Dopamine release from the locus coeruleus to the dorsal hippocampus promotes spatial learning and memory

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Dopamine neurotransmission in the dorsal hippocampus is critical for a range of functions from spatial learning and synaptic plasticity to the deficits underlying psychiatric disorders such as attention-deficit hyperactivity disorder. The ventral tegmental area (VTA) is the presumed source of dopamine in the dorsal hippocampus. However, there is a surprising scarcity of VTA dopamine axons in the dorsal hippocampus despite the dense network of dopamine receptors. We have explored this apparent paradox using optogenetic, biochemical, and behavioral approaches and found that dopaminergic axons and subsequent dopamine release in the dorsal hippocampus originate from neurons of the locus coeruleus (LC). Photostimulation of LC axons produced an increase in dopamine release in the dorsal hippocampus as revealed by high-performance liquid chromatography. Furthermore, optogenetically induced release of dopamine from the LC into the dorsal hippocampus enhanced selective attention and spatial object recognition via the dopamine D1/D5 receptor. These results suggest that spatial learning and memory are energized by the release of dopamine in the dorsal hippocampus from noradrenergic neurons of the LC. The present findings are critical for identifying the neural circuits that enable proper attention selection and successful learning and memory.

dopamine | locus coeruleus | hippocampus | memory | attention

Dopamine is a neurotransmitter released throughout the brain to encode salience and facilitate the formation of associative memory (1, 2). When released into the dorsal hippocampus, dopamine binds to D1/D5 receptors to promote attention, episodic memory formation, spatial learning, and synaptic plasticity (3–5). Successful spatial learning requires that hippocampal place cells, location-encoding pyramidal neurons (6), display consistent and stable patterns of neural activity, a process that can be enhanced by selective attention to spatial cues and by dopamine agonists (7, 8). Conversely, dopamine receptor blockade attenuates the ability of spatial attention to stabilize the firing pattern of hippocampal place cells (8). The role of dopamine in driving attentional processes is highlighted by the fact that methylphenidate, one of the most common treatments for attention-deficit hyperactivity disorder (ADHD), improves attention by increasing synaptic availability of dopamine in the hippocampus, as well as in the prefrontal cortex and striatum (9–11). These findings suggest that dopamine is critical for the selective attention underlying spatial learning and memory.

For decades, the ventral tegmental area (VTA) has been the presumed source of hippocampal dopamine. However, in recent years, the source of dopamine in the dorsal hippocampus has become less clear. McNamara et al. (12) argued that a dopaminergic projection from the VTA to the dorsal hippocampus promoted hippocampal reactivation during sleep and stabilized memory. However, only 10% of the sparse projection from the VTA to the hippocampus contains dopamine, raising the question of whether this weak VTA projection could be solely responsible for activating the dense network of dopamine receptors found in the dorsal hippocampus (13–16). Moreover, whereas the ventral aspect of the hippocampus, an area associated with learned fear and anxiety (17), receives significant dopaminergic projections from the VTA (13, 14), the dorsal region of the hippocampus, which houses the place cells required for spatial memory (7), has relatively sparse dopaminergic innervation from the VTA. We therefore set out to identify the neurons that provide dopaminergic tone to the dorsal hippocampus and to assess their involvement in spatial learning and attention.

Dopamine is removed from hippocampal synapses by the norepinephrine transporter (18), which suggests that dopamine may also be released from norepinephrine-containing neurons outside of the VTA. Although indirect action via the VTA had not been ruled out, electrical and pharmacological stimulation of the noradrenergic locus coeruleus (LC) increased dopamine levels in the prefrontal cortex and modulated hippocampal synaptic transmission (19, 20). Also, dopamine-mediated enhancement of excitatory transmission in the hippocampus can be reversed by tyrosine hydroxylase (TH) knockdown in the LC, but not in the VTA (21). Smith and Greene (21) therefore suggested that dopamine may be released from the LC into the dorsal hippocampus. A major caveat of the study is that the synaptic transmission effects are produced in vitro by amphetamine, a

Significance

Successful completion of daily activities relies on the ability to select the relevant features of the environment to pay attention to and remember. Disruptions of these processes can lead to disorders, such as attention-deficit hyperactivity disorder and age-related memory loss. To devise therapeutic strategies, we must understand the neural circuits underlying normal cognition. One important pathway is the signaling of dopamine, a reinforcement-related neurotransmitter, in the hippocampus, a spatial learning and memory center. Surprisingly, the brain region supplying dopamine to the dorsal hippocampus is unclear. This study provides direct evidence that the noradrenergic locus coeruleus coreleases dopamine in the dorsal hippocampus and provides insight into dopamine function in selective attention and spatial learning and memory.

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drug known to increase extracellular dopamine concentrations (22). The finding provides no insight as to whether dopamine is released from the LC under more physiological, nondrug conditions, a question we test directly in our study.

Taken together, there are some groups who argue that the VTA is the main source of dopamine to the dorsal hippocampus (12–14) and others who support the idea that dopamine is released from LC neurons (19–21). These conflicting hypotheses leave unanswered the question of which brain region supplies the dorsal hippocampus with dopaminergic tone. We combined optogenetic, biochemical, and behavioral methods to identify the major source of dopamine in the dorsal hippocampus and then examined the involvement of this dopaminergic pathway in selective attention and spatial learning and memory.

Results

VTA Dopamine Neurons Project More Widely to the Ventral Versus Dorsal Hippocampus. To identify the neurons that release dopamine in the dorsal aspect of the hippocampus, we first selectively labeled and then stimulated dopamine axons in the dorsal hippocampus using the neuronal specificity afforded by optogenetics. Using the Cre-Lox method for gene expression, we used mice that expressed Cre under control of the dopamine transporter with an internal ribosome entry site (IRES-Cre mice). We then transfected dopaminergic cell bodies in the VTA and their axonal projections by injecting a floxed adenoassociated virus coding for Channelrhodopsin-2 (ChR2), a light-sensitive ion channel coupled to enhanced yellow fluorescent protein (EYFP), a fluorescent reporter gene (Fig. 1 *A* and *B*). The genetic precision of optogenetics eliminates the possibility of nonspecific binding and injection site spillover that can accompany classical immunohistochemical techniques. To map dopamine axonal projections, we imaged ChR2-EYFP–expressing VTA dopamine axons and immunostained for TH (Fig. 1 *C* and *D*), an enzyme in the pathway for dopamine synthesis that highlights catecholaminergic structures (23). Confirming the efficacy of our cell type-specific labeling method, we observed fibers in expected VTA projection sites, including the striatum and amygdala (Fig. S1*A*), as well as in the ventral hippocampus (Fig. 1*D*). However, we found very sparse expression of VTA dopamine axons in the cortical area 1 (CA1) region of the dorsal hippocampus (Fig. 1*C*). We observed a significantly greater number of VTA axons in the ventral versus dorsal hippocampus (Fig. 1*E*).

We repeated the procedure of tracing the VTA to the dorsal hippocampus projection using Th-IRES-Cre mice and similar to results in DAT-IRES-Cre mice, we found scarce expression of VTA dopamine axons in the dorsal hippocampus. However, upon inspection of the midbrain, we also observed ChR2-EYFP viral expression in neurons outside of VTA dopamine cells, and therefore eliminated the dataset (Fig. S1*B*). Indeed, it has previously been reported that Cre-induced reporter expression in the midbrain of Th-IRES-Cre and Th-Cre mice is nonspecific for VTA dopamine neurons (24).

Catecholamine Neurons of the LC Project to the Dorsal Hippocampus. To explore the source of dopamine to the dorsal hippocampus, we used retrograde tracing to locate the cell bodies of catecholamine neurons that project directly to the dorsal hippocampus (Fig. 1 *F* and *G*). We injected red retrobeads into the dorsal CA1 region of the hippocampus in naive C57BL/6 mice and did not label dopamine cell bodies in the VTA, providing further evidence that the catecholamine projection from the VTA to the dorsal hippocampus is sparse at best (Fig. 1*G*). In contrast, the retrobeads fluorescently labeled significantly more catecholamine neurons in the LC (Fig. 1*H*), another brain region related to attention and arousal (25, 26). Unlike the VTA, which contains neurons that release dopamine, cells of the LC are thought to produce dopamine solely as an intermediate in the synthesis of norepinephrine (27). We now hypothesize that LC neurons

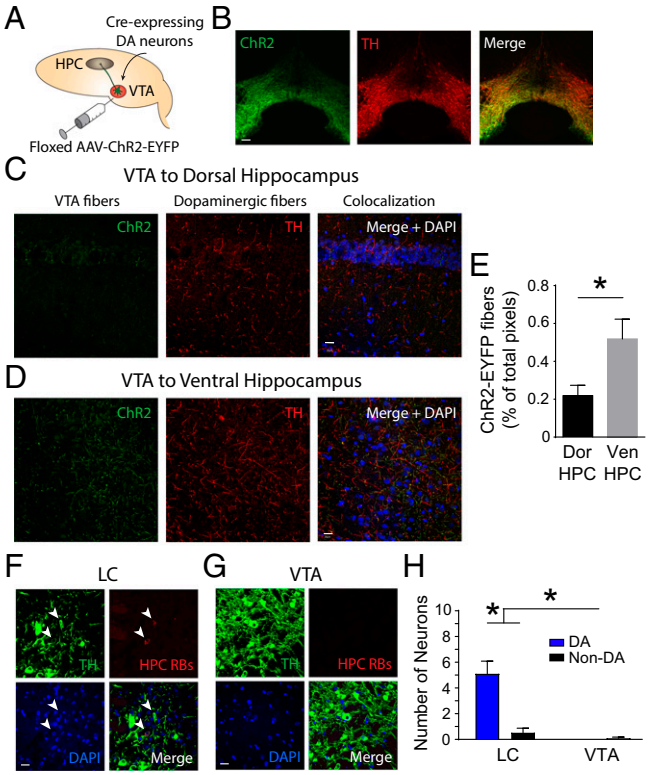


Fig. 1. Dopaminergic projection from the VTA to the CA1 region of the dorsal hippocampus is very sparse in dopamine transporter (DAT)-IRES-Cre mice. (*A*) Schematic of the experimental approach depicts infection of VTA dopamine (DA) neurons and axons with ChR2-EYFP to allow for axonal tracing. AAV, adenoassociated virus; HPC, hippocampus. (*B*) ChR2-EYFP expression was restricted to VTA dopamine neurons. ChR2 (green) illustrates ChR2-EYFP expression, TH (red) is a dopamine marker, and Merge indicates combined signals. (Scale bar: 100 μm.) (*C*) ChR2$^+$ fibers (green) indicate the dearth of dopamine-containing axons in the dorsal hippocampus originating from the VTA. (Scale bar: 20 μm.) (*D*) ChR2$^+$ VTA dopamine axons are abundant in the ventral hippocampus. DAPI (blue) is a nuclear marker. (Scale bar: 20 μm.) (*E*) Significantly more ChR2$^+$ fibers are found in the ventral hippocampus (Ven HPC = 0.52 ± 0.10%) versus the dorsal hippocampus (Dor HPC = 0.22 ± 0.05%) (Mann–Whitney test, $^*P = 0.022$). (*F*) Neurons of the LC project to the dorsal hippocampus as indicated by (red) neurons retrogradely labeled from the dorsal hippocampus. TH (green), retrobeads from the dorsal hippocampus (HPC RBs, red), and DAPI (blue) are shown. (Scale bar: 20 μm.) (*G*) VTA was devoid of dorsal hippocampus-projecting dopamine neurons. (Scale bar: 20 μm.) (*H*) Significantly more dopamine-containing neurons in the LC versus the VTA project to the dorsal hippocampus [two-way ANOVA, LC DA = 4.92 ± 0.95, LC non-DA = 0.50 ± 0.34, VTA DA = 0, VTA non-DA = 0.08 ± 0.08; $F(1,44) = 19.82$; $n = 12$; $^*P < 0.001$].

corelease dopamine along with norepinephrine in the dorsal hippocampus.

To determine whether the catecholamine terminals of the LC are appropriately positioned to influence hippocampal activity, we next isolated and mapped the catecholamine pathway from the LC to the dorsal hippocampus in Th-IRES-Cre mice. The LC projection to the hippocampus (28), specifically to the ventral hippocampus, has been documented (29); however, the anatomical localization of LC catecholamine terminals within distinct dorsal hippocampal subregions has not been well characterized. Using ChR2-EYFP as a neuronal label, we found that catecholamine neurons in the LC indeed send numerous axons to the dorsal hippocampus (Fig. 2 *A* and *B*). Given that place cells are found in the CA1 and CA3 regions in the dorsal hippocampus (6), we quantified LC terminal expression in these hippocampal subregions (schematic in Fig. 2*C*). We found dense LC catecholamine

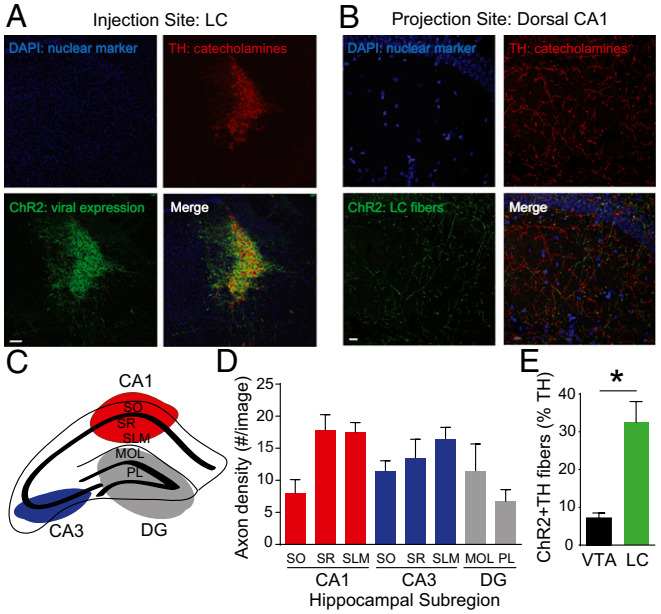


Fig. 2. Distribution of LC catecholamine axons in the dorsal hippocampus of Th-IRES-Cre mice. (*A*) ChR2 successfully infected catecholamine-containing neurons in the LC. (Scale bar: 100 μm.) (*B*) ChR2$^+$ LC catecholamine axons are found in the dorsal hippocampus. (Scale bar: 20 μm.) (*C*) Dorsal hippocampal subregions. (*D*) LC catecholamine fiber density in hippocampal subregions (one-way ANOVA, Kruskal–Wallis test, $P = 0.024$; $n = 4$). MOL, molecular layer of the dentate gyrus (DG); PL, polymorphic layer of the DG; SLM, stratum lacunosum-moleculare; SO, stratum oriens; SR, stratum reticulatum. DAPI (blue) is a nuclear marker, ChR2 (green) indicates ChR2$^+$ LC axons, TH (red) indicates catecholamine-containing fibers, and Merge indicates combined signals. (*E*) LC catecholamine axon density is significantly greater than the density of VTA dopamine axons in the dorsal hippocampus (LC: 32.53 ± 5.48%, VTA: 7.20 ± 1.32%, Mann–Whitney test, $*P < 0.001$).

fiber expression throughout the dorsal CA1 region (Fig. 2*D*). This finding suggests that LC catecholamine fibers terminate in close proximity to the dendritic branches of hippocampal place cells, neurons that encode position in space and are tuned by selective attention to environmental cues (7, 8). We next compared the density of LC catecholamine axons with the density of VTA dopamine axons in the dorsal CA1 region and found that LC axons are substantially denser than VTA fibers (Fig. 2*E*).

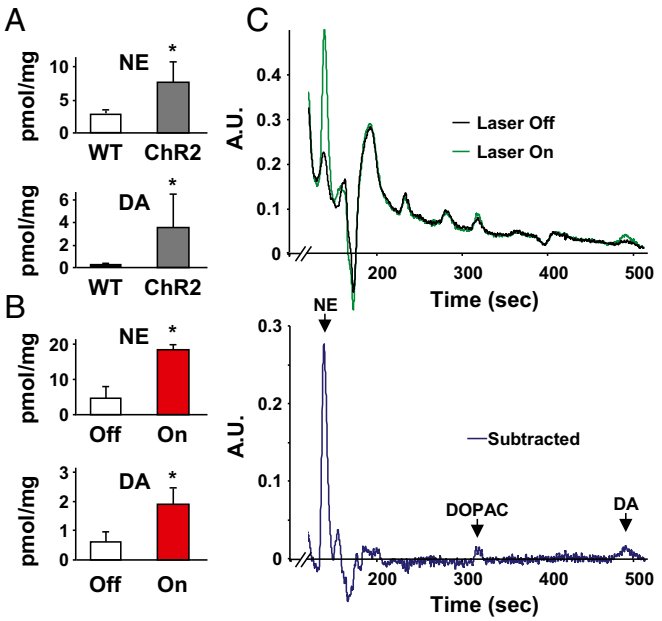
Previous studies support the hypothesis that LC neurons are capable of coreleasing dopamine in the dorsal hippocampus. Electrical stimulation of the LC produces increased tissue content and pharmacological action of dopamine in the hippocampus (20, 30). However, these studies did not rule out network action via the VTA and failed to demonstrate release of dopamine from noradrenergic LC axons into the dorsal hippocampus. An increase in dopamine tissue content may suggest intracellular accumulation of norepinephrine synthesis products, but does not provide evidence that these neurons are capable of dopamine release. We attempted to address these unresolved questions using the genetic and anatomical specificity afforded by optogenetics.

Dopamine Is Released from Axons of the LC in the Dorsal Hippocampus. To assess directly whether and to what extent dopamine is released from axons of the LC, we measured extracellular dopamine concentrations in acute hippocampal slices using high-performance liquid chromatography (HPLC). Dorsal hippocampus coronal brain sections (250 μm) were prepared from Th-IRES-Cre mice in which LC catecholamine neurons and fibers were infected with the floxed ChR2-EYFP virus. We then photostimulated LC axons in the hippocampus ex vivo and compared dopamine release in the extracellular fluid of stimulated hippocampal slices with identically handled wild-type controls lacking ChR2 expression. Optical stimulation of LC catecholamine terminals in the dorsal hippocampus evoked significantly higher levels of both dopamine and norepinephrine release compared with controls (Fig. 3*A*). Similarly, optically stimulated slices (laser on) showed significantly higher extracellular concentrations of dopamine and norepinephrine than nonstimulated slices expressing ChR2-EYFP (laser off; Fig. 3 *B* and *C*). These findings confirm that both neurotransmitters were released from LC axons into the dorsal hippocampus.

We next lesioned catecholamine neurons of the LC selectively using a floxed Caspase-3 virus (University of North Carolina Vector Core) (31) and measured a 72.7% reduction of dopamine and a 77.2% reduction of norepinephrine tissue levels in the hippocampus (Fig. 3 *D* and *E*). The ratio of norepinephrine to dopamine remained the same in lesioned and control samples (Fig. 3*E*). We then stimulated nonspecific neurotransmitter release into the extracellular fluid by incubating slices in potassium chloride to promote release of dopamine and norepinephrine from all axons in the dorsal hippocampus. A selective lesion of LC catecholamine neurons reduced the extracellular dopamine concentration by 67.9% and norepinephrine by 83.8% (Fig. S2). This finding suggests that the majority of the dopaminergic tone present in dorsal hippocampal tissue and actively released into the extracellular space originated from neurons of the LC. Together, these results provide a direct demonstration that dopamine is released from LC axons into the dorsal hippocampus.

Optogenetic Activation of LC Catecholamine Axons in the Dorsal Hippocampus Promotes Selective Attention and Spatial Learning via the Dopamine D1/D5 Receptor. To assess the behavioral significance of the dopamine pathway from the LC to the hippocampus, we examined mice in a spatial object recognition task while activating ChR2-EYFP$^+$ LC axons in the dorsal hippocampus (Fig. 4*A*). We allowed mice to explore a square arena with distinct walls containing two identical objects during a 5-min training session. One day later, we challenged these mice to explore the arena during a 5-min test session in which the least preferred object was moved to another position. During the training session for this task, both ChR2-EYFP–expressing Th-IRES-Cre animals and their wild-type controls received intrahippocampal optical stimulation. We then assessed performance by determining the difference index, a normalized measure indicating the relative amount of time spent exploring the displaced object. Increased attention to the displaced object during the test session generates a higher difference index and correlates positively with the degree of successful spatial learning and memory. In ChR2-EYFP–expressing animals, optical activation of hippocampal LC catecholamine terminals during training significantly increased the average difference index in the test session. This enhancement of spatial learning only occurred in optically stimulated animals, and not in wild-type controls (Fig. 4*B*). These results demonstrate that photostimulation of the LC-to-hippocampus catecholamine pathway promoted spatial learning and converted nonlearners into learners.

The LC-to-hippocampus projection was previously thought to be strictly noradrenergic (28); therefore, the function of dopamine released from LC fibers into the dorsal hippocampus was not known. Given the dearth of catecholamine innervation from the VTA, we now asked whether the LC actively releases behaviorally relevant dopamine concentrations into the dorsal hippocampus. To address this question, before the optical stimulation training session, we infused into the dorsal hippocampus one of three compounds: (*i*) saline; (*ii*) SCH23390, a dopamine D1/D5 receptor antagonist we have previously used to block the effects



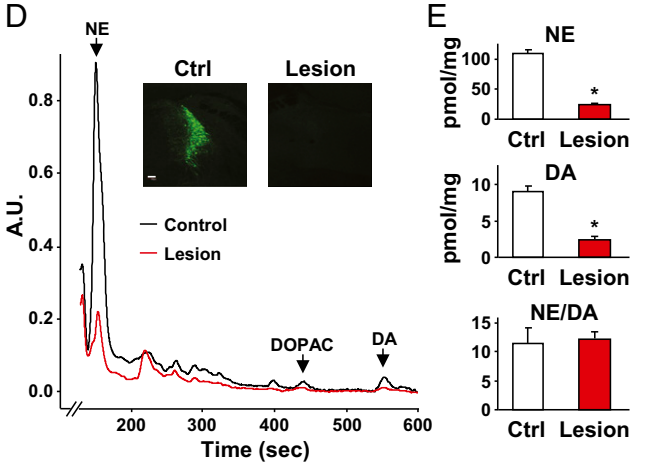


Fig. 3. Dopamine is released from LC axons in the hippocampus. (*A*) Average dopamine and norepinephrine concentrations in the extracellular fluid of photostimulated hippocampal slices from mice expressing ChR2 in LC axons and their non-ChR2–expressing littermates [wild-type (WT): DA = 0.11 ± 0.05, norepinephrine (NE) = 3.05 ± 0.22, $n = 3$; ChR2: DA = 3.57 ± 1.14, $n = 7$; Student's t test, $^*P = 0.017$). (*B*) Higher dopamine and norepinephrine levels were also measured in the supernatant of ChR2-expressing hippocampal slices that were optically stimulated (On) versus unstimulated (Off) controls (nonstimulated: DA = 0.60 ± 0.18, NE = 4.75 ± 1.65, $n = 4$; stimulated: DA = 1.88 ± 0.29, NE = 18.25 ± 0.85, $n = 4$; Student's t test, $^*P = 0.029$). (*C*) Representative HPLC traces show clear peaks for norepinephrine, dihydroxyphenylacetic acid (DOPAC), and dopamine in the extracellular fluid of stimulated slices versus nonstimulated controls. A.U., arbitrary units. (*D*) HPLC trace of hippocampal tissue samples from animals with a unilateral, Caspase-3–induced lesion of catecholamine neurons in the LC. (*Insets*) TH staining of the LC region. (Scale bar: 100 μm.) (*E*) Both dopamine and norepinephrine tissue levels in the dorsal hippocampus were nearly eliminated by lesioning LC catecholamine neurons. (Control: DA = 9.06 ± 0.64, NE = 108.96 ± 6.32, $n = 5$; Lesion: DA = 2.47 ± 0.49, NE = 24.85 ± 1.22, $n = 5$; Student's t test, $P < 0.001$). The ratio of norepinephrine to dopamine was the same in control and lesioned hemispheres (Control: 11.67± 2.49, Lesion: 12.29 ± 1.16; $^*P > 0.05$).

of attention on place cell stability (8); or (*iii*) propranolol, a beta-adrenergic receptor antagonist. Site-specific delivery (Fig. S3) of the dopamine D1/D5 receptor antagonist blocked the memory-enhancing effects of LC-to-hippocampus photostimulation, consistent with the hypothesis that LC neurons do, in fact, release dopamine into the dorsal hippocampus to promote spatial learning and memory (Fig. 4*C*). The norepinephrine antagonist had no significant effect on the LC-to-hippocampus optical stimulation-induced learning enhancement (Fig. 4*C*). Given that the difference index is a normalized measure of activity and that total exploration times in antagonist experiments were not significantly different from baseline experiments, it is highly unlikely that this effect was due to alterations in locomotion (Fig. 4*D*).

Optogenetic Activation of LC Catecholamine Axons in the Dorsal Hippocampus Increases the Rate of Spatial Learning and Memory. To explore the role of the catecholaminergic projection from the LC to the dorsal hippocampus in spatial learning and memory further, we also performed the Barnes Maze task. In this hippocampus-dependent paradigm, animals explore a circular platform with equidistant holes surrounding the periphery (Fig. 5*A*). One hole leads to an escape box, which, when entered, turns off the aversive bright lights. Animals received optical stimulation of LC axons in the dorsal hippocampus during each of the daily training sessions. After 6 d of training, the escape box was removed and optical stimulation was discontinued to test the memory retention for the target hole. Optical stimulation

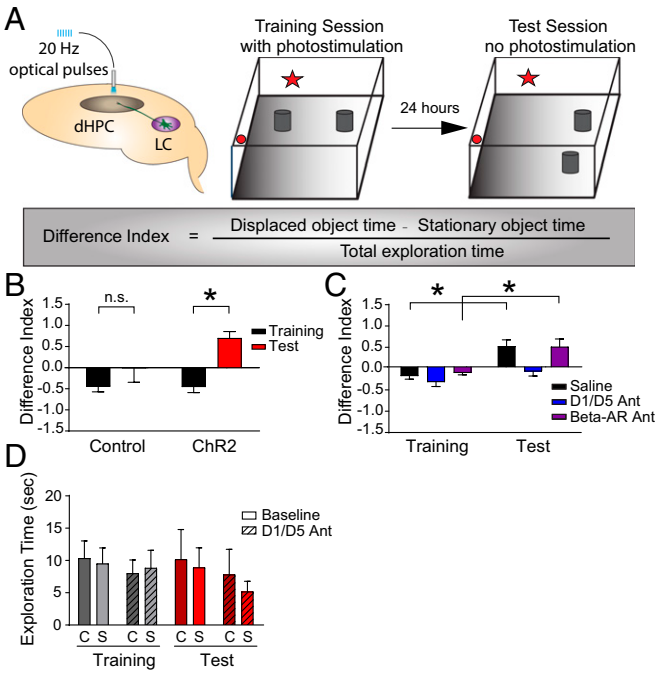


Fig. 4. Dopamine release from the LC to the dorsal hippocampus improved spatial object recognition via dopamine D1/D5 receptors. (*A*) Experimental paradigm. dHPC, dorsal hippocampus. (*B*) ChR2-stimulated animals (heterozygous Th-IRES-Cre, $n = 10$) show significantly enhanced spatial learning compared with control animals (wild-type littermates, $n = 6$). Difference index: mean ± SEM; ChR2 ($n = 10$): Training = −0.45 ± 0.14, Test = 0.70 ± 0.15; Control ($n = 6$): Training = −0.45 ± 0.12, Test = −0.01 ± 0.34; two-way ANOVA, $F(1,14) = 16.78$; $^*P = 0.001$, Sidak's multiple comparison test. n.s., not significant. (*C*) Intrahippocampal injection of SCH23390, a dopamine D1/D5 receptor antagonist, but not propranolol, a beta-adrenergic receptor antagonist (AR Ant), reversed optically stimulated LC-hippocampus–induced enhancement of performance versus saline controls. D1/D5 Ant ($n = 6$): Training = −0.35 ± 0.10, Test = −0.11 ± 0.10; Saline ($n = 7$): Training = −0.21 ± 0.08, Test = 0.48 ± 0.15; Beta-AR Ant ($n = 4$): Training = −0.13 ± 0.05, Test = 0.47 ± 0.18; two-way ANOVA, treatment factor $F(2,38) = 6.840$; $^*P = 0.004$, Sidak's multiple comparison test. (*D*) Total object exploration time during 5 min of training did not change significantly in the presence of the D1/D5 antagonist. C, Control; S, ChR2 optical stimulation ($P > 0.05$).

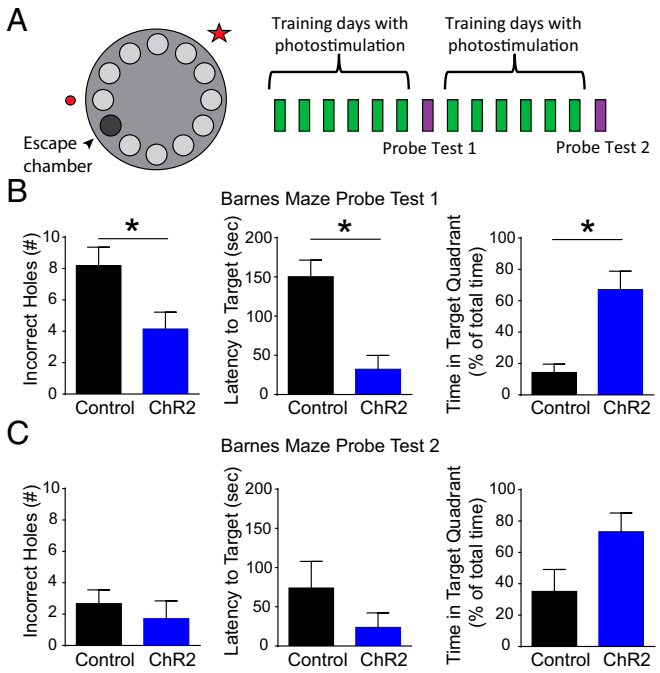


Fig. 5. Optogenetic activation of LC catecholamine axons in the dorsal hippocampus increased the rate of learning in the Barnes Maze task. (*A*) A schematic of the Barnes Maze and training protocol is shown. Small light gray circles represent empty holes, and the dark gray circle indicates the position of the hole leading to a goal box in which the animal can escape to turn the lights off. Memory retention was tested by probe sessions during which the escape box and optical stimulation were removed. (*B*) On probe day 1, optically stimulated animals learned the task significantly better than wild-type controls [Control ($n = 6$), ChR2 ($n = 7$): incorrect holes visited before reaching target hole: $^*P = 0.030$, latency to reach target hole from trial start: $^*P = 0.005$, time spent in target quadrant: $^*P = 0.015$]. (*C*) By probe day 2, both controls and optically stimulated animals learned the task well, as indicated by no significant difference between the two groups ($P > 0.05$).

resumed for the next 6 d of training to the same target hole, followed by a second probe day. Animals were connected to a fiber optic cable that did not deliver photostimulation on probe days. LC-to-hippocampus–stimulated animals learned this task significantly better than controls during probe test 1, as indicated by a fewer number of errors, shorter latency to the target hole, and more time spent in the target quadrant (Fig. 5*B*). By probe test 2, control animals had sufficiently learned the task, yielding no significant difference between the two groups (Fig. 5*C*). These findings demonstrate that in a task learned by control animals, activation of LC axons in the dorsal hippocampus of ChR2-expressing mice significantly increased the rate of learning. These results reinforce earlier findings and support the idea that dopamine release from the LC to the dorsal hippocampus is a significant component of the spatial learning and memory circuit.

Optogenetic Activation of LC Catecholamine Axons in the Dorsal Hippocampus Had No Effect on a Modified Conditioned Place Preference Task. To assess whether photostimulation of the LC-to-hippocampus catecholamine pathway is inherently reinforcing, we performed a conditioned place preference task modified to increase involvement of hippocampus-dependent spatial learning circuits. Animals explored a circular chamber with cues surrounding the walls for spatial orientation. We delivered photostimulation each time the animal explored an unmarked target quadrant of the arena (Fig. 6*A*). This spatial version of conditioned place preference was designed to assay whether the LC-to-hippocampus pathway provided reinforcement in the absence of selective attention to a specific object or goal. Optically stimulated animals did not display a significant change in the amount of time spent in the unmarked target region versus wild-type controls (Fig. 6*B*). This finding is consistent with the argument that LC-to-hippocampus stimulation enhances selective attention to specific stimuli, thereby energizing spatial learning and memory. This result demonstrates that dopamine signaling in the dorsal hippocampus did not produce an inherently rewarding or reinforcing signal but, instead, promoted selective attention to salient environmental cues.

Discussion

These findings provide direct evidence that efferents from the LC are not purely noradrenergic, but corelease dopamine in the dorsal hippocampus. The LC dopamine signal drives the selective attention underlying spatial learning and memory. Numerous reports suggest that the VTA is the main source of dopamine to the dorsal hippocampus (12–14), whereas others suggest that the LC supplies the hippocampus with dopamine (19–21). The present results reconcile the discrepancy between dense dopamine receptor expression and sparse VTA dopamine axon networks in the dorsal hippocampus and support the hypothesis that dopaminergic tone in the dorsal hippocampus arises from neurons of the LC.

Previous work by McNamara et al. (12) demonstrated that activation of VTA dopamine axons in the dorsal hippocampus promoted spatial memory for a reward location. Our work adds to these findings by suggesting that dopamine release from LC neurons energizes the attention component of the spatial environment in the absence of reward and supports the acquisition of spatial memory. Given that catecholamine neurons of the LC contribute 73% of all measurable dopaminergic tone in the dorsal hippocampus, we argue that dopamine release from the LC may have more significant influence over dorsal hippocampus neuronal activity than the VTA.

Our findings that LC neurons corelease dopamine in the dorsal hippocampus are consistent with the earlier finding that LC neurons also corelease dopamine in the prefrontal cortex and drive dopamine-dependent activity in the hippocampus (19, 21). Moreover, the current findings provide anatomical support for the documented functional distinctions between the dorsal and ventral hippocampus (17). The density of VTA dopamine terminals in the ventral hippocampus, but sparse expression in the dorsal

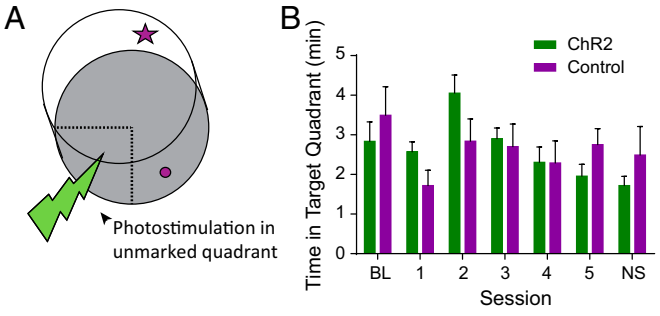


Fig. 6. Optogenetic activation of LC catecholamine axons in the dorsal hippocampus had no effect on a modified conditioned place preference task. (*A*) Schematic of the circular chamber in which animals received photostimulation of the catecholaminergic pathway from the LC to the dorsal hippocampus when traversing through the unmarked target quadrant. (*B*) ChR2-stimulated animals ($n = 10$) did not spend significantly more time in the target quadrant than wild-type controls ($n = 6$), two-way ANOVA, $F(1,14) = 0.00$, $P > 0.05$. The numbers 1–5 represent 5 d of exploration with optical stimulation in the target region. BL, baseline session with no optical stimulation; NS, no stimulation day.

hippocampus, suggests that hippocampal subregion-dependent behaviors are driven by different neuromodulatory pathways. Specifically, LC catecholamine release may be the primary source of synaptic modulation responsible for tuning the space-encoding place cells found in the dorsal hippocampus. In contrast, VTA dopamine innervation in the ventral hippocampus may provide the incentive salience and motivational drive underlying emotion-based learning.

The present findings also expand the list of dopamine-releasing brain regions to include the LC. Numerous studies postulate dopamine release from the LC into the hippocampus, but rely on pharmacological blockade of the dopamine D1/D5 receptor to infer the presence of dopamine. However, the possibility exists that the D1/D5 receptor is activated by a ligand other than dopamine or undergoes receptor–receptor interactions. Norepinephrine has a K_d of 50 μM at the dopamine D1 receptor (32), and the possibility exists that norepinephrine action at the dopamine D1 receptor is responsible for the effects reported by Smith and Greene (21). In fact, norepinephrine promotes cAMP accumulation in embryonic retinas via the dopamine D1/D5 receptor (33), and norepinephrine-induced increases in adenyl cyclase activity in retinal homogenates can be reversed by the dopamine D1/D5 receptor antagonist (34). In the present study, we use biochemical approaches to directly demonstrate dopamine corelease from LC axons in the dorsal hippocampus.

One implication of our finding is that the peripheral signals that carry information about arousal, novelty, stress, and sleep-wake states may drive the release of dopamine in the dorsal hippocampus as well as other LC projection sites, such as the prefrontal cortex and amygdala. The current results further suggest that LC dopamine release may play a role in energizing spatial learning and memory by increasing attention to salient features of the environment. Activation of the LC-to-hippocampus pathway did not produce place preference in the absence of salient environmental objects, suggesting that photostimulation is not inherently reinforcing but may promote selective attention to relevant cues in the given setting. Finally, insofar as this work relates to attention and memory it may prove helpful for identifying therapeutic targets of dopamine- and norepinephrine-related disorders, such as ADHD.

Materials and Methods

Details on mice and procedures regarding optogenetics, behavioral paradigms, immunohistochemistry, and HPLC are detailed in *SI Materials and Methods*. Animal experiments were carried out following the guidelines of the Institutional Animal Care and Use Committee of Columbia University.

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