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Noradrenaline activation of hippocampal dopamine D_1 receptors promotes antidepressant effects

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Dopamine D_1 receptors (D_1Rs) in the hippocampal dentate gyrus (DG) are essential for antidepressant effects. However, the midbrain dopaminergic neurons, the major source of dopamine in the brain, only sparsely project to DG, suggesting possible activation of DG D_1Rs by endogenous substances other than dopamine. We have examined this possibility using electrophysiological and biochemical techniques and found robust activation of D_1Rs in mouse DG neurons by noradrenaline. Noradrenaline at the micromolar range potentiated synaptic transmission at the DG output and increased the phosphorylation of protein kinase A substrates in DG via activation of D_1Rs and β adrenergic receptors. Neuronal excitation preferentially enhanced noradrenaline-induced synaptic potentiation mediated by D_1Rs with minor effects on β-receptor–dependent potentiation. Increased voluntary exercise by wheel running also enhanced noradrenaline-induced, D_1R-mediated synaptic potentiation, suggesting a distinct functional role of the noradrenaline–D_1R signaling. We then examined the role of this signaling in antidepressant effects using mice exposed to chronic restraint stress. In the stressed mice, an antidepressant acting on the noradrenergic system induced a mature-to-immature change in the DG neuron phenotype, a previously proposed cellular substrate for antidepressant action. This effect was evident only in mice subjected to wheel running and blocked by a D_1R antagonist. These results suggest a critical role of noradrenaline-induced activation of D_1Rs in antidepressant effects in DG. Experience-dependent regulation of noradrenaline–D_1R signaling may determine responsiveness to antidepressant drugs in depressive disorders.

monoamine | dentate gyrus | mossy fiber | stress | exercise

Hippocampal dopamine D_1 receptors (D_1Rs) have been implicated in regulation of learning and memory (1, 2). Accumulating evidence also suggests a critical involvement of hippocampal D_1Rs, especially those expressed in the dentate gyrus (DG) neurons, in antidepressant action (3–7). D_1R activation induces robust synaptic potentiation at the synapse formed by the mossy fiber (MF) axons of the dentate granule cells (GCs) (8). Pharmacological and physical antidepressant treatments augment this D_1R-dependent synaptic potentiation (3, 4) and increase D_1R expression in the DG (4, 5). Furthermore, D_1R activation contributes to antidepressant-induced behavioral changes and neuronal plasticity in the DG (5). Midbrain dopaminergic neurons are the major source of brain dopamine and have been thought to be responsible for activation of dopamine receptors in the DG (9). However, projection of these dopaminergic neurons to the hippocampus including DG is relatively sparse (10–13). In addition, the dopamine content in the hippocampus is much lower than other monoamines (14–16). Therefore, dopamine may mediate only a part of dopamine-receptor–dependent hippocampal functions, and endogenous substances other than dopamine may also be involved.

Noradrenergic fibers from the locus coeruleus abundantly project to the hippocampus (13, 17). The noradrenaline content in the hippocampus is 10- to 100-fold higher than dopamine (14–16). Previous studies have shown that optogenetic manipulation of the noradrenergic projection to the hippocampus modulated performance of learning tasks in a D_1R-dependent manner (13, 18, 19). It is likely that noradrenaline can activate D_1Rs as an endogenous agonist, thereby regulating hippocampal functions. However, none of the previous studies focused on this possibility, because it is generally believed that noradrenaline activates D_1Rs at tens of micromolar concentrations (20), a range that may exceed physiological levels.

In the present study, we examined whether noradrenaline could modulate hippocampal neuronal signaling by activating D_1Rs using electrophysiological and biochemical techniques, with a particular focus on the dentate GCs and their MF synaptic output. We found that noradrenaline at the micromolar range causes robust changes in neuronal transmission in all hippocampal subregions examined and increases protein phosphorylation in the DG

Significance

Dopamine receptors in the brain are important targets for the therapeutic treatment of psychiatric disorders. Dopamine receptors are generally thought to be activated by dopamine. In the present study, however, we reveal that noradrenaline can also robustly activate dopamine D_1 receptors in the mouse hippocampus. Noradrenaline-activated D_1 receptor signaling was highly sensitive to the neuronal activity and experience of mice. Chronic stress and voluntary exercise synergistically augmented noradrenaline–D_1 receptor signaling. This augmented noradrenaline–D_1 receptor signaling promoted the induction of hippocampal neuronal plasticity by an antidepressant drug acting on the noradrenergic system. Our results suggest that noradrenaline–D_1 receptor signaling increases the efficacy of antidepressant treatment and therefore can be a unique therapeutic target for augmenting antidepressant medication.

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via D_1R activation. Notably, noradrenaline–D_1R signaling at the MF synapse was activated by submicromolar noradrenaline and substantially enhanced in an activity- or experience-dependent manner. A possible involvement of this noradrenaline–D_1R signaling in antidepressant effects was further examined. Antidepressant treatments can change the phenotype of mature dentate GCs into an immature-like state, a process characterized as "dematuration" of GCs (5, 21–24). We found that experience-dependent augmentation of noradrenaline–D_1R signaling promotes the induction of dematuration by a noradrenergic antidepressant. Our results suggest that D_1R signaling activated by noradrenaline plays a critical role in regulating the antidepressant efficacy.

Results

Noradrenaline Activates Hippocampal Dopamine D_1 Receptors at Micromolar Concentrations. Dopamine D_1Rs regulate synaptic transmission and neuronal excitability in the somatodendritic region and at the axonal output of the dentate GCs (8, 25). We first examined the effects of noradrenaline at the MF axon to CA3 pyramidal cell synapse (Fig. 1*A*). Dopamine potentiates MF synaptic transmission by activating presynaptic D_1Rs (4, 8). Previous studies have shown that noradrenaline had little effect on basal MF synaptic transmission in rat hippocampal slices (26) or inhibited synaptic transmission via α adrenergic receptors in hippocampal slice cultures (27). We found that noradrenaline (10 μM) potentiated excitatory postsynaptic potentials (EPSPs) at the MF synapse in mouse hippocampal slices, with only minor effects on presynaptic fiber volleys (Fig. 1 *B* and *C*). This synaptic potentiation was accompanied by a decrease in a presynaptic form of synaptic facilitation induced by repetitive stimulation (Fig. 1*D*), suggesting that noradrenaline potentiated MF synaptic transmission via presynaptic mechanisms. Noradrenaline-induced synaptic potentiation was partially blocked by an antagonist of β adrenergic receptors, but not of α adrenergic receptors (Fig. 1 *E* and *F*). Antagonists of dopamine D_1Rs also partially blocked noradrenaline-induced synaptic potentiation (Fig. 1 *E* and *F*). The D_1-antagonist–resistant potentiation was suppressed by the broad β receptor antagonist propranolol or the β_1-receptor–specific antagonist CGP20712 (Fig. 1 *G* and *H*), indicating that noradrenaline-induced potentiation is mediated by dopamine D_1 and β_1 adrenergic receptors. Noradrenaline had minimal effects at low micromolar concentrations (Fig. 1*C*). However, this is due to uptake of noradrenaline into noradrenergic nerve terminals in the hippocampus, because addition of the noradrenaline transporter inhibitor nisoxetine unveiled robust D_1R-dependent potentiation by low micromolar noradrenaline (Fig. 1*I*). These results revealed that noradrenaline can activate dopamine D_1Rs at the MF output of the GCs at the micromolar concentration range. As compared with noradrenaline, dopamine had larger potentiating effects on both EPSPs and fiber volleys (Fig. 1*J*).

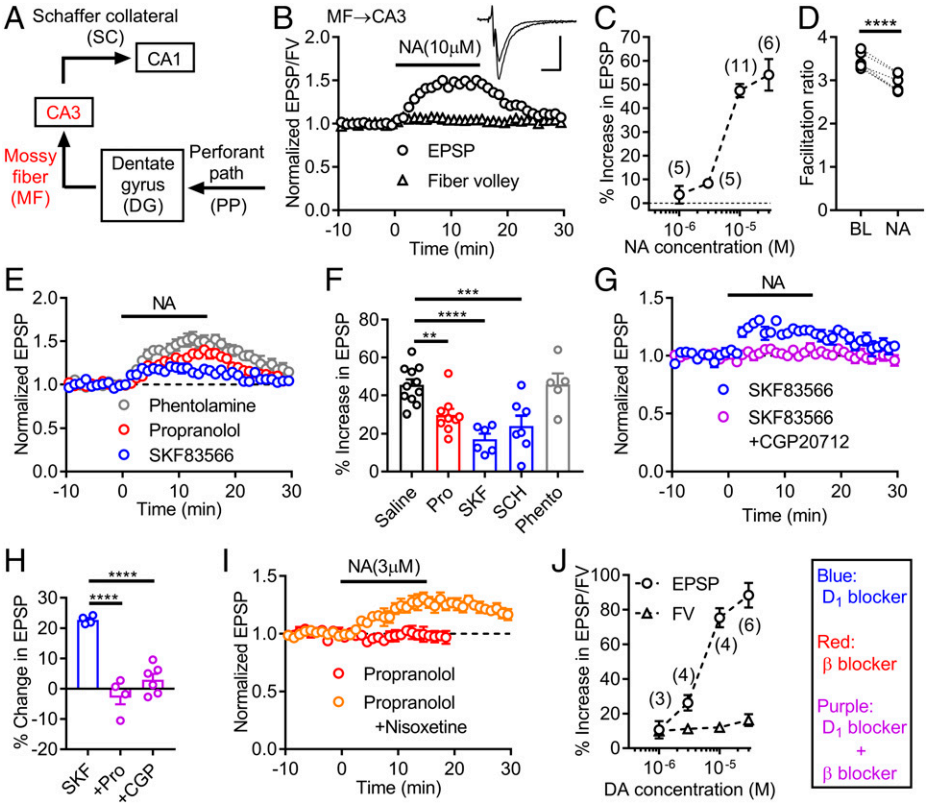

Fig. 1. Activation of D_1 receptors by noradrenaline potentiates mossy fiber synaptic transmission in the hippocampus. (*A*) Schematic diagram of hippocampal excitatory circuit highlighting mossy fiber (MF)–CA3 connection. (*B*) Effects of noradrenaline (NA) applied at the bar on presynaptic fiber volley (FV) and EPSPs at MF–CA3 synapse. Sample traces show averaged field potentials before and during NA application (scale bars: 10 ms, 0.2 mV). (*C*) Dependence of synaptic potentiation on NA concentrations. (*D*) Decrease in triple-pulse facilitation at 200-ms intervals by NA. BL: baseline. Paired *t* test (t_5 = 11.77, ****P < 0.0001). (*E*) NA-induced synaptic potentiation in D_1 antagonist SKF83566 (SKF) (200 nM), β antagonist propranolol (Pro) (10 μM) or α antagonist phentolamine (Phento) (20 μM). (*F*) Summary of antagonist effects on NA-induced synaptic potentiation. SCH: SCH23390 (D_1 antagonist, 50 nM). One-way ANOVA ($F_{4,33}$ = 10.2, P < 0.0001) followed by Dunnett's test (**P = 0.0085, ***P = 0.0009, ****P = 0.0001). (*G*) Block of SKF-resistant potentiation by β_1 antagonist CGP20712 (CGP) (100 nM). (*H*) Summary of β antagonist effects on SKF-resistant potentiation. One-way ANOVA ($F_{2,11}$ = 35.66, P < 0.0001) followed by Dunnett's test (****P = 0.0001). (*I*) D_1-receptor–dependent synaptic potentiation by low micromolar NA in presence (n = 6) and absence (n = 3) of nisoxetine (1 μM). (*J*) Dependence of FV and EPSP potentiation on dopamine (DA) concentrations. The number of data is shown in the graph in *C* and *J*. Data are presented as means ± SEM in all figures with or without individual values.

Since dopamine-induced potentiation at the MF synapse is almost exclusively mediated by D_1Rs (4, 8), dopamine is more effective in activating D_1Rs than noradrenaline (see also Fig. 3*F*).

We next examined the effects of noradrenaline at the somatodendritic region of the GCs. Population spikes (PSs) were evoked by stimulating the perforant path input to the DG (*SI Appendix*, Fig. S1*A*). Noradrenaline at 10 μM strongly potentiated PSs and had only small potentiating effects on EPSPs (*SI Appendix*, Fig. S1*B*). The β and α_1 antagonist labetalol suppressed potentiation of EPSPs and reduced PS potentiation (*SI Appendix*, Fig. S1 *C–F*). Propranolol had similar effects (*SI Appendix*, Fig. S1 *D* and *F*), confirming an involvement of β receptors. The D_1 antagonist SKF83566 applied alone had no significant effects (*SI Appendix*, Fig. S1 *C–F*). In the presence of labetalol, however, SKF83566 suppressed potentiation of PSs without affecting EPSPs (*SI Appendix*, Fig. S1 *C–F*). In the presence of SKF83566 and labetalol, noradrenaline caused inhibition of PSs that was suppressed by the α receptor antagonist phentolamine, and block of α and β receptors isolated D_1R-mediated potentiation of PSs that was abolished by D_1R antagonists (*SI Appendix*, Fig. S1 *C, D, G,* and *H*). The D_1R-mediated potentiation of PSs was observed along the longitudinal axis of the hippocampus with no clear differences between dorsal and ventral regions (*SI Appendix*, Fig. S1*I*). These results indicate that activation of D_1Rs by noradrenaline potentiates PSs in the GCs but has no detectable effects on EPSPs. In the CA1 region, noradrenaline potentiated PSs without affecting EPSPs (*SI Appendix*, Fig. S2 *A* and *B*). This PS potentiation was largely suppressed by the β antagonist (*SI Appendix*, Fig. S2 *C* and *D*). As in the DG, SKF83566 significantly suppressed potentiation of PSs only in the presence of the β antagonist, revealing α-receptor–dependent inhibition of PSs (*SI Appendix*, Fig. S2*D*). Isolated D_1R-dependent potentiation was detected in the presence of α and β receptor antagonists (*SI Appendix*, Fig. S2 *C* and *D*). Taken together, these results indicate that spike generation in both DG GCs and CA1 pyramidal cells is facilitated by noradrenaline via activation of D_1 and β receptors and inhibited via activation of α receptors. The intact β receptor functioning masked the effect of the D_1R antagonist on noradrenaline-induced potentiation of PSs, suggesting an overlap or redundancy in signaling pathways between D_1 and β receptors (see *Discussion*). In addition to the electrophysiological studies, we conducted a biochemical assay of D_1R activation by noradrenaline. Noradrenaline strongly increased the phosphorylation of downstream substrates of protein kinase A signaling, GluA1, DARPP32, and ERK2, in slices of the hippocampal DG, but not of the striatum (Fig. 2 *A, C,* and *E*). The increased phosphorylation in the DG was blocked by addition of propranolol and SKF83566 (Fig. 2 *B, D,* and *F*). However, neither propranolol nor SKF83566 alone had significant effects, again suggesting the overlap in the signaling pathway. Taken together, these results indicate that noradrenaline at the micromolar range activates dopamine D_1 receptors expressed in the hippocampus, but not in the striatum.

Activity-Dependent Augmentation of Noradrenaline–D_1R Signaling. A unique feature of D_1R signaling at the MF synapse is marked augmentation by neuronal excitation (4). To further characterize the D_1R signaling activated by noradrenaline, we next examined the effect of electroconvulsive treatment (ECT) that causes massive neuronal excitation and consistently augments dopamine-induced synaptic potentiation at the MF synapse (Fig. 3*F*) (4). In mice treated with ECT, noradrenaline-induced potentiation at the MF synapse was significantly augmented (Fig. 3 *A* and *B*). The reduction of synaptic facilitation by noradrenaline was also augmented by ECT, suggesting presynaptic mechanisms in the effect of ECT (Fig. 3*C*). Unexpectedly, ECT also changed the sensitivity to the antagonists. While the D_1R antagonist SKF83566 strongly reduced noradrenaline-induced potentiation in both control and ECT-treated mice, the β antagonist propranolol applied alone reduced the synaptic potentiation only in control mice (Fig. 3 *A* and *B*). In ECT-treated mice, however, propranolol suppressed the SKF83566-resistant potentiation (Fig. 3*E*), suggesting that the effect of propranolol in the absence of SKF83566 was masked by intact D_1R functioning. These results suggest that the β receptor signaling overlaps with a part of the D_1R signaling at the MF synapse only after ECT. In addition, the overlapping β receptor signaling was partly mediated by β_2 receptors (Fig. 3 *D* and *E*). Therefore, ECT reorganized D_1- and β-receptor–dependent signaling at the MF synapse. Since synthetic β receptor agonists potentiated the MF synaptic transmission via β_2 receptors in naive mice (*SI Appendix*, Fig. S3), ECT appeared to increase the sensitivity of β_2 receptors to noradrenaline. A comparison of the effects of noradrenaline and dopamine revealed that noradrenaline was more effective in activating D_1Rs after ECT, and there was an apparent increase in potency of noradrenaline (Fig. 3*F*). In the presence of the noradrenaline uptake inhibitor, even submicromolar noradrenaline was able to activate D_1Rs after ECT (Fig. 3*G*), excluding a possibility that altered noradrenaline uptake caused the apparent increase in potency. These results indicate that ECT facilitated noradrenaline–D_1R signaling at the MF synapse, possibly via the reorganization of the D_1 and β receptor signaling, rather than simply augmenting D_1R-dependent synaptic modulation. In the DG, ECT did not significantly increase D_1R-dependent potentiation of PSs, although it changed the time course of potentiation (*SI Appendix*, Fig. S4*A*). In CA1, ECT tended to reduce D_1R-dependent potentiation of PSs (*SI Appendix*, Fig. S4*B*). Therefore, the activity-dependent facilitation of the noradrenaline–D_1R signaling is specific to the MF system.

Interaction between D_1 and β Receptors. Above results showed the significant overlap between D_1 and β receptor signaling. G-protein–coupled receptors, including dopamine receptors, can form a heteromeric complex (28, 29). Heteromeric D_1 and β receptors may be a molecular basis for such overlap in signaling. To examine formation of D_1–β heteromers, we performed a coimmunoprecipitation assay (Fig. 4*A*). FLAG-tagged D_1Rs were coexpressed with HA-tagged β_1 or β_2 receptors in HEK293T cells. After immunoprecipitation with a FLAG antibody, blotting with an HA antibody detected β_1 and β_2 receptors coprecipitated with D_1Rs (Fig. 4*B*). Furthermore, interaction between native D_1 and β receptors was examined using whole hippocampal tissue lysates (Fig. 4*D*). After immunoprecipitation with a β_1 receptor antibody, a D_1R antibody detected coprecipitated D_1R in control and ECT-treated mice (Fig. 4*E* and *SI Appendix*, Fig. S5*B*). There was no clear difference in the D_1–β_1 coprecipitation between control and ECT-treated mice, while ECT increased D_1R expression levels (*SI Appendix*, Fig. S5*A*). These results suggest that D_1 and β receptors can form the heteromeric receptor complex. In HEK293T cells expressing D_1Rs alone, noradrenaline increased cAMP production at the micromolar range (Fig. 4*C*). Therefore, the complex formation is not required for noradrenaline responsiveness and may regulate efficacy and/or potency of D_1R activation by noradrenaline (see *Discussion*).

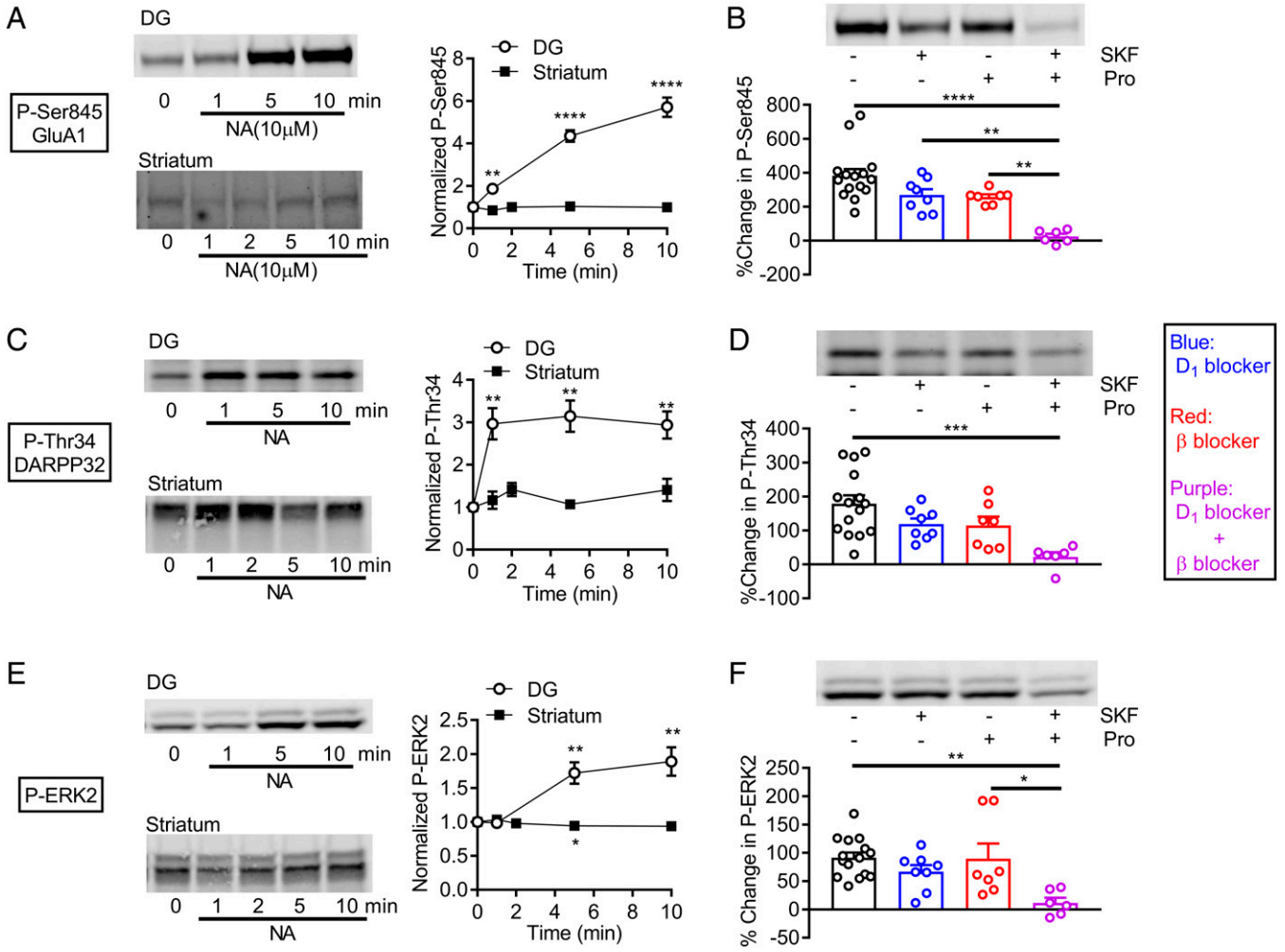

Fig. 2. D_1-receptor–dependent protein phosphorylation by noradrenaline in the dentate gyrus. (*A*) Effects of NA on GluA1 phosphorylation in dentate gyrus (DG) ($n = 7$ for each point) and striatum ($n = 4$). Typical immunoblots for detection of phospho-Ser845 (left) and quantified data (right). One-sample *t* test (**$P = 0.0019$, ****$P < 0.0001$, compared with 1). (*B*) Effects of SKF83566 (500 nM) and propranolol on NA-induced GluA1 phosphorylation. Typical immunoblots (top) and quantified data (bottom). Two-way ANOVA (SKF effect, $F_{1,32} = 19.28$, $P = 0.0001$; Pro effect, $F_{1,32} = 22.07$, $P < 0.0001$) followed by Tukey's test (**$P < 0.005$, ****$P < 0.0001$). (*C*) Effects of noradrenaline on DARPP32 phosphorylation (phospho-Thr34) in slices of DG and striatum. One-sample *t* test (**$P < 0.005$). DG: $n = 6$, striatum: $n = 4$. (*D*) Antagonist effects on NA-induced phosphorylation of DARPP32 in DG. Two-way ANOVA (SKF effect, $F_{1,32} = 8.549$, $P = 0.0063$; Pro effect, $F_{1,32} = 9.497$, $P = 0.0042$) followed by Tukey's test (***$P = 0.0006$). (*E*) Effects of noradrenaline on ERK2 phosphorylation in slices of DG and striatum. One-sample *t* test (*$P < 0.05$, **$P < 0.01$). DG: $n = 7$, striatum: $n = 4$. (*F*) Antagonist effects on NA-induced phosphorylation of ERK2 in DG. Two-way ANOVA (SKF effect, $F_{1,32} = 11.65$, $P = 0.0018$) followed by Tukey's test (*$P = 0.0123$, **$P = 0.0025$). Phospho-proteins were normalized to total proteins and then data were normalized to values obtained with time 0 (*A*, *C*, and *E*) or control without noradrenaline (*B*, *D*, and *F*).

Experience-Dependent Augmentation of Noradrenaline–D_1R Signaling Facilitates Antidepressant Effects. The noradrenergic system has been implicated in stress-induced alterations of the central nervous system and antidepressant effects (30), and D_1Rs in the dentate GCs contribute to antidepressant effects (5, 7). Finally, we examined an involvement of the noradrenaline–D_1R signaling in antidepressant effects, focusing on possible influence of stress on this signaling. Mice were subjected to 4 wk of restraint stress (Fig. 5*A*). Noradrenaline-induced MF synaptic potentiation mediated by D_1Rs was recorded in the presence of propranolol. Chronic restraint stress had no significant effect on this noradrenaline–D_1R signaling at the MF synapse (Fig. 5*C*). On the other hand, increased voluntary exercise by wheel running (Fig. 5*A*), which often causes neuronal changes opposite to those induced by stress and has antidepressant-like effects (31), significantly increased the noradrenaline–D_1R signaling (Fig. 5 *B* and *C*). Low micromolar noradrenaline was able to induce significant D_1R-dependent synaptic potentiation in mice subjected to wheel running (*SI Appendix*, Fig. S6*A*). Unexpectedly, chronic stress enhanced the effect of wheel running on the noradrenaline–D_1R signaling (Fig. 5 *B* and *C*). Chronic stress combined with wheel running enhanced the D_1R-antagonist–sensitive component of noradrenaline-induced synaptic potentiation (*SI Appendix*, Fig. S7), confirming the enhancing effect on the noradrenaline–D_1R signaling. The basal synaptic efficacy was not affected by chronic stress or wheel running (Fig. 5*D*). In addition, chronic stress combined with wheel running had no significant effect on serotonin 5-HT_4-receptor–dependent synaptic modulation that shares the intracellular signaling pathway with the D_1R-dependent modulation (32) (Fig. 5 *E* and *F*). These results indicate that chronic stress and voluntary exercise synergistically and specifically enhance the noradrenaline–D_1R signaling.

We then examined a contribution of this synergistic enhancement of the noradrenaline–D_1R signaling to dematuration of GCs, a proposed cellular substrate for antidepressant action (5, 21–24). Dematuration is characterized by altered expression of maturation stage markers and the appearance of immature-like functional properties in GCs. Prominent frequency facilitation at the MF synapse represents the functional maturity of GCs and is

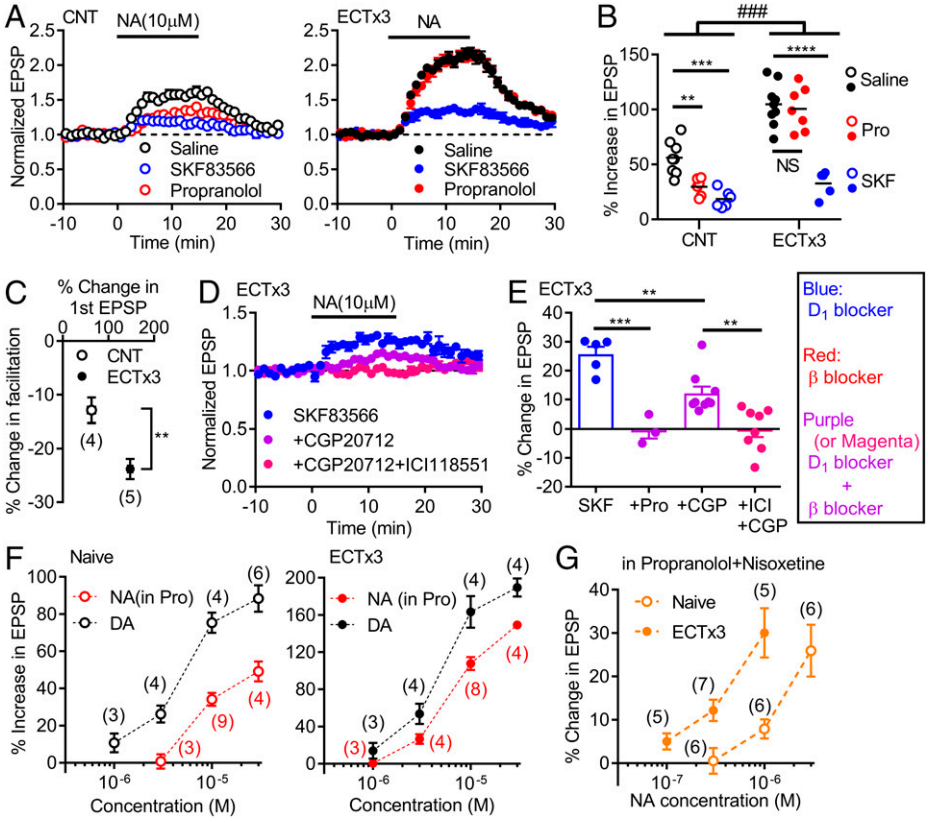

Fig. 3. Activity-dependent enhancement of noradrenaline–D_1 receptor signaling. (*A*) Effects of NA in control mice (CNT) and mice treated with three times of ECT (ECTx3) in normal saline or in antagonists. (*B*) Summary of data shown in *A*. Two-way ANOVA (ECT effect, $F_{1,35} = 83.22$, $P < 0.0001$; drug effect, $F_{2,35} = 42.98$, $P < 0.0001$; interaction, $F_{2,35} = 10.13$, $^{###}P = 0.0003$) followed by Tukey's test (NS: not significant, $^{**}P = 0.0083$, $^{***}P = 0.0002$, $^{****}P < 0.0001$). (*C*) Effects of ECTx3 on reduction of triple-pulse facilitation by NA ($t_7 = 3.644$, $^{**}P = 0.082$). (*D*) Effects of β receptor antagonists on SKF-resistant component of NA-induced potentiation in ECT-treated mice. (*E*) Summary of β antagonist effects on SKF-resistant component of NA-induced potentiation. CGP (100–200 nM), ICI: ICI118551 (β_2 antagonist, 100–200 nM). One-way ANOVA ($F_{3,21} = 17.37$, $P < 0.0001$) followed by Bonferroni's test ($^{**}P < 0.01$, $^{***}P = 0.0001$). (*F*) Dependence of D_1-mediated synaptic potentiation on NA and DA concentrations in naive and ECT-treated mice. The DA data in naive mice are the same as those in Fig. 1*J*. (*G*) Dependence of D_1-mediated potentiation on NA concentrations in nisoxetine. The number of data is shown in the graph in *C*, *F*, and *G*.

attenuated by serotonergic antidepressants (21, 22). We used the noradrenergic antidepressant desipramine, which acts as a highly potent noradrenaline reuptake inhibitor (33). Acute desipramine significantly augmented D_1R-dependent synaptic potentiation induced by low micromolar noradrenaline (*SI Appendix*, Fig. S6*B*). Chronic treatment with desipramine (Fig. 6*A*) did not change frequency facilitation when applied alone or in combination with wheel running (Fig. 6 *B* and *C*). In mice exposed to chronic restraint stress, desipramine alone had no significant effect. However, desipramine combined with wheel running significantly reduced frequency facilitation in stressed mice (Fig. 6 *B* and *C*). The reduction of facilitation was blocked by SKF83566 (Fig. 6*D*), suggesting an involvement of D_1Rs. Furthermore, we found that the expression level of the mature GC

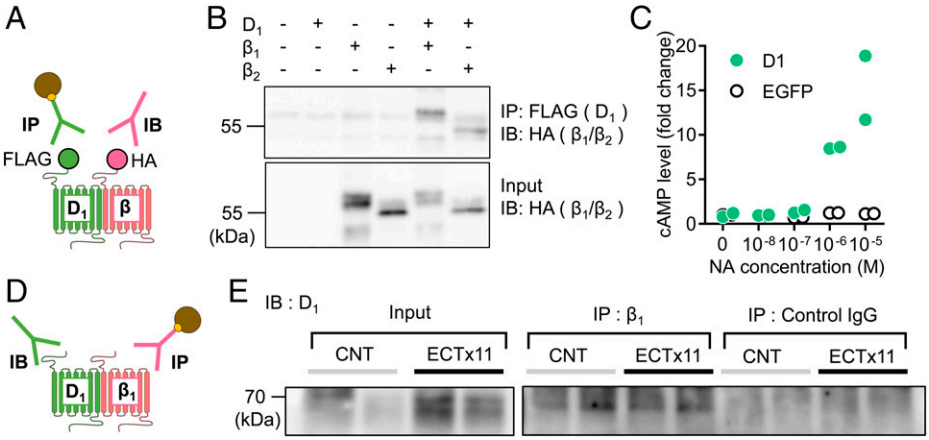

Fig. 4. Interaction between D_1 and β receptors. (*A*) Schematic diagram showing experimental design of coimmunoprecipitation assay using tagged receptors. IP: immunoprecipitation, IB: immunoblot. (*B*) Coimmunoprecipitation of FLAG-tagged D_1 and HA-tagged β_1 or β_2 receptors expressed in HEK293T cells. (*C*) NA-induced cAMP production in HEK293T cells expressing EGFP or D_1 receptors ($n = 2$ each). (*D*) Experimental design of coimmunoprecipitation assay of native receptors. (*E*) Coimmunoprecipitation of hippocampal D_1 and β_1 receptors in control and mice treated with 11 times of ECT (ECTx11). IgG: immunoglobulin G. Experiments were repeated three times with similar results (*SI Appendix*, Fig. S5*B*).

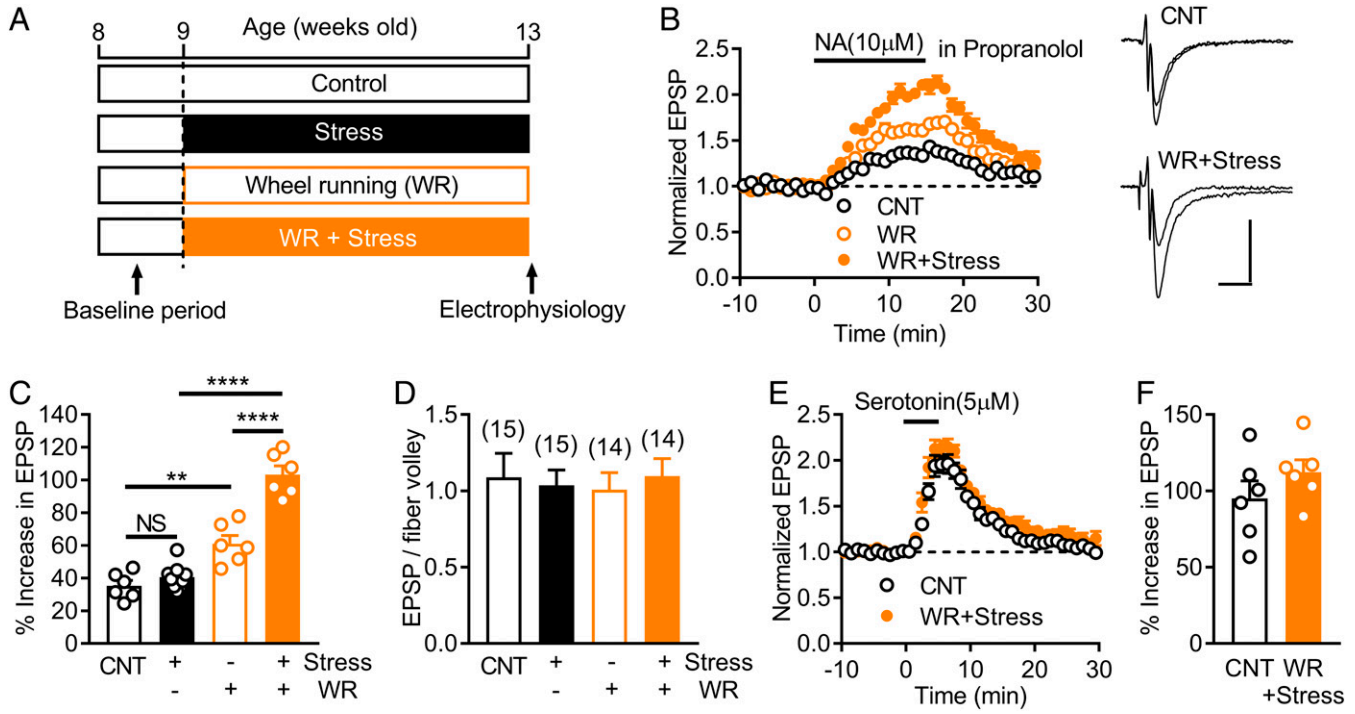

Fig. 5. Synergistic augmentation of noradrenaline–D_1 receptor signaling by chronic stress and exercise. (*A*) Timeline of chronic restraint stress and increased voluntary exercise by wheel running (WR). (*B*) Effects of chronic restraint stress and WR on NA-induced MF synaptic potentiation mediated by D_1 receptors. Recordings were made in the presence of propranolol. Sample traces show averaged field potentials before and during NA application (scale bars: 10 ms, 0.2 mV). (*C*) Summary of effects of stress and WR on D_1-mediated potentiation. Two-way ANOVA (stress effect, $F_{1,23} = 35.29$, $P < 0.0001$; WR effect, $F_{1,23} = 122.9$, $P < 0.0001$; interaction, $F_{1,23} = 21.36$, $P = 0.0001$) followed by Tukey's test (**$P = 0.0012$, ****$P < 0.0001$). (*D*) Lack of effects of stress and WR on the basal MF synaptic efficacy assessed by EPSP to fiber volley ratios. The number of data is shown in the graph. (*E*) Effects of stress and WR on serotonin-induced MF synaptic potentiation. (*F*) Summary of serotonin-induced potentiation.

marker calbindin was significantly reduced by desipramine combined with wheel running in stressed mice (Fig. 6*E*). In mice coadministered with SKF83566, the calbindin expression level was higher than that in mice treated with desipramine alone (Fig. 6*F*). These results suggest that the synergistic enhancement of the noradrenaline–D_1R signaling in stressed mice subjected to wheel running facilitated the induction of GC dematuration by the noradrenergic antidepressant. We also examined behavioral effects of desipramine in stressed mice. While chronic stress did not change anxiety- or depression-related behavior in commonly used behavioral paradigms (*SI Appendix*, Fig. S8), it significantly reduced voluntary activity in home cages (Fig. 6*G*). Desipramine combined with wheel running reversed the reduced activity in stressed mice, and SKF83566 blocked this effect (Fig. 6*H*), suggesting a beneficial effect of the desipramine treatment combined with wheel running on stress-induced behavioral impairment.

Discussion

We have revealed that noradrenaline can activate dopamine D_1Rs in the mouse hippocampus, but not in the striatum, at the submicromolar to micromolar concentration range. This noradrenaline–D_1R signaling was strongly augmented by neuronal excitation, a process that may include reorganization of D_1- and β-receptor–dependent signaling. Chronic stress and voluntary exercise synergistically enhanced the noradrenaline–D_1R signaling, thereby facilitating antidepressant-induced dematuration of DG neurons. Our results suggest that experience-dependent plasticity of the noradrenaline–D_1R signaling determines the responsiveness to antidepressant drugs.

Previous in vivo microdialysis studies have estimated that the basal extracellular noradrenaline concentration in the rodent hippocampus is 14–35 nM (34, 35). In general, transmitter concentrations near the release sites are much higher than the basal extracellular levels. While microdialysis studies estimated that the basal glutamate concentration in the hippocampus was 1–3 μM (36, 37), an electrophysiological study suggested that the peak glutamate concentration outside synapses could reach up to about 200 μM (38). Therefore, noradrenaline concentrations may reach micromolar levels around noradrenergic terminals. We showed that micromolar noradrenaline can activate D_1R in all hippocampus subregions examined. In the presence of intact noradrenaline uptake activity, low micromolar noradrenaline activated D_1Rs at the MF synapse in mice subjected to wheel running (*SI Appendix*, Fig. S6*A*), but not in naive mice (Fig. 1*I*), suggesting a critical influence of experience on the sensitivity of D_1Rs to noradrenaline. Our finding is consistent with the previous observations that the noradrenergic projection regulated performance of hippocampus-dependent learning tasks via D_1Rs (13, 18, 19). However, these studies favored an alternative interpretation that dopamine coreleased from the noradrenergic fibers activated D_1Rs. On the basis of the biosynthetic pathway of catecholamines, high-frequency firing of noradrenergic fibers is predicted to increase the vesicular content of dopamine relative to noradrenaline (19). The amount of dopamine released from optogenetically activated noradrenergic fibers projecting to the hippocampus is about 10% of noradrenaline after prolonged high-frequency firing (18). Therefore, assuming micromolar levels of noradrenaline as estimated above, hippocampal D_1Rs near noradrenergic terminals may be primarily activated by noradrenaline, although our results do not exclude the possibility of D_1R activation by coreleased dopamine (also see below).

The overlap or redundancy in D_1 and β receptor signaling was suggested by pharmacological experiments. We observed

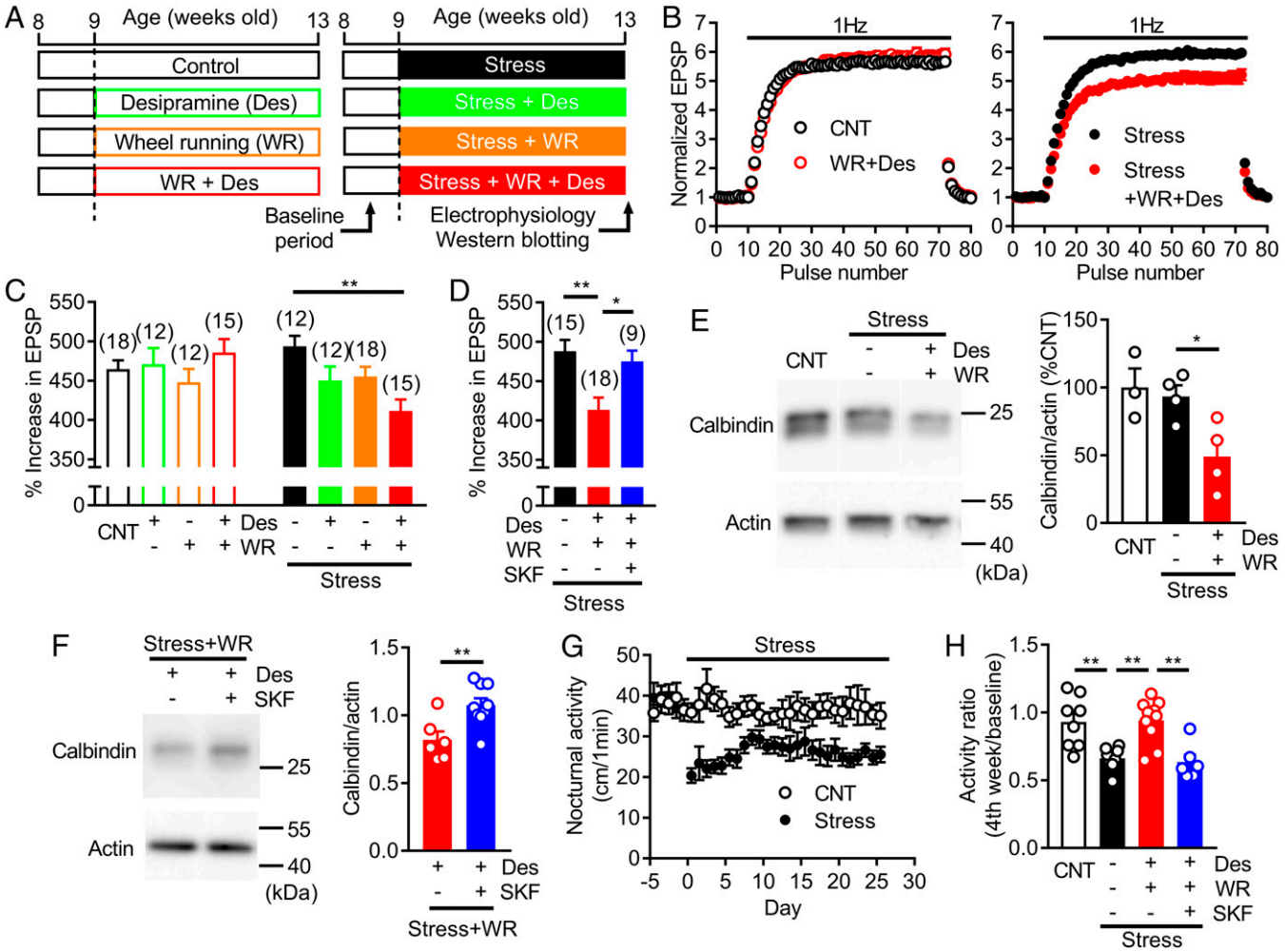

Fig. 6. Experience-dependent augmentation of noradrenaline–D_1 receptor signaling facilitates effects of noradrenergic antidepressant. (*A*) Timeline of restraint stress, WR, and desipramine (Des) treatment. (*B*) Effects of chronic Des (30 mg/kg/day for 4 wk) and WR on 1-Hz frequency facilitation at MF synapse in nonstressed (left) and stressed (right) mice. (*C*) Summary of effects of Des and WR on frequency facilitation in nonstressed and stressed mice. Two-way ANOVA (stress effect, $F_{1,106} = 1.757$, $P = 0.1879$; treatment effect, $F_{3,106} = 1.657$, $P = 0.1809$; interaction, $F_{3,106} = 4.424$, $P = 0.0057$) followed by Dunnett's test (**$P = 0.0011$). (*D*) Effects of SKF (1 mg/kg/day) on reduction of frequency facilitation caused by Des and WR in stressed mice. One-way ANOVA ($F_{2,39} = 7.508$, $P = 0.0017$) followed by Dunnett's test (*$P = 0.0247$, **$P = 0.0015$). (*E*) Effects of Des and WR on calbindin expression in DG. Typical immunoblots (left) and quantified data (right). One-way ANOVA ($F_{2,8} = 5.809$, $P = 0.0276$) followed by Dunnett's test (*$P = 0.0399$). (*F*) Effects of SKF on calbindin expression in DG of Des-treated mice ($t_{13} = 3.164$, **$P = 0.0075$). (*G*) Effects of chronic stress on nocturnal home cage activity. (*H*) D_1-receptor–dependent reversal of stress-induced decrease in home cage activity by Des and WR. One-way ANOVA ($F_{3,28} = 9.789$, $P = 0.0001$) followed by Bonferroni's test (**$P < 0.01$). The number of data is shown in the graph in *C* and *D*.

that the D_1R antagonist failed to cause significant effects in the absence of the β receptor antagonist or vice versa. Especially, at the MF synapse in ECT-treated mice, the effect of the β antagonist propranolol was detectable only after blocking D_1Rs (Fig. 3), suggesting that D_1R activation completely occluded the effect of β receptor activation. Both D_1 and β receptors are coupled with Gs–cAMP signaling pathways. It has been suggested that the cAMP signaling can be compartmentalized (39, 40). If D_1 and β receptors are localized close to each other, activation of either of them may saturate the local cAMP signaling, which could explain our results showing that blocking D_1 or β receptors alone had insignificant effects. Such colocalization can be best exemplified by the formation of heteromeric D_1 and β receptor complex. Indeed, our coimmunoprecipitation studies suggested the formation of the D_1–β receptor complex. As discussed below, we speculate that the formation of the heteromeric receptor complex is essential for regulation of the noradrenaline–D_1R signaling. At the MF synapse, the overlap of D_1 and β receptor signaling was seen in ECT-treated mice, but not in control mice (Fig. 3). ECT causes a marked increase in D_1R expression levels (4), which may be required to induce colocalization of D_1 and β receptors.

The sensitivity of D_1Rs to noradrenaline was different between brain regions. Micromolar noradrenaline caused robust activation of D_1Rs in the hippocampus, but not in the striatum. A bioluminescence resonance energy transfer assay showed that the ligand affinity of recombinant human D_1Rs depends on the subtype of Gα subunit. The D_1R coupled to Gαolf has a lower affinity for noradrenaline than that coupled to Gαs (41). Gαolf is the predominant subtype of Gα coupled with D_1Rs in the striatum (42), which may explain the failure in activation of striatal D_1Rs by micromolar noradrenaline. At the MF synapse, the D_1R sensitive to submicromolar noradrenaline was induced by ECT. ECT also produced the overlap between D_1 and β signaling and increased the sensitivity of β_2 receptors to noradrenaline. Heteromeric β_1–β_2 receptors show a higher affinity to synthetic agonists than homomeric β_1 receptors (29). Since we found that D_1Rs can interact with β_1 and β_2

receptors, a heteromeric complex including D_1 and β_2 or β_1–β_2 receptors is a candidate for the D_1R with the higher affinity for noradrenaline.

ECT augmented the noradrenaline-induced D_1R-dependent synaptic potentiation by about 200% at the maximal concentration of 30 μM, while dopamine-induced synaptic potentiation was increased by about 100% (Fig. 3*F*). Since D_1Rs solely mediate dopamine-induced potentiation at the MF synapse even after ECT (4), it is conceivable that ECT increased the efficacy of D_1R activation by noradrenaline. While ECT strongly up-regulates D_1R expression (*SI Appendix*, Fig. S5*A*) (4), an increase in the number of D_1Rs would equally enhance noradrenaline- and dopamine-induced potentiation. Therefore, we hypothesize that the reorganization of D_1 and β signaling, most probably the formation of the heteromeric D_1–β receptor complexes, underlies the increase in the efficacy as well as potency of D_1R activation by noradrenaline.

We observed coimmunoprecipitation of D_1 and β receptors in the hippocampal tissue lysates from both control and ECT-treated mice. Coimmunoprecipitation of these receptors in control mice supports the idea that the heteromeric receptor complex is the molecular basis for the overlap in the D_1 and β receptor signaling observed by electrophysiological and biochemical studies in naive mice (*SI Appendix*, Fig. S1 and Fig. 2). Although we hypothesized that ECT caused the reorganization of D_1 and β signaling at the MF synapse via formation of the heteromeric receptor complex, there was no obvious difference in the coimmunoprecipitation between control and ECT-treated mice. Since ECT did not significantly increase noradrenaline-induced potentiation of PSs mediated by D_1Rs in the DG and CA1 (*SI Appendix*, Fig. S4), the reorganization of D_1 and β signaling may be specifically induced at the MF system. An increase in heteromeric receptor complexes specific to the MF synapse, if any, may not be detected by coimmunoprecipitation in whole hippocampal tissue lysates.

Many lines of evidence suggest a critical role of the hippocampal DG in depression and antidepressant medication (7, 43, 44). We have previously demonstrated that antidepressant drugs and ECT induce immature-like phenotypes in the dentate GCs and at their output MF synapse and proposed this dematuration as the common neuronal mechanism underlying pharmacological and physical antidepressant treatments (5, 21–24). Although serotonergic antidepressants consistently induce GC dematuration in nonstressed mice (5, 21, 23, 45), the noradrenergic antidepressant desipramine failed to induce dematuration even when combined with exercise in nonstressed mice. This is probably related to lower functional levels of endogenous catecholamines at the MF system as compared with serotonin (46). Stress facilitated the induction of dematuration by desipramine, most likely via augmentation of the noradrenaline–D_1R signaling. Since desipramine inhibits uptake of dopamine as well as noradrenaline, D_1R activation by dopamine coreleased from noradrenergic fibers may also contribute to the induction of dematuration by desipramine. As described above, the amount of dopamine that can be released from noradrenergic fibers in the hippocampus is predicted to be at most 10% of noradrenaline. In the presence of desipramine, noradrenaline at 3 μM, a concentration that may be reached around noradrenergic terminals as estimated above, caused strong D_1R-dependent synaptic potentiation in mice subjected to stress and wheel running (*SI Appendix*, Fig. S6*B*). In the same condition, a 10-fold lower concentration of dopamine also induced robust potentiation, although it was smaller in magnitude than that by noradrenaline (*SI Appendix*, Fig. S6*B*). Therefore, while the noradrenaline–D_1R signaling is likely to play a major role in the antidepressant effect of desipramine, dopamine could also make a significant contribution, depending on its vesicular content. Since the dopamine transporter is expressed, if any, at a very low level in the hippocampus (47, 48), the noradrenaline transporter may be essential for clearance of dopamine released from the dopaminergic fibers in addition to the noradrenergic fibers. Indeed, systemic administration of the noradrenaline transporter inhibitor can raise extracellular dopamine levels in the hippocampus (49). However, the dopamine level is much less sensitive to the noradrenaline transporter inhibitor as compared with the extracellular noradrenaline level (50). Given sparse dopaminergic innervation to the DG and CA3 region (11–13), it is unlikely that dopamine solely mediates D_1R receptor activation involved in the effects of desipramine, although we cannot exclude this possibility. Dematuration by the serotonergic antidepressant fluoxetine requires 5-HT_4 receptors (21, 23), which are abundantly expressed in the GCs and along the MF tract (23, 51). Since both 5-HT_4 and D_1Rs are coupled with Gs, cAMP-dependent signaling is likely to be a common downstream pathway for the induction of dematuration. The noradrenaline–D_1R signaling was observed at the somatodendritic and axonal regions of GCs. At present, it is unknown whether somatodendritic or axonal D_1Rs are required for the induction of dematuration. Experience- or activity-dependent augmentation of noradrenaline–D_1R signaling was prominent at the MF synapse. The extent of fluoxetine-induced dematuration was significantly correlated with the magnitude of 5-HT_4-receptor–mediated potentiation at the MF synapse (21). Therefore, it is possible that axonal cAMP signaling plays a critical role in GC dematuration.

Although chronic restraint stress combined with wheel running enhanced the noradrenaline–D_1R signaling at the MF synapse, it did not significantly change 5-HT_4-receptor–dependent synaptic potentiation (Fig. 5). The exact mechanism underlying this selective enhancement is unknown. The dentate GCs show an acute increase in the c-fos expression, a measure of neuronal activity, after voluntary exercise (52, 53). The D_1R expression in the DG is highly sensitive to neuronal activity. While single ECT strongly up-regulates D_1R expression levels (4), repeated ECT does not significantly change 5-HT_4 receptor expression levels (46). This differential sensitivity to neuronal activity can explain the selective enhancement of the noradrenaline–D_1R signaling. In addition to up-regulation of the D_1R expression, neuronal activity could increase the efficacy of D_1R activation by noradrenaline as demonstrated in ECT-treated mice. Furthermore, chronic restraint stress may boost the activity-dependent up-regulation of the D_1R signaling via corticosterone (22), resulting in the marked enhancement of the noradrenaline–D_1R signaling by restraint stress combined with wheel running.

The central noradrenergic system has long been thought to be an important target of antidepressant drugs, although it is not precisely understood how the noradrenergic system is involved in the pathology of depression and antidepressant effects. Antidepressant drugs have been assumed to reverse dysfunction of monoaminergic systems caused by stress and/or other risk factors for depression (30, 54). However, our finding implies that antidepressant drugs make use of stress-induced adaptive changes in the noradrenaline–D_1R signaling to induce dematuration of GCs. Stress and antidepressant treatment can activate shared cellular responses that mediate resilience to stress (55, 56). The noradrenaline–D_1R signaling may play a role as

stress resilience that facilitates the antidepressant efficacy in depressive disorders. Activation of D_1Rs contributes to the effects of the serotonergic antidepressant fluoxetine in the DG (5), including GC dematuration and increased adult neurogenesis, another candidate cellular substrate for antidepressant action (44). Therefore, the noradrenaline–D_1R signaling may be commonly involved in the effects of different classes of antidepressant drugs. Chronic corticosterone treatment that mimics chronic stress exposure augments D_1R signaling at the MF synapse and concomitantly facilitates the induction of dematuration by fluoxetine (22). Chronic corticosterone also facilitates the fluoxetine-induced increase of adult neurogenesis in the DG (57), and antidepressant drugs more consistently increase adult neurogenesis in stressed animals than in naive animals (58). These lines of evidence, including the present study, suggest that antidepressant drugs activate the process of adaptive neuronal plasticity that is initiated by chronic stress rather than simply reversing stress-induced alterations in the brain. Our present finding suggests that the enhancement of the noradrenaline–D_1R signaling plays a pivotal role in linking stress with antidepressant-induced plasticity. The enhancement of the noradrenaline–D_1R signaling by stress was conditional, requiring increased voluntary activity. This synergistic experience-dependent regulation of the noradrenaline–D_1R signaling, possibly influenced by other unknown factors, may determine the antidepressant responsiveness, which suggests a unique target for therapeutic treatments of depressive disorders refractory to antidepressant medication.

Materials and Methods

Adult male C57BL/6J mice were used in all experiments. Animal use and procedures were approved by the Animal Care and Use Committee of Nippon Medical School, Tokyo University of Science, and Kurume University. Electrophysiological recordings were made using acute hippocampal slices (8, 46). In protein phosphorylation analyses, the regions of the dentate gyrus or the striatum were dissected from coronal brain slices (5, 59). Bilateral electroconvulsive treatment was administered to mice anesthetized with isoflurane (24). HEK293T cells transfected with plasmids carrying FLAG-tagged D_1 receptors (60) and/or HA-tagged β_1/β_2 receptors were used for cAMP measurements and receptor coimmunoprecipitation analyses. Mice were treated with oral desipramine, exposed to chronic restraint stress or wheel running, or subjected to combination of these treatments. These mice were used for electrophysiological experiments, home cage activity monitoring (61), and western blotting. For detailed methods, see *SI Appendix*.

Data, Materials, and Software Availability. All study data are included in the article and/or supporting information.

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