Gain in sensitivity and loss in temporal contrast of STDP by dopaminergic modulation at hippocampal synapses

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Spike-timing-dependent plasticity (STDP) is considered a physiologically relevant form of Hebbian learning. However, behavioral learning often involves action of reinforcement or reward signals such as dopamine. Here, we examined how dopamine influences the quantitative rule of STDP at glutamatergic synapses of hippocampal neurons. The presence of 20 μM dopamine during paired pre- and postsynaptic spiking activity expanded the effective time window for timing-dependent long-term potentiation (t-LTP) to at least −45 ms, and allowed normally ineffective weak stimuli with fewer spike pairs to induce significant t-LTP. Meanwhile, dopamine did not affect the degree of t-LTP induced by normal strong stimuli with spike timing (ST) of +10 ms. Such dopamine-dependent enhancement in the sensitivity of t-LTP was completely blocked by the D1-like dopamine receptor antagonist SCH23390, but not by the D2-like dopamine receptor antagonist sulpiride. Surprisingly, timing-dependent long-term depression (t-LTD) at negative ST was converted into t-LTP by dopamine treatment; this conversion was also blocked by SCH23390. In addition, t-LTP in the presence of dopamine was completely blocked by the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid, indicating that D1-like receptor-mediated modulation appears to act through the classical NMDA receptor-mediated signaling pathway that underlies STDP. These results provide a quantitative and mechanistic basis for a previously undescribed learning rule that depends on pre- and postsynaptic ST, as well as the global reward signal.

dopamine receptor | synaptic plasticity | learning | memory | reward

Activity-dependent synaptic plasticity through long-term potentiation (LTP) and long-term depression (LTD) is believed to serve as the cellular substrate for various forms of learning and memory (1, 2). For neurons experiencing spiking activity, it has been found that the direction of subsequent synaptic plasticity can be determined by near coincident pre- and postsynaptic firing: LTP is induced when presynaptic firing precedes postsynaptic spike, and paring in the converse order results in LTD (3, 4). Such spike-timing-dependent plasticity (STDP) has been widely used in modeling naturally occurring synaptic plasticity (5). Meanwhile, several lines of evidence show that the quantitative rule of STDP can vary across synapses in different brain areas, and even on the same dendrites, or axons (3, 4). Also, the STDP rule can be dynamically regulated by the activity of adjacent synapses (6) or by the activation of β-adrenergic receptors and M1 muscarinic cholinergic receptor (7, 8). Therefore, rather than being a stereotypic learning rule, it is clear that STDP is influenced or modulated by various intrinsic and external factors.

Behavioral learning often involves reward processes. In such learning, the activity of dopamine neurons has been shown to code for prediction error and uncertainty (9). Animal experiments have shown that blockade of dopamine receptors impairs learning and memory, and dopamine receptor agonists can improve learning and memory (10, 11). The hippocampus, a brain structure that receives extensive dopaminergic projections from the ventral tegmental area (VTA) and the substantia nigra (SN) (12, 13), has a key role in learning and memory (14, 15). It was found that the activation of D1-like dopamine receptors in the hippocampus facilitates the induction of classical LTP in the Schäffer collateral-CA1 pathway (16, 17), and inhibits depotentiation of recently potentiated synapses in CA1 area and dentate gyrus (18, 19). Blockade of D1-like dopamine receptors impairs late-phase LTP (20). The effects of dopamine on LTD in the hippocampus have been less clear. The activation of D1-like dopamine receptors was found to facilitate LTD in some experiments (16, 21), but reverse LTD in others (22). These results suggest that dopamine signaling system can modulate LTP and LTD, and that such modulation could potentially be involved in learning and memory.

Although the effects of dopamine on classical LTP and LTD have been extensively investigated in the hippocampus, much less is known about how dopamine might modulate the temporally specific rules of STDP in hippocampal synapses. In particular, it is not clear whether the magnitude, the sensitivity, or the temporal specificity of STDP is altered by dopamine. Answers to these questions are important for understanding how the reward system may interact with Hebbian mechanisms in behavioral learning and for implementing computational models of reinforcement learning. Recent studies suggest that in the striatum GABAergic medium spiny neurons exhibit different forms of dopamine-dependent STDP (23, 24). However, this important issue has not been examined in excitatory synapses on pyramidal neurons that are the most common in the brain. Here, we addressed this issue using cultured hippocampal neurons where quantitative rules of STDP have been established (25, 26). We found that dopamine enhanced the sensitivity of timing-dependent (t)-LTP induction by expanding the timing window and lowering the number of repetitive pairings required for effective induction. Also, activation of dopamine receptors converted t-LTD into t-LTP, and thus, dramatically altered the timing requirement for STDP. Pharmacological experiments suggested that these effects were mediated by D1-like dopamine receptors. Similar to STDP in the absence of dopamine, all forms of t-LTP in the presence of dopamine also required the activation of NMDA receptors, suggesting that dopamine signaling biased STDP mechanisms toward potentiation. These results reveal a new temporally specific learning rule, which is determined by the interaction among pre- and postsynaptic spike timing (ST) and the global reward signal.

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Results

Dopamine Extends the Timing Window for t-LTP. Experiments were carried out using cultured hippocampal neurons where STDP has been characterized (25, 26). Before investigating how dopamine modulates synaptic plasticity, we first tested the effect of dopamine on basal synaptic transmission. In previous experiments, it was found that high concentration (250 μM) of dopamine induced a late-phase potentiation in the CA1 area of hippocampus 1 h after application (27). Interestingly, at the Schäffer collateral-CA1 pathway, acute application of low concentration (1–10 μM), but not high concentration of dopamine (100 μM), reduced the AMPA receptor-mediated synaptic currents (28). In our experiments, 20 μM dopamine was applied along with 40 μM L-ascorbic acid that can partially attenuate the oxidation of dopamine to the bath solution. This concentration is commonly used for in vitro studies, and is comparable with the effective dopamine concentration in vivo (29). With ≈12–15 min of dopamine application (Fig. S1*A*), no significant effect was found on basal glutamatergic transmission either during wash in (0.94 ± 0.04, $P > 0.26$) or after wash out (0.97 ± 0.02, $P > 0.1$). However, NMDA receptor-mediated current was significantly reduced by 20 μM dopamine (0.90 ± 0.05, $P < 0.05$; Fig. S1*B*), and recovered after wash out (1.03 ± 0.04, $P > 0.48$; Fig. S1*B*), consistent with previous reports (28, 30).

To examine the effects of dopamine on STDP induction (see *Materials and Methods*), dopamine was washed in 2–3 min before repetitive pre- and postsynaptic spike parings, and washed out 2–3 min after the pairings. In the presence of dopamine, significant LTP was induced by 60 spike pairs with ST $\Delta t = +45$ ms (1.21 ± 0.03, $P < 0.01$ vs. unity, $P < 0.01$ vs. control; Fig. 1 *A*, *C*, and *F*), although 45 ms is beyond the normal timing window for LTP induction in hippocampal neurons (25). In contrast, no LTP was induced by the same pairing protocol in the absence of dopamine (1.02 ± 0.01, $P > 0.13$ vs. unity; Fig. 1 *B*, *C*, and *F*). However, for paring protocol with $\Delta t = +10$ ms that normally induces t-LTP (1.20 ± 0.05, $P < 0.01$ vs. unity; Fig. 1 *D* and *F*), dopamine had no significant effect on the magnitude of t-LTP induction (1.27 ± 0.04, $P < 0.05$ vs. unity, $P > 0.37$ vs. control; Fig. 1 *D* and *F*). Also, a longer timing interval ($\Delta t = +100$ ms) failed to induce LTP either under control conditions or in the presence of dopamine (control, 1.02 ± 0.01, $P > 0.20$ vs. unity; dopamine, 1.02 ± 0.02, $P > 0.27$ vs. unity, $P > 0.96$ vs. control; Fig. 1 *E* and *F*). Therefore, dopamine effectively extends the timing window for t-LTP induction from ≈20 ms (25) to at least 45 ms.

Dopamine Converts t-LTD into t-LTP. An important feature of classical STDP is its asymmetric ST window: spike pairing with positive timing result in t-LTP, whereas spike pairing with negative timing result in t-LTD. To test the effect of dopamine on t-LTD, we first used post-pre spike pairs ($\Delta t = -10$ ms) that normally induces significant synaptic depression (0.87 ± 0.02, $P < 0.01$ vs. unity; Fig. 2 *A*, *C*, and *F*). Surprisingly, in the presence of dopamine, the same spike pairing pattern resulted in significant potentiation (1.12 ± 0.04, $P < 0.05$ vs. unity, $P < 0.01$ vs. control; Fig. 2 *B*, *C*, and *F*). At longer intervals beyond the timing window for t-LTD under normal conditions (25), the presence of dopamine had no significant effect on the plasticity outcome [$\Delta t = -45$ ms: control, 0.98 ± 0.02, $P > 0.36$ vs. unity; dopamine, 1.04 ± 0.03, $P > 0.18$ vs. unity, $P > 0.08$ vs. control (Fig. 2 *D* and *F*); $\Delta t = -100$ ms: control, 0.99 ± 0.01, $P > 0.47$ vs. unity; dopamine, 1.0 ± 0.02, $P > 0.96$ vs. unity, $P > 0.73$ vs. control (Fig. 2 *E* and *F*)]. Therefore, dopamine allows spike pairs with negative timing to induce t-LTP rather than t-LTD. Together, these results reveal a bell-shape curve that contrasts dramatically with the canonical STDP window (Fig. 3).

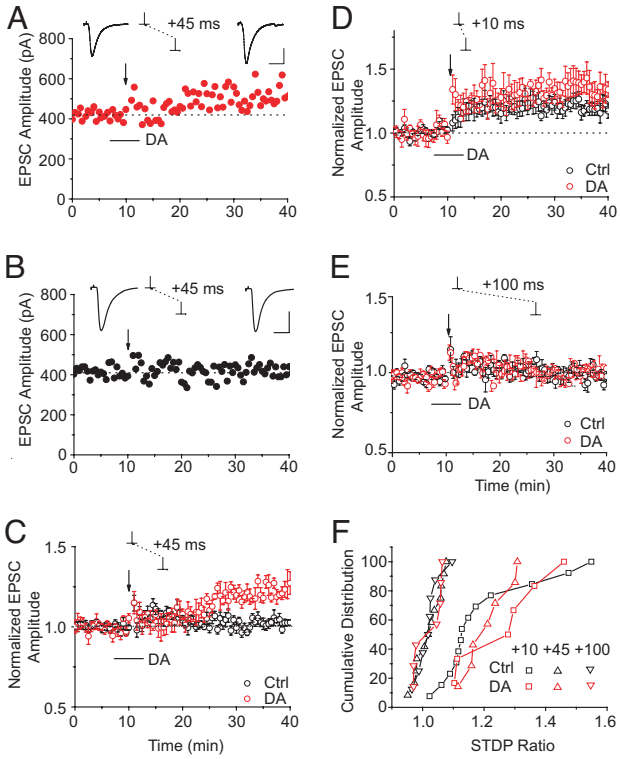

Fig. 1. Dopamine extended the timing window for t-LTP. (*A* and *B*) Results from 2 typical experiments with ST $\Delta t = +45$ ms in the presence (*A*) or absence (*B*) of dopamine. Data points shown are peak amplitudes from monosynaptic EPSCs elicited by test stimuli (0.03 Hz) before and after spike pairing. Insects show traces of EPSCs (average of 20 consecutive events) 0–10 min before (*Left*) and 20–30 min (*Right*) after STDP induction. (Scale bar: 200 pA, 10 ms.) (*C*) Summary of experiments in the presence (red, $n = 7$) or absence (black, $n = 12$) of dopamine showing the effects of dopamine on t-LTP induction at $\Delta t = +45$ ms. (*D* and *E*) Summary of experiments of t-LTP induction at $\Delta t = +10$ ms (*D*; control, $n = 13$, dopamine, $n = 6$) or $\Delta t = +100$ ms (*E*; control, $n = 8$, dopamine, $n = 7$). Either in the presence (red) or absence (black) of dopamine, reliable t-LTP was induced at $\Delta t = +10$ ms with or without dopamine (*D*), but not at $\Delta t = +100$ ms (*E*). (*F*) Cumulative histogram of all experiments described above. In the above panels, arrows indicate the time when the paring protocol was applied, black bars indicate the periods when the drug was present, DA indicates dopamine, Ctrl indicates control conditions, error bars are SEM.

Dopamine Reduces the Paring Repetition Threshold for t-LTP. Expanded timing window for t-LTP suggested that dopamine might have caused the neurons to be more sensitive to paired stimuli. We hypothesized that with dopamine, fewer spike pairs might be needed to induce t-LTP. To test this hypothesis, different number of repetitive spike pairs (at 1 Hz) were delivered to the pre- and postsynaptic neurons with $\Delta t = +10$ ms. In the presence of dopamine, significant t-LTP was reliably induced with 5 or 10 spike pairs; without dopamine, the same number of spike pairs failed to induce t-LTP [10 pairs: control, 1.03 ± 0.02, $P > 0.21$ vs. unity; dopamine, 1.20 ± 0.05, $P < 0.01$ vs. unity, $P < 0.01$ vs. control (Fig. 4 *A*–*C*); 5 pairs: control,1.02 ± 0.02, $P > 0.34$ vs. unity; dopamine, 1.09 ± 0.04, $P < 0.05$ vs. unity, $P < 0.05$ vs. control (Fig. 4*D*)]. The moderate t-LTP induced by 20 spike pairs was also significantly enhanced by dopamine (control, 1.10 ± 0.03, $P < 0.01$ vs. unity; dopamine, 1.21 ± 0.04, $P < 0.01$ vs. unity, $P < 0.05$ vs. control; Fig. 4*E*). However, more spike pairs in the presence of dopamine did not induce stronger t-LTP compared with control groups [60 spike pairs: control, 1.20 ± 0.05; dopamine, 1.27 ± 0.04, $P > 0.37$ vs. control (Fig. 3); 120 spike pairs: control, 1.17 ± 0.03, $P < 0.01$ vs. unity; dopamine, 1.18 ± 0.03, $P < 0.01$ vs. unity, $P > 0.83$ vs. control (Fig. 4*F*)]. In

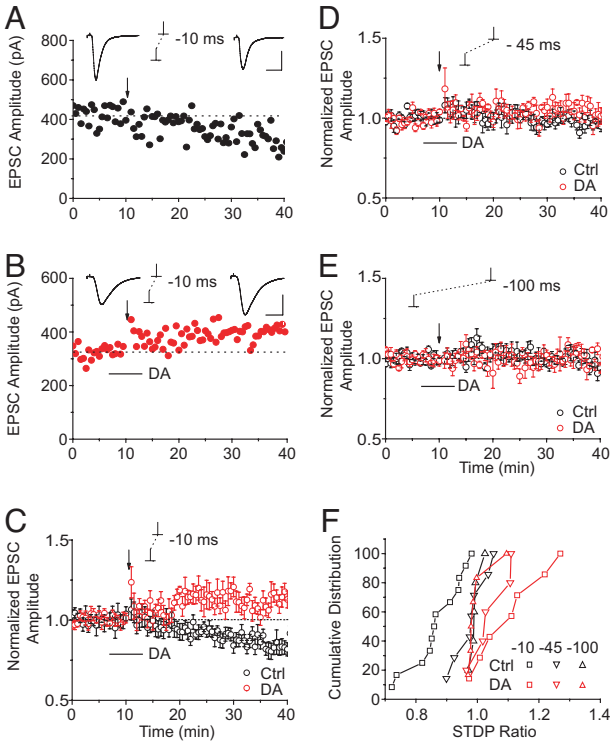

Fig. 2. Dopamine converted t-LTD into t-LTP. (*A* and *B*) Example experiments of t-LTD induction in the absence (*A*) and presence (*B*) of dopamine with $\Delta t = -10$ ms. (Scale bar, 200 pA, 10 ms.) (*C–E*) Summary of experiments of t-LTD induction in the presence (red) and absence (black) of dopamine with $\Delta t = -10$ ms (*C*; control, $n = 12$, dopamine, $n = 7$), $\Delta t = -45$ ms (*D*; control, $n = 5$, dopamine, $n = 5$), or $\Delta t = -100$ ms (*E*; control, $n = 5$, dopamine, $n = 6$). (*F*) Cumulative histogram of experiments with 60 spike pairs at different Δt.

summary, dopamine shifts the dependence of t-LTP on stimulation intensity such that fewer spike pairs are required to induce reliable LTP, which suggests an enhanced sensitivity in the LTP-mediating signaling mechanisms in the presence of dopamine (Fig. 4 *G* and *H*). Meanwhile, the saturating level of t-LTP induction is not altered by the presence of dopamine.

Modulation of STDP by Dopamine Is Mediated by D1-Like Receptors. Dopamine receptors are classified into 2 groups, D1-like receptors (D1/D5 receptors), which activate adenylate cyclase (AC), and D2-like receptors (D2/D3/D4 receptors), which inhibit AC (31). The activation of D1-like receptors has a central role in

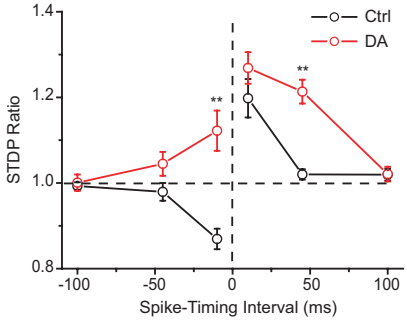

Fig. 3. Summary of STDP induction in the presence of dopamine showing altered ST window. Each data point is the averaged STDP ratio (mean ± SEM) for all experiments with a given Δt. **, significant difference ($P < 0.01$, Student's *t* test) in STDP induction between DA and corresponding control experiments.

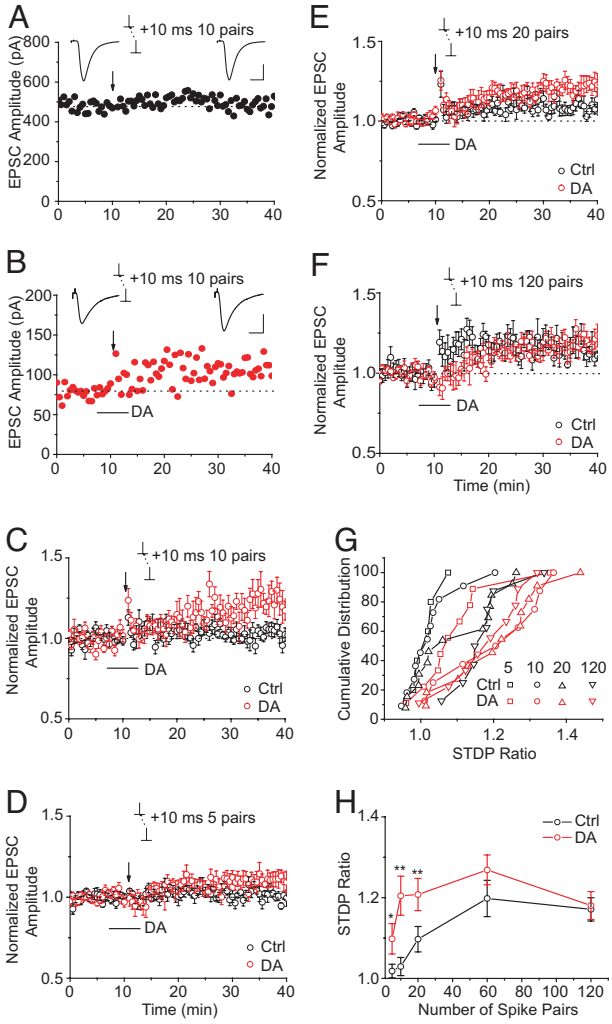

Fig. 4. Dopamine reduced the induction threshold for t-LTP. (*A* and *B*) Example experiments of t-LTP induction with 10 spike pairs in the absence (Scale bar: 200 pA, 10 ms.) (*A*); or presence of dopamine (Scale bar: 50 pA, 10 ms.) (*B*). (*C–F*) Summary of experiments of t-LTP induction with 10 (*C*; control, $n = 11$, dopamine, $n = 8$), 5 (*D*; control, $n = 5$, dopamine, $n = 9$), 20 (*E*; control, $n = 13$, dopamine, $n = 11$), and 120 (*F*; control, $n = 8$, dopamine, $n = 9$) spike pairs. (*G*) Cumulative histogram of experiments with different numbers of spike pairs. (*H*) Summary of averaged STDP ratios (mean ± SEM) for experiments with different numbers of spike pairs. * and **, significant difference ($P < 0.05$ for * and $P < 0.01$ for **, Student's *t* test) in STDP induction between DA and corresponding control experiments. For comparison, some data points were replotted here.

facilitating LTP induction and maintenance in many brain areas, such as the hippocampus (17), the basal ganglia (32), and the prefrontal cortex (33). To examine how D1-like dopamine receptors are involved in the observed modulation of STDP, we added the D1/D5 receptor antagonist SCH23390 to the perfusion solution 1 min before dopamine treatment; 10 μM SCH23390 blocked the dopamine-facilitated t-LTP induction with a longer prepost spike interval ($\Delta t = +45$ ms and 60 spike pairs, 1.02 ± 0.01, $P > 0.27$ vs. control, $P < 0.05$ vs. dopamine only; Fig. 5 *A* and *D*) or with fewer spike pairs ($\Delta t = +10$ ms and 10 spike pairs, 1.03 ± 0.03, $P > 0.73$ vs. control, $P < 0.05$ vs. dopamine only; Fig. 5 *B* and *D*). Also, SCH23390 rescued the t-LTD ($\Delta t = -10$ ms and 60 spike pairs) that was converted into t-LTP by dopamine (0.90 ± 0.04, $P < 0.05$ vs. unity, $P > 0.52$ vs. control, $P < 0.01$ vs. dopamine only; Fig. 5 *C* and *D*). To exclude the possibility that SCH23390 might directly block t-LTP induc-

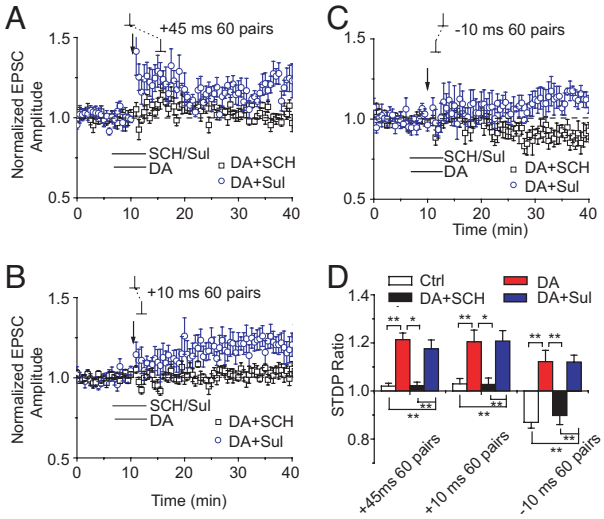

Fig. 5. Modulation of STDP by dopamine was mediated by D1-like receptors. (*A* and *B*) Effects of SCH23390 (SCH; black) and sulpiride (Sul; blue) on dopamine facilitated t-LTP with 60 spike pairs at $\Delta t = +45$ ms (*A*; SCH, $n = 6$, Sul, $n = 6$) and with 10 spike pairs at $\Delta t = +10$ ms (*B*; SCH, $n = 6$, Sul, $n = 8$). Facilitated t-LTP was blocked by SCH23390, but not by sulpiride. (*C*) Effects of SCH23390 and sulpiride on dopamine reverted t-LTP with 60 spike pairs at $\Delta t = -10$ ms (SCH, $n = 5$, Sul, $n = 6$). Timing-dependent LTD was rescued by SCH23390, but not by sulpiride. (*D*) Summary of the above experiments with control and DA data replotted for comparison. *, $P < 0.05$ and **, $P < 0.01$ (Student's *t* test).

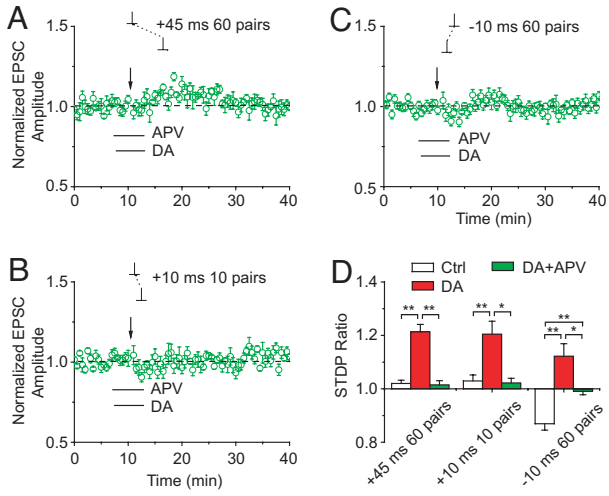

Fig. 6. Modulation of STDP by dopamine required the activation of NMDA receptors. (*A–C*) Effects of APV on dopamine facilitated t-LTP induction with 60 spike pairs at $\Delta t = +45$ ms (*A*; $n = 4$), 10 pairs at $\Delta t = +10$ ms (*B*; $n = 5$) or 60 pairs at $\Delta t = -10$ ms (*C*; $n = 7$). (*D*) Summary of the above experiments with control and DA data replotted for comparison.

tion rather than blocking the modulatory effect of dopamine, SCH23390 was added alone when neurons were stimulated with 60 spike pairs at $\Delta t = +10$ ms. Under this condition, t-LTP induction in SCH23390 alone was not different from the control group (1.17 ± 0.04, $P < 0.01$ vs. unity, $P > 0.13$ vs. control; Fig. S2).

To determine the role of D2-like receptors in STDP modulation, we tested the effect of D2-like receptor selective antagonist sulpiride (50 μM) on dopamine-facilitated t-LTP induced by 60 spike pairs at $\Delta t = +45$ ms (1.18 ± 0.04, $P < 0.01$ vs. control, $P > 0.41$ vs. dopamine only; Fig. 5 *A* and *D*), by 10 spike pairs at $\Delta t = +10$ ms (1.21 ± 0.04, $P < 0.01$ vs. control, $P > 0.88$ vs. dopamine only; Fig. 5 *B* and *D*) and by 60 spike pairs at $\Delta t = -10$ ms (1.12 ± 0.03, $P < 0.01$ vs. control, $P > 0.97$ vs. dopamine only; Fig. 5 *C* and *D*). No significant change in dopamine-facilitated t-LTP was observed. Also, sulpiride alone did not facilitate t-LTP induction by 10 spike pairs at $\Delta t = 10$ ms (0.97 ± 0.02, $n = 5$, $P > 0.09$ vs. control, $P < 0.01$ vs. dopamine only). Together, these data suggested that the modulatory effects of dopamine on STDP were mediated by D1-like, but not D2-like receptors (Fig. 5*D*).

Dopamine-Facilitated t-LTP Is NMDA Receptor-Dependent. Our previous work showed that STDP in cultured hippocampal neurons, as in conventional forms of LTP and LTD (2), requires functional NMDA receptors (25, 34). The effects of dopamine on STDP could be mediated by modulation of NMDA receptor-dependent mechanisms or, alternatively, could be achieved by invoking parallel pathways that target downstream expression mechanisms. To test these hypotheses, 25 μM of NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (APV) was added 1 min before the addition of dopamine for 3 induction conditions that led to dopamine-facilitated t-LTP or dopamine-converted t-LTP (from t-LTD). In all 3 types of experiments, APV completely blocked any significant synaptic modification [$\Delta t = +45$ ms and 60 pairs, 1.02 ± 0.02, $P > 0.42$ vs. unity, $P < 0.01$ vs. dopamine only (Fig. 6 *A* and *D*); $\Delta t = +10$ ms and 10 pairs, 1.02 ± 0.02, $P > 0.32$ vs. unity, $P < 0.05$ vs. dopamine only (Fig. 6 *B* and *D*); $\Delta t = -10$ ms and 60 pairs, 0.99 ± 0.01, $P > 0.52$ vs. unity, $P < 0.01$ vs. control, $P < 0.05$ vs. dopamine only (Fig. 6 *C* and *D*)]. Therefore, it is likely that dopamine exerts its effects on STDP by modulating rather than bypassing the classical NMDA-receptor-dependent signaling pathways.

Discussion

STDP has been demonstrated in a wide range of systems and experimental preparations (3, 4). Because of its requirement for naturally occurring spiking activity of neurons, STDP is likely to represent a physiologically relevant form of synaptic plasticity (3, 5). The quantitative rules of STDP, especially its asymmetric ST window, have been implemented in many theoretical models (35, 36). However, our results indicate that such a quantitative rule for a given synaptic connection can rapidly change into a dramatically different form under the influence of neuromodulator dopamine. Besides the substantial gain in the sensitivity of t-LTP, dopamine also causes the inversion of t-LTD into t-LTP, thus, the loss of the temporal contrast distinct of STDP. These results imply a previously unknown intricacy in the cellular signaling mechanisms that underlie STDP.

Substantial amounts of experimental evidence have shown that D1-like receptors are critical for conventional LTP in the hippocampus. Blockade of D1-like receptors results in the impairment of LTP, whereas activation of D1-like receptors facilitates LTP (18–20). Recent work shows that the activation of different classes of dopamine receptors enables bidirectional STDP in the GABAergic medium spiny neurons in the striatum (23, 24). However, our experiments suggested that in glutamatergic hippocampal synapses, the activation of D1-like receptors not only facilitated the existing t-LTP induction by lowering the induction threshold and broadening the effective positive ST window, but also inverted t-LTD induced by negative timing. Notably, it was observed that pulsatile dopamine application caused reversal of LTD induced by pairing cortical stimuli with postsynaptic depolarization in corticostriatal synapses on medium-sized spiny neurons (37), indicating potential common signaling mechanisms. Meanwhile, the magnitude of t-LTP induced with normal strong stimuli (e.g., $\Delta t = +10$ ms and 60 pairs) was not enhanced by the presence of dopamine. Also, in contrast to other previous results found in rats (16), blockade of D1-like receptors in our experiment did not affect normal induction of synaptic plas-

ticity. The discrepancy could be due to the difference in induction protocols, or could reflect an adaptation process of cellular signaling underlying STDP in cultured neurons that have been chronically deprived from dopamine inputs. Notably, even in similar hippocampal slices, it has remained controversial whether D1-like receptor activation facilitates or blocks LTD induction (16, 21, 22). Thus, in a sense, cultured neurons provide us an opportunity for dissecting cellular mechanisms without the complexity caused by unidentified modulatory components of native circuits.

How does D1-like receptor signaling influence the outcome of STDP? It has long been known that NMDA receptors are crucial for many forms of synaptic plasticity including STDP (1, 2). Indeed, in our system, NMDA receptor-mediated synaptic currents were partially inhibited by dopamine (Fig. S1*B*), similar to previous findings (28, 30). It is commonly believed that downstream of NMDA receptor activation, the level of the transient increase in intracellular calcium determines the outcome of synaptic plasticity: A transient high increase in calcium leads to LTP, whereas a transient low increase in calcium leads to LTD (38). From this classical picture, one would expect that inhibition of NMDA receptor-mediated currents by dopamine would cause less calcium influx and, thus, attenuating t-LTP while favoring t-LTD. However, rather than attenuating t-LTP, we observed facilitated t-LTP induction in the presence of dopamine, and even inversion of t-LTD into t-LTP. Therefore, the effects of dopamine on STDP were unlikely to be due to its partial inhibition of NMDA receptor function. Meanwhile, we found that NMDA receptors were required for the modulated STDP that was abolished by NMDA receptor antagonist APV. Therefore, it is likely that dopamine exerted its action on an NMDA receptor-mediated signaling pathway rather than bypassing that signaling pathway.

It has been suggested that, in addition to the magnitude, the time course of calcium influx is also important for STDP (39). Consistent with this idea, STDP rules can be altered by manipulating the shape and their back propagation of dendritic action potentials (40, 41). Also, it is well known that dopamine can modulate calcium channels, potassium channels, and even calcium release from intracellular stores (31, 42–44). Indeed, it has been observed that dopamine increased the propagation of dendritic action potential in a subpopulation of hippocampal neurons (45). More direct experiments, such as calcium imaging, are needed to delineate whether and how changes in dynamics of intracellular calcium are responsible for the modulation of STDP by dopamine.

D1-like receptors are known to activate AC (31) and subsequently protein kinase A, which is known to have an important role in synaptic potentiation (46). Also, dopamine- and cAMP-regulated phosphoprotein (DARPP)-32, is activated via D1-like receptors activation, and DARRP-32 is known to potently inhibit protein phosphatase 1 (PP1) (47), and thus, promotes activation of CaMKII (48). Previous studies have suggested that the induction of STDP involves modular competition between the CaMKII-mediated t-LTP signal and the calcineurin/PP1-mediated t-LTD signal (26, 34, 39, 49). Therefore, it is possible that through the action of D1-like receptors, dopamine application biases the modular competition toward LTP; thus, causing the enhancement of t-LTP, as well as the inversion of t-LTD.

Alternatively, STDP could be the sum of rather independent LTP and LTD components (49), and that dopamine exclusively blocks the LTD component. This hypothesis is especially appealing if these 2 components are expressed independently at different loci. For example, visual cortical synapses exhibit postsynaptic t-LTP and presynaptic t-LTD, probably through the interaction between presynaptic NMDA receptor signaling and retrograde endocannabinoid signaling (50, 51). In hippocampal neurons, we have previously found that t-LTD does not require endocannabinoid signaling, and that STDP signaling is likely to involve nonlinear modular competition before the final expression (34, 39). However, we cannot exclude potential involvements of presynaptic NMDA receptors, and the possibility that dopamine interferes with the expression of depression thereby unmasking potentiation. The intricate interactions among these, as well as other cellular signals underlying STDP and their modulation by dopamine remain to be explored.

Last, at the systems level, an important fact is that learning can happen in a very brief period, indicating that relevant synaptic modifications should be very sensitive to input stimuli. Meanwhile, the external environment, as well as the internal neuronal activity, is very noisy; thus, requiring the rules of synaptic modification to be highly selective. Our results suggest that under the influence of dopamine, the STDP rule can take a very different form with substantially enhanced sensitivity for potentiation and diminished temporal contrast. This effect implies a new form of plasticity rule, in which the plasticity outcome is determined by 3 input components: the local pre- and postsynaptic STs and the global reward signal dopamine. Under this 3-component rule, the learning rate for specific events that trigger the activation of dopamine inputs will be selectively enhanced; thus, allowing for both reliable and efficient encoding of relevant information into memory engrams (52). Indeed, various rules of modulated Hebbian learning have been suggested and implemented in computational models (53, 54). Although more experiments are needed to fully characterize the modulatory effects of dopamine, as well as other neuromodulators on STDP, we can expect that the implementation of such multicomponent learning rules will enhance the computational power of neural network models. Also, quantitative studies of how dopamine and other modulators affect synaptic plasticity could lead to new insights into neural circuit functions in learning and memory, as well as related brain disorders such as schizophrenia and drug addiction (55, 56).

Materials and Methods

Cell Culture. Low-density cultures of dissociated embryonic rat hippocampal neurons were prepared according to a previously described protocol (25), which was approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Hippocampi were removed from embryonic day 18 rats, and were treated with trypsin for 15 min at 37 °C, followed by washing and gentle trituration. The dissociated cells were plated on poly-L-lysine coated glass coverslips in 35-mm Petri dishes with 30,000–60,000 cells per dish. The culture medium was DMEM (BioWhittaker) supplemented with 10% heat inactivated bovine calf serum (HyClone), 10% Ham's F12 with glutamine (BioWhittaker), 50 units/mL penicillin-streptomycin (Sigma), and 1× B-27 (Invitrogene/Gibco). One-third culture medium was replaced with the same medium supplemented with 20 mM KCl 24 h after plating. At 9–15 days in vitro, pairs of neurons with glutamatergic connection of 50–500 pA were selected for experiment.

Electrophysiologic Recordings. Double perforated whole-cell recording were carried out with patch clamp amplifiers (Axon 700A; Axon Instruments) at room temperature. The pipette solution contains: 136.5 mM K-gluconate/17.5 mM KCl/9 mM NaCl/1 mM $MgCl_2$/10 mM Hepes/0.2 mM EGTA/200 μg/mL amphotericin B, pH 7.3. The external bath solution was a Hepes-buffered saline (HBS): 150 mM NaCl/3 mM KCl/3 mM $CaCl_2$/2 mM $MgCl_2$/10 mM Hepes/5 mM glucose, pH 7.4. To record NMDA current, the pipette solution contained 136.5 mM CsOH, 17.5 mM CsCl, 9 mM NaCl, 1 mM $MgCl_2$, 10 mM Hepes, and 0.2 mM EGTA (pH 7.3). The external bath solution for recording of NMDA current was Mg^{2+}-free HBS with 10 μM CNQX and 10 μM glycine. Dopamine hydrochloride, R(+)−SCH23390, (S)−(−)−sulpiride, L-ascorbic acid, CNQX, glycine, and APV were purchased from Sigma. All drugs were prepared in DMSO or water, and then diluted (1:1,000) in external bath solution when being used. Throughout the experiment, the culture was perfused with fresh bath solution at a constant rate of 1 mL/minute. Signals were filtered at 5 kHz, and sampled at 10 kHz by a 16-bit digital board (PCI-6035, National Instruments) interfaced with custom program based on Igor Pro (WaveMetrics). The pipette resistance was ≈2 MΩ. Series resistance (20–40 MΩ) and input impendence (300–500 MΩ) were monitored by a hyperpolarizing pulse (5 mV, 10 ms). Data were accepted for analysis only in the case that series resistance and input impedance did not change >10% throughout the experiment. Trials

showing significant run-up or run-down during the control period (>5% in 10 min) were also excluded.

To minimize the complication of connectivity with other neurons that were not monitored by recording, we examined only pairs of neurons that grew on isolated glial island. Neighboring neurons that may have connections to the pair were removed with a suction pipette. Only monosynaptic connections between 2 glutamatergic neurons were studied, polysynaptic connections were identified based on the latency of evoked postsynaptic current (EPSC) (>5 ms) and were excluded.

Pre- and postsynaptic neurons were both voltage clamped at −70 mV in the experiment. Test stimuli were delivered every 30 s with brief step depolarization of presynaptic neurons (100 mV, 1–2 ms). During STDP induction, the postsynaptic neuron was current clamped, and stimulation was 1–2 nA current injection for 2 ms, sufficient to induce a spike. A stable 10-min baseline of synaptic response was first obtained before the application of an STDP induction protocol consisting of pre- and postsynaptic spike pairs at 1 Hz. ST Δt was defined as the time interval between the presynaptic spike and the postsynaptic spike. Dopamine was washed in 2–3 min before repetitive pre- and postsynaptic spike pairings, and washed out 2–3 min after the pairings. SCH23390, sulpiride or APV were washed in 1 min before the dopamine treatment, and washed out 2–3 min after the spike pairings.

STDP ratio was calculated from the averaged EPSC amplitude from 0–10 min before and between 15–30 min after the stimulation paradigm. Comparisons were made using unpaired student's t test for means. Values were reported as means ± SEM.

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