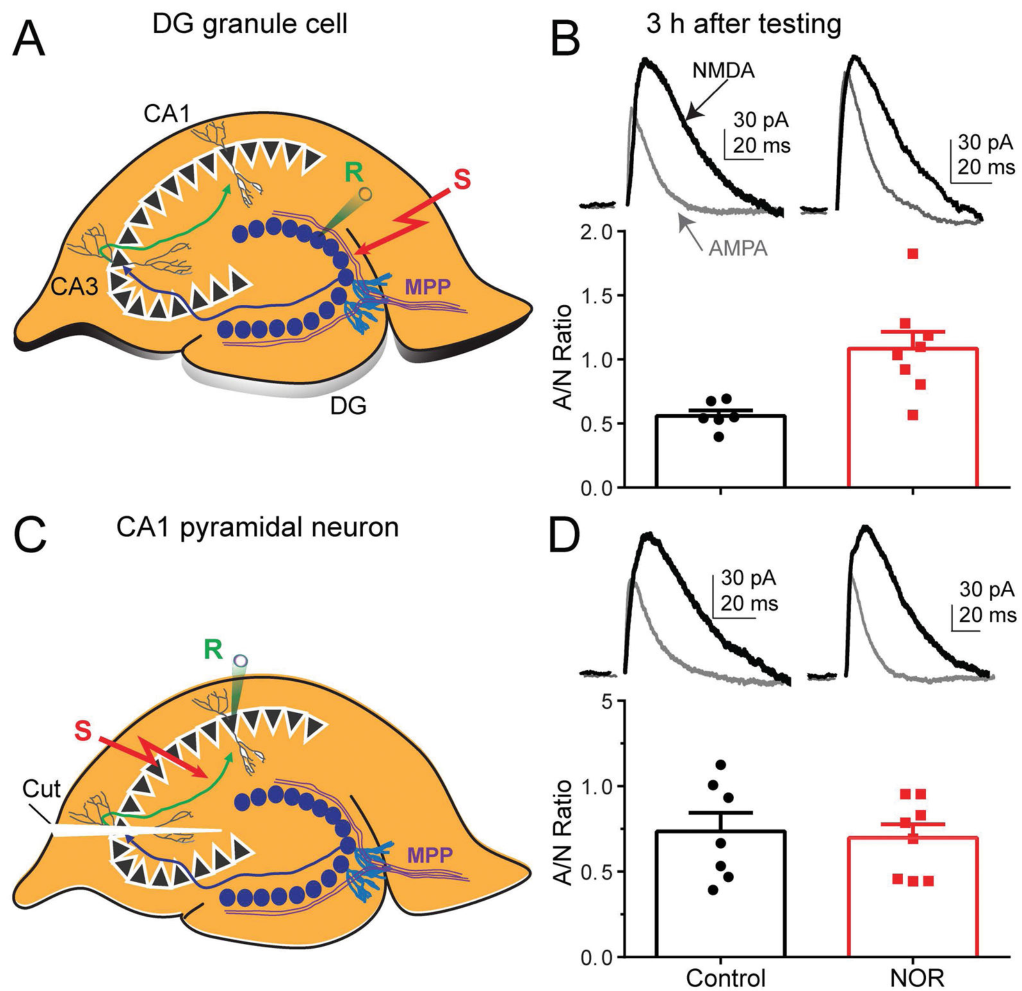

Figure 3. NOR test increased the AMPA/NMDA ratio in DG granule cells

(A) Diagram illustrating a whole-cell recording from a DG granule cell in the hippocampal slice. We placed the patch-clamp recording electrode (R) onto a granule cell, and the stimulating electrode (S) into the medial perforant path (MPP). (B, top) Representative traces of AMPA-receptor (gray traces) and NMDA-receptor (black traces) mediated whole-cell currents recorded from granule cells obtained from wild-type mice 3 h after NOR testing. (B, bottom) The tabulated individual plasticity values and averaged AMPA/NMDA ratios from recorded granule cells in controls (open black bar) or from mice that explored a novel object (open red bar). (C) Diagram illustrating a whole-cell recording from a CA1 pyramidal neuron in the hippocampal slice. We placed the patch-clamp recording electrode (R) onto a pyramidal neuron and the stimulating electrode (S) into the Schaffer collateral path. The white triangle (labelled, Cut) indicates that connections between CA3 and CA1 were cut to prevent the spread of recurrent electrical activity into the CA1. (D, top) Representative traces of AMPA-receptor (gray traces) and NMDA-receptor (black traces) mediated whole-cell currents recorded from CA1 pyramidal neurons obtained from wild-type mice 3 h after NOR testing. (D, bottom) The tabulated individual plasticity values and averaged AMPA/NMDA ratios from recorded pyramidal neurons in controls (open black bar) or from mice that explored a novel object (open red bar).

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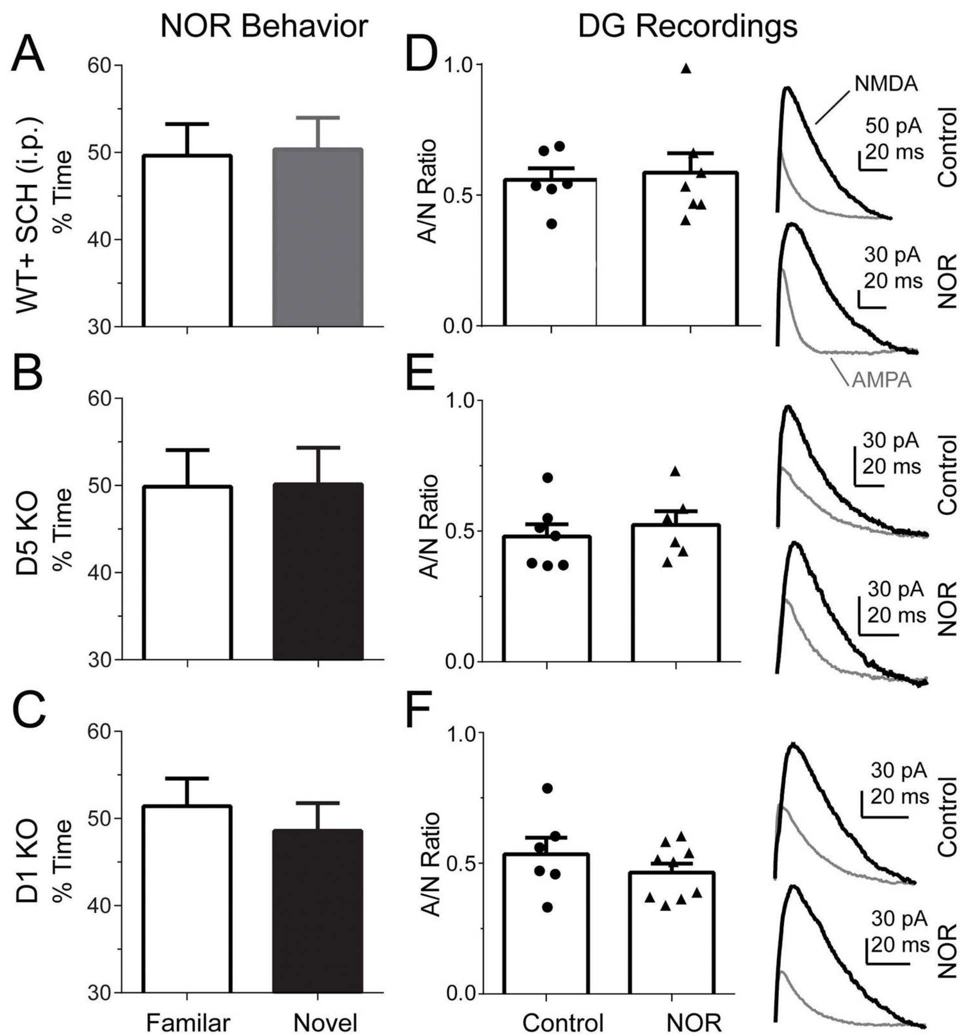

Figure 4. D1-like receptor inhibition or D1 and D5 KO on performance in the NOR test and AMPA/NMDA ratio in the dentate gyrus

The percentage of time spent exploring one familiar object (open bars) and a novel object (solid bars) in wild-type mice injected with the D1-like receptor antagonist, SCH, (A); or D5-receptor KO mice (B); or D1-receptor KO mice (C). The tabulated individual plasticity values and averaged AMPA/NMDA ratios (left panel) and representative traces of AMPA-receptor (gray traces) and NMDA-receptor (black traces) mediated whole-cell currents (right panel) recorded in granule neurons from mice injected with the D1-like receptor antagonist, SCH, (D); or from D5-receptor KO mice (E); or from D1-receptor KO mice (F). The hippocampal slices were obtained from mice 1.5 h after NOR testing, and the currents were measured about 3 h after testing. The number of mice or slices and the statistics are given in the text. The baselines for the currents (right panel) were left off to conserve space.