# Uncoupling the D1-*N*-Methyl-D-Aspartate (NMDA) Receptor Complex Promotes NMDA-Dependent Long-Term Potentiation and Working Memory

Qiang Nai, Shupeng Li, Szu-Han Wang, Jing Liu, Frank J.S. Lee, Paul W. Frankland, and Fang Liu

**Background:** Although dopamine D1 receptors are involved in working memory, how D1 receptors contribute to this process remains unclear. Numerous studies have shown that D1 receptors have extensive functional interaction with *N*-methyl-D-aspartate (NMDA) receptor. Our group previously demonstrated that D1 receptors were able to regulate NMDA receptor functions through direct protein–protein interactions involving the carboxyl terminals of D1 receptors and NMDA receptor NR1a and NR2A subunits respectively. In this study, we explored the effects of the D1–NR1 interaction on NMDA receptor-dependent long-term potentiation (LTP) and working memory by using the TAT-conjugated interfering peptide (TAT-D1-t2).

**Methods:** Miniature excitatory postsynaptic currents are recorded in rat hippocampal primary cultures. Coimmunoprecipitation and calcium/calmodulin-dependent protein kinase II (CaMKII) activity are measured in hippocampal slices and hippocampal neurons under the specified experimental conditions, respectively. Working memory was assessed using a delayed match-to-place protocol in the Morris Water Maze following administration of the TAT-D1-t2 peptide.

**Results:** Electrophysiology experiments showed that activation of D1 receptor upregulates NMDA receptor-mediated LTP in a CaMKII-dependent manner. Furthermore, D1 receptor agonist stimulation promotes the NR1-CaMKII coupling and enhances the CaMKII activity; and the D1 receptor-mediated effects can be blocked by the application of the TAT-D1-t2 peptide. Interestingly, animals injected with TAT-D1-t2 peptide exhibited significantly impaired working memory.

**Conclusions:** Our study showed a critical role of NMDA-D1 direct protein–protein interaction in NMDA receptor–mediated LTP and working memory and implicated the involvement of CaMKII in this process.

**Key Words:** CaMKII, LTP, protein–protein interaction, working memory

Both dopamine D1 receptors (D1R) and glutamate *N*-methyl-D-aspartate (NMDA) receptors (NMDAR) are involved in the induction and expression of long-term potentiation (LTP) and have been implicated in working memory (1,2). Functional interactions between D1R and NMDAR have long been shown to play critical roles in locomotor activity, positive reinforcement, attention, and working memory (3–6). Activation of D1R enhances NMDA currents through a protein kinase A (PKA)-dependent pathway that most likely involves the phosphorylation and activation of dopamine and cyclic adenosine monophosphate-regulated phosphoprotein with molecular weight 32 kDa (DARPP-32) (7). Similar results are demonstrable on the NMDAR-mediated component of synaptic transmission (8–10). In the hippocampus, dopamine has been shown to produce a synapse-specific enhancement of LTP through D1/D5 receptors and cyclic adenosine monophosphate (cAMP) (11). Furthermore, we have demonstrated that D1R can inhibit NMDAR currents and NMDAR-mediated excitotoxicity through direct protein–protein interactions (12). In addition, several studies have suggested that NMDAR may modulate D1R-mediated functions, because blockade of the NMDAR activity led to the attenuation of D1R's ability to modulate neuronal activity (9,13) and in the induction of immediate early gene expression (14,15). There are also studies suggesting that the activation of NMDAR recruits D1R to the cell plasma membrane and enhances D1-mediated cAMP accumulation through the D1-NMDA protein–protein interaction (16,17). Furthermore, reduced function of both NMDAR and D1R in the prefrontal cortex is thought to contribute significantly to the negative symptoms associated with schizophrenia, including impairment of working memory and cognitive function (18,19).

Extensive research effort has been invested in identifying the exact molecular mechanisms underlying how D1-NMDA interaction exerts functional modulation of LTP and working memory. Most research effort has focused on the intracellular signaling pathways involving PKA/DARPP-32/protein phosphatase type 1, intracellular calcium levels, or both. There are also a few reports demonstrating the functional role of D1-NMDA direct coupling in the upregulation of D1R function by increasing the synaptic expression of D1R (20,21). We have reported that activation of D1R uncouples D1–NR1a interaction while promoting the formation of a NR1: calmodulin (CaM): phosphatidylinositol 3-kinase (PI-3) complex that leads to the activation of PI-3 kinase–dependent cell survival pathway (17). Interestingly, NR1a subunit interacts with D1, CaM, and calcium/calmodulin-dependent protein kinase II (CaMKII) (17,22) through the same region located at the carboxyl terminals. The D1R, CaM, CaMKII, and PI-3 kinase have all been implicated in LTP and memory (21,23,24), and the NR1a-CaMKII direct coupling is associated with NMDA-dependent LTP (22). Thus, in this study, we investigated the role of the D1–NR1 interaction in NMDA-dependent LTP by examining the effects of disrupting the direct protein–

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protein interaction between D1R and NR1 subunit of NMDAR using the interfering peptide, as well as the potential involvement of downstream signaling pathways such as PI-3 kinase and CaMKII. Furthermore, we investigated whether disruption of the D1–NR1a interaction in mice could affect working memory. We found that uncoupling of D1–NR1a interaction abolished the D1R-induced upregulation of LTP in the rat hippocampal cultures. This impairment is associated with a deficit of association between NR1 and CaMKII and decreased CaMKII enzyme activity. Most important, this impairment is also manifested with the selective impairment of working memory.

## Methods and Materials

### Primary Cell Culture

Hippocampal neurons from fetal (E18) Wistar rats were cultured as described previously (23). The cells were plated on glass coverslips coated with .1 mg/mL poly-d-lysine in borate buffer (25). The cultures were maintained by feeding twice a week by replacing half medium with fresh feeding medium. After 6 days of plating, 5 μmol/L Ara-C was added to stop the growth of glial cells.

### Electrophysiology

Miniature excitatory postsynaptic currents (mEPSCs) were recorded from cultured hippocampal neurons 17 days after plating under whole-cell patch clamp configuration as described previously (26). Cells were voltage clamped at −70 mV. The extracellular solution contained (in mmol/L) NaCl, 140; $CaCl_2$, 1.3; KCl, 5.0; N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 25; glucose, 33; tetrodotoxin, .0005; strychnine, .001; bicuculline methiodide, .02; at pH 7.4 and osmolarity 325–335 $mosmol^{-1}$. The intracellular solution consisted of (in mmol/L): CsCl, 140; ethylene glycol bis-2-aminoethyl ether-N,N′,N″,n′-tetraacetic acid (EGTA), 2.5; $MgCl_2$, two; HEPES, 10; tetraethylammonium, two; $K_2$ adenosine triphosphate (ATP), four; pH 7.3; and osmolarity 300–310 $mosmol^{-1}$. In some experiments, TAT or D1-t2 peptide as well as enzyme inhibitors (KN-62 and wortmannin) were also included in the intracellular solution.

Recordings were made at room temperature (21°–23°C). The recordings with series resistance larger than 10 MΩ or varying more than 10% were rejected. Series resistance was not compensated. Recordings were made approximately 30 to 40 min before the application of 6-chloro-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol hydrobromide (SKF) 81297 to establish the baseline level, whereas only the last 10 min of this period was reflected in the figures.

Synaptic activities were recorded using an Axopatch 200 B (Axon Instruments, Union City, California), signals were filtered at 2 kHz, digitized at 10 kHz, and stored in laboratory computer. Data were analyzed using Mini Analysis Software (Synaptosoft, Decatur, Georgia). The mEPSC frequency and amplitude for each time point were obtained from a 2-min recording. The trigger level for event detection was 3 times higher than that of baseline noise. Eye inspection was performed to eliminate the false events.

Physiology data were further analyzed with OriginPro 8 (OriginLab, Northampton, Massachusetts). Mann–Whitney Rank–Sum Test (SigmaStat, Aspire Software International, Ashburn, Virginia) was used to test the difference between percentage data.

**Acute Hippocampal Slices.** Acute hippocampal slice (350 μm) were prepared from Sprague-Dawley rats using a McIlwain tissue chopper (Mickle Laboratory Engineering, Gomshall, United Kingdom). Hippocampus was dissected out and left for 5 min in ice-cold artificial cerebrospinal fluid (aCSF) containing (mmol/L) NaCl, 126; KCl, 2.5; $MgCl_2$, 1; $CaCl_2$, 1; $KH_2PO_4$, 1.25; NaHCO3, 26; and glucose, 20 that was bubbled continuously with carbogen (95% $O_2$/5% $CO_2$) to adjust the pH to 7.4. Freshly cut slices were placed in an incubating chamber with carbogenated aCSF and recovered from stress at 37°C for 1 hour. Slices were then treated with 1 μmol/L TAT or TAT-D1-t2 peptide for 30 min followed by 10 μmol/L SKF-81297 treatment for 30 min. Slices were harvested for coimmunoprecipitation and Western blotting analysis.

**Coimmunoprecipitation and Western Blotting.** Coimmunoprecipitation and Western blot analyses were performed as previously described (12). Samples from rat brain hippocampus were prepared as described before. Solubilized extracts (500 to ~700 μg of protein) were incubated in the presence of primary anti-NR1 (Pharmingen, San Diego, CA), anti-CaMKII (Chemicon, Billerica, Massachusetts), or mouse immunoglobulin G (1 to ~2 μg), followed by the addition of 20 μL of protein A/G agarose (Santa Cruz Biotechnology, Santa Cruz, CA). Pellets were boiled and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. In each experiment, 20 to 50 μg of tissue-extracted protein was used as control. Blots were incubated with the appropriate primary antibody; the membrane was incubated with horseradish peroxidase–conjugated secondary antibody (diluted in 1% milk in Tris-buffered saline with Tween; Sigma). The proteins were visualized with enhanced chemiluminescence reagents (Amersham Biosciences, Piscataway, New Jersey).

**CaMKII Activity Assay.** CaMKII assay was performed mainly according to the SignaTECT calcium/calmodulin-dependent protein kinase Assay System (Promega, Madison, Wisconsin). Briefly, hippocampal neurons were first incubated with 1 μmol/L TAT, TAT-D1-t2, or TAT-D1-t3 peptide for 30 min at 37°C then treated with 10 μmol/L SKF-81297 for 30 min 37°C. Cells were lysed by a buffer containing 20 mmol/L Tris-HCl (pH 8.0), 2 mmol/L ethylenediamine tetraacetate, 2 mmol/L EGTA, 20 μg/mL soybean trypsin inhibitor, 10 μg/mL aprotinin, 5 μg/mL leupeptin, 2 mmol/L dithiothreitol, 25 mmol/L benz amidin, 1 mmol/L phenylmethylsulfonyl fluoride for 30 min at 4°C. Cell debris was removed by 15 min of centrifugation at 14,000 *g*. CaMKII activity was measured on the basis of phosphorylation of specific biotinylated peptide substrate by the transfer of ($\gamma$-$^{32}$P) ATP by CaMKII. Assay components were mixed on ice, and reactions were started by the addition of the sample to reactants containing ($\gamma$-$^{32}$P) ATP. Samples were incubated at 30°C for 2 min and reactions were stopped by adding 12.5 μL of termination buffer. Ten microliters terminated reaction was spotted on a biotin capture membrane. Following several washes, papers were exposed to a Kodak film or transferred to vials, and associated radioactivity was measured by using liquid scintillation counting. All samples in each experiment were assayed in triplicate. Data are expressed as the means ± SEM. Normalized values were analyzed by Student's *t* test ($n = 3$; $p < .05$).

**Mouse Surgery.** Male offspring from a cross between C57Bl/6NTacfBr (C57B6) and 129Svev (129) mice (Taconic, Germantown, New York) were used in these experiments. These mice were bred in our colony at the Hospital for Sick Children and were maintained on a 12-hour light–dark cycle with free access to food and water. All mice were at least 12 weeks of age at the

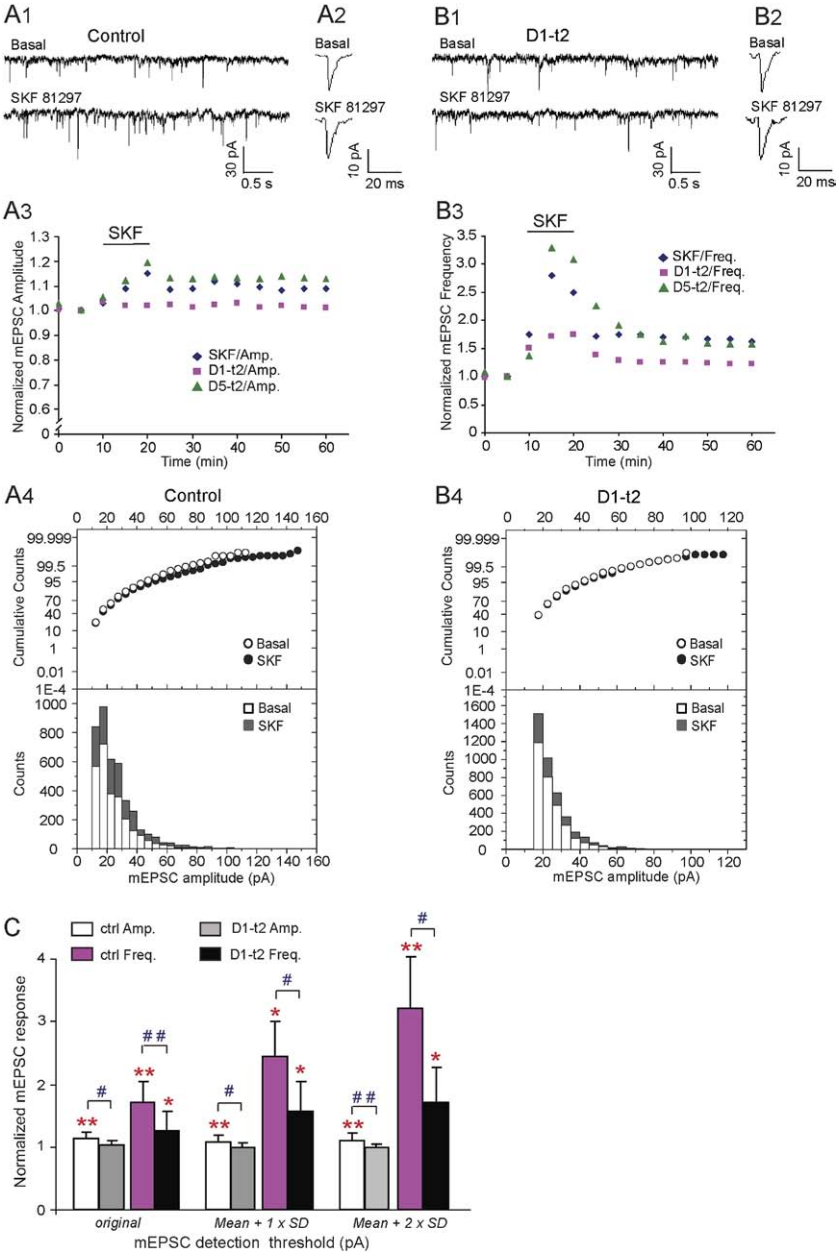


**Figure 1. (A1)** D1R-mediated increase in miniature excitatory postsynaptic currents (mEPSCs) in primary cultures of hippocampal neurons. Traces showing the basal and 20 min after application of D1 receptors (D1R)-specific agonist 6-chloro-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol hydrobromide (SKF) 81297 (10 μmol/L, 10 min). **(A2)** SKF 81297 induced a persistent increase in the incidence of mEPSCs. **(A3, B3)** The mEPSC amplitude was also increased by SKF 81297 as shown by the individual events. **(A4)** Persistent enhancement of the mEPSC amplitude and frequency by SKF 81297. Histogram and probability curves showing the enhancing effect of D1R activation on mEPSC amplitude. **(B1–4)** SKF 81297 tended to increase preferentially the proportion of events with larger amplitudes ($n = 2614$ events for basal, and $n = 4110$ events for SKF 81297 from 10 cells; $^{*}p = .0001$, Kolmogorov-Smirnov [K-S] Test). **(B1–4, A3)** Peptide D1-t2 (10 μmol/L), but not the D5-t2 peptide (10 μmol/L) included in the recording pipet inhibited the enhancing effects of SKF 81297. The difference detected by the K-S test was most likely due to the events with large amplitude (i.e., > 100 pA) that were not completely blocked by the D1-t2 peptide ($n = 3076$ events for basal, and $n = 4034$ events for SKF 81,297 from six cells; $p = .03$, K-S test). **(C)** Further comparison between the recordings with or without D1-t2 in the recording pipets after SKF 81297 application with larger arbitrary mEPSC detecting amplitude thresholds for each cell (mean + 1XSD and mean + 2XSD) revealed the preferential effects of SKF 81297 on the occurrence of mEPSCs with larger amplitudes. SKF 81297 increased the mEPSC amplitude to 110.6% ± 10.6% of basal level ($^{**}p < .01$, $n = 10$, paired $t$ test). The change in the mean mEPSC amplitude was inhibited by inclusion of D1-t2 peptide in the recording pipet (102.4% ± 7.2% of basal; $p > .05$, $n = 6$, paired $t$ test; $^{\#}p < .05$, between D5-t2 and D1-t2 treatments, Whitney Rank-Sum Test). The mEPSC frequency was increased by SKF 81297 in the absence (171.8% ± 31.5% of basal; $^{**}p < .01$, $n = 10$, paired $t$ test) or presence of D1-t2 (126.2% ± 31.9%; $^{*}p < .05$, $n = 6$, paired $t$ test); however, inclusion of D1-t2 peptide markedly inhibited the enhancing effect of SKF 81297 on the mEPSC frequency ($^{\#\#}p < .01$, Whitney Rank-Sum Test). Analysis of the mEPSCs with larger detecting amplitude thresholds (mean + 1XSD and mean + 2XSD) revealed that D1R activation preferentially enhanced the frequency of mEPSCs with larger amplitude (244.2% ± 56.2% of basal level for control vs. 146.0% ± 48.0% of basal level for D1-t2 with mean ± 1XSD; 321.8 ± 82.1% vs. 155.0 ± 55.7% of respective basal level with mean ± 2XSD. $^{\#}p < .05$, Whitney Rank-Sum test).

start of experiments, and behavioral procedures were conducted during the light phase of the cycle. Experiments were conducted blind to the treatment condition of the mouse and according to local animal care protocols.

Under chloral hydrate anesthesia and using standard stereotaxic procedures, stainless-steel guide cannulae (22 gauge) were implanted bilaterally above the dorsal hippocampus (dHPC; −2.0 mm, ± 1.6, −2.1 mm). Mice were allowed to recover for at least 10 days following surgery.

**Behavior Apparatus.** Behavioral testing was conducted in a circular water maze tank (120 cm in diameter, 50 cm deep), located in a dimly lit room. The pool was filled to a depth of 40 cm with water made opaque by adding white nontoxic paint. Water temperature was maintained at 28 ± 1°C by a heating pad located beneath the pool. A circular escape platform (10 cm diameter) was submerged .5 cm below the water surface. The pool was surrounded by curtains, at least 1 m from the perimeter of the pool. The curtains were white and had distinct cues painted on them.

**Behavioral Procedures and Peptide Infusion.** Before the commencement of training, mice were individually handled for 2 min each day for 1 week. On each training day, mice received six training trials with the platform in a fixed location (presented in two blocks of three trials; interblock interval was 30 min; intertrial interval was ~ 15 sec). The 30-min interblock delay was used to encourage the animal to maintain information that is an essential property of working memory. Across training days, the platform position was varied, making it necessary for mice to integrate new spatial information each day. On each trial they were placed into the pool, facing the wall, in one of four start locations. The order of these start locations was pseudo-randomly varied throughout training. The trial was complete after the mouse found the platform or 60 sec had elapsed. If the mouse failed to find the platform on a given trial, the experimenter guided the mouse onto the platform.

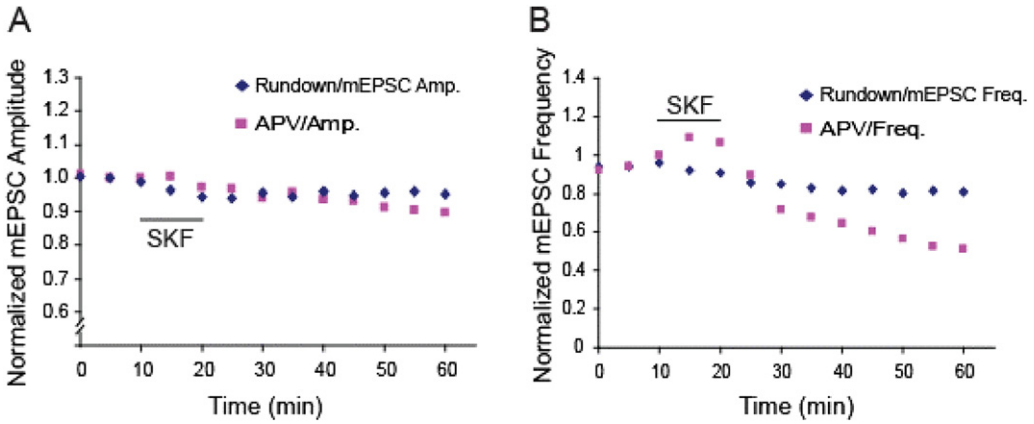


**Figure 2.** Coapplication of 10 μmol/L DL-2-Amino-5-phosphonopentanoic acid (D-APV) blocked the effects of SKF 81297. The mEPSC amplitude and frequency in the presence of D-APV 20 min after drug application were compared with the respective values after rundown of miniature excitatory postsynaptic currents (mEPSCs). **(A)** D-APV did not markedly alter the mEPSC amplitude (93.6% ± 2.4% of basal amplitude for APV, $n = 6$ vs. 95.8% ± 4.5% of basal amplitude for rundown, $n = 4$; $p > .05$, Whitney Rank-Sum Test). **(B)** However, D-APV markedly decreased the mEPSC frequency (68.7% ± 4.9% of basal frequency for APV, $n = 6$ vs. 86.8% ± 2.0% of basal frequency for rundown, $n = 4$; $p < .05$, Whitney Rank-Sum Test).

For the peptide group, 1 hour before the first block of training on Days 4 and 6, mice received .5-μL infusions of either TAT-D1-t2 or TAT control peptide at a rate of .2 μL/min in counterbalanced manner (i.e., half the mice received TAT on Day 4 and TAT-D1-t2 on Day 6 and vice versa). The injection cannula (28 gauge) was left in place for 2 min following the infusion, and the mice were returned to their home cages before commencing Block 1 of training. Thirty minutes after Block 1, mice were returned to the water maze for modified Block 2 training where they received multiple trials until they reach a criterion of three consecutive trials, each less than 20 sec. In Block 2, continuous trials, starting from different start location (sequence counterbalanced across mice), were given until the mice reach the criterion. We did not use an a priori cutoff for trial number because all mice reached the criterion in a reasonable number of trials (i.e., typically < 10 trials). This "trial to criterion" index has previously been shown to be a sensitive index of performance (27).

**Histology.** At the completion of the experiments, mice were overdosed with chloral hydrate and perfused transcardially with saline, followed by 4% polyformaldehyde (PFA) solution to fix the brain tissue. Brains were removed, postfixed in 4% PFA solution, and cut into 50-μm coronal sections on a cryostat. Sections were mounted on slides and stained with cresyl violet; cannula placements were examined under a light microscope.

## Results

### Activation of D1R Upregulated NMDAR-Dependent LTP of mEPSCs in Hippocampal Neurons Through the D1–NR1 Direct Protein–Protein Interaction

The glycine-induced LTP model in hippocampal primary cultures is a validated model of LTP, similar to the electrically evoked EPSCs in CA1 neurons in hippocampal slices as previously described (23,26,28). Before determining the effect of D1R activation on synaptic activity, we used the glycine (200 μmol/L; 3 min) to validate our experimental system (data not shown). Once confirmed, we then tested whether activation of D1R can modulate mEPSC in hippocampal primary culture. SKF 81297 (10 μmol/L; 10 min), a D1-specific agonist, significantly enhanced synaptic transmission by increasing the mEPSC frequency and

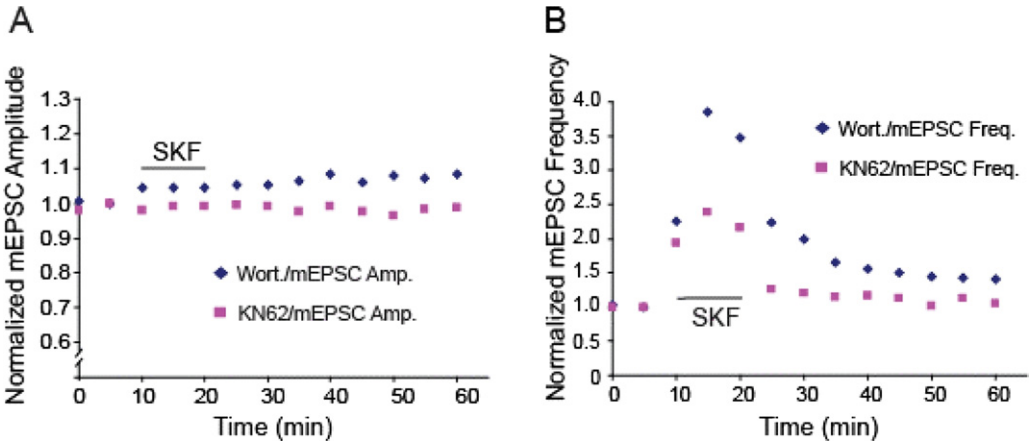


**Figure 3.** Involvement of calcium/calmodulin-dependent protein kinase II (CaMKII) in the D1R-mediated increase in mEPSCs in primary cultures of hippocampal neurons. **(A)** Inclusion of CaMKII inhibitor KN-62 (10μmol/L, 98.9% ± 1.8% of basal level, $n = 9$; $p > .05$, paired $t$ test) in the recording pipets, but not the PI-3 kinase inhibitor wortmannin (100 nmol/L, 108.2% ± 19.4% of basal level, $n = 6$; $p < .01$, paired $t$ test), inhibited the potentiation in mEPSC amplitude. **(B)** KN-62 blocked the potentiation of mEPSC frequency by SKF 81297 (114.1% ± 9.6%, $n = 9$; $p > .05$, paired $t$ test), but wortmannin failed to do so (157.5% ± 17.7% of basal level, $n = 6$; $p < .05$, paired $t$ test). Abbreviations as in Figure 1.

amplitude (Figure 1, A1–A4, B3), indicating that D1R activation modulates LTP similarly in culture and brain-slice preparations (29). To examine whether the observed effects of SKF 81297 are mediated by a D1–NR1 interaction, we included the interfering peptide D1-t2, which inhibits the interaction between D1R and NR1 subunit in the recording pipets (12). We also included a peptide corresponding to a homologous region of the dopamine D5 receptor carboxyl tail as the control peptide D5-t2. We found that D1-t2, but not the D5-t2 peptide, impaired the persistent enhancement in synaptic transmission induced by SKF 81297 (Figure 1, B1–B4). Although the interfering D1-t2 peptide couples to the NR1 subunit within its C1 region of carboxyl tail (29,30), we have previously shown in hippocampal neurons that application of the D1-t2 peptide by itself does not affect NMDAR channel activity (12), indicating the critical role of the D1–NR1 interaction in this process. Our data also suggested that activation of D1R preferentially increased the occurrence of mEPSCs with larger amplitude (Figure 1, A4, C). Furthermore, coapplication of 10 μmol/L DL-2-Amino-5-phosphonopentanoic acid with SKF 81297 blocked the enhancement of the mEPSCs by SKF 91287 (Figure 2A and 2B), suggesting the requirement for NMDAR activation in the observed LTP of mEPSCs.

### D1R Upregulated LTP of mEPSCs Through a CaMKII-Dependent Pathway

A number of protein kinases such as CaMKII and PI-3 kinase have been implicated in LTP (24,31,32). Interestingly, both kinases have also been shown to interact with D1R and NMDAR, respectively. Thus, in this study we tested the potential involvement of CaMKII and PI-3 kinase. As shown in Figure 3A and 3B, CaMKII-specific inhibitor KN-62, but not PI-3 kinase inhibitor wortmannin, blocked the D1-mediated upregulation of NMDAR-dependent synaptic transmission, implicating the involvement of CaMKII in this process.

Previous studies have suggested that the CaMKII activity is associated with its direct interaction with NR1 subunit (22). Our previous data have shown that activation of D1R dissociates the D1–NR1 coupling (12). The fact that D1R and CaMKII couple to the same region of NR1a subunit led to our hypothesis that the observed enhancement of mEPSCs upon D1R stimulation is the consequence of enhanced NR1-CaMKII coupling as a result from the dissociation of D1–NR1. As shown in Figure 4A, consistent with our hypothesis, activation of D1R with SKF 81297 significantly increased the NR1-CaMKII coupling; an effect can be blocked by the application of TAT-D1-t2. Furthermore, we examined the role of the D1-NR1 interaction on CaMKII activity in primary cultures of hippocampal neurons. Consistent with the LTP result, SKF 81297 significantly enhanced CaMKII activity (Figure 4B and 4C), whereas pretreating hippocampal neurons with the TAT-D1-t2 peptide abolished the D1R-mediated upregulation of CaMKII activity, indicating the involvement of D1-NR1 interaction. In control experiments, we used TAT alone as well as TAT-D1-t3 peptide, which is able to disrupt the D1-NR2A interaction as we previously identified (12).

### Uncoupling of D1-NR1a Interaction Led to Working Memory Deficits

Dopaminergic signaling plays a critical role in modulating working memory in the hippocampus (33,34). To test whether specifically disrupting the D1–NR1 interaction impairs working memory, we trained mice in a delayed match-to-place (DMP) memory version of the water maze (27). During training, mice learned to navigate to a new escape location each day (Figure 5A). On each day, mice received two blocks of training (three trials each), separated by a 30-min delay. Following 3 days of training, escape latencies were significantly reduced in the second block compared with the first block [$F(1,10) = 14.38$, $p < .01$], indicating that mice were able to maintain spatial information across the 30-min interblock delay (Figure 5B).

To examine the effects of specifically disrupting D1–NR1 interactions on spatial working memory, TAT-D1-t2 or TAT peptide was infused into the mice hippocampus before block 1 of training. In mice treated with the TAT peptide, escape latencies were reduced in Block 2 relative to block 1 (paired $t_{10} = 3.84$, $p < .005$, planned comparison). In contrast, in TAT-D1-t2-treated mice latencies in Blocks 1 and 2 remained similar (paired $t_{10} = .75$, $p = .47$, Figure 5C). The absence of behavioral savings indicates that disrupting the D1–NR1 interaction impairs spatial working memory. Although the drug-by-block interaction in latency measure only reaches marginal significance (2 × 2 analysis of variance, $F(1,10) = 3.68$, $p = .08$), this is not

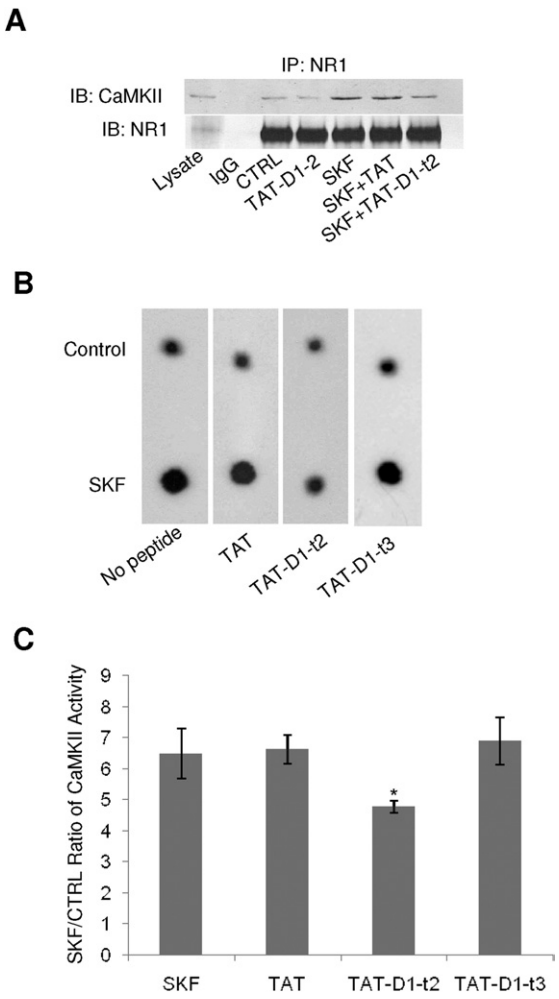


**Figure 4.** D1R-mediated CaMKII activity. **(A)** SKF 81297 (10 μmol/L) stimulation enhanced the association of CaMKII and NR1 subunit, which could be blocked by 30 min preincubation of 10 μmol/L TAT-D1-t2 peptide. **(B)** SKF 81297 (10 μmol/L) mediated activation of CaMKII was measured in hippocampal neurons in presence or absence of peptides. Preincubation of TAT-D1-t2 peptide inhibited the activation of CaMKII. **(C)** Histogram summarizes the effects of TAT-D1-t2 and SKF 81297 on CaMKII activity (*$p < .05$). Abbreviations as in Figures 1–3.

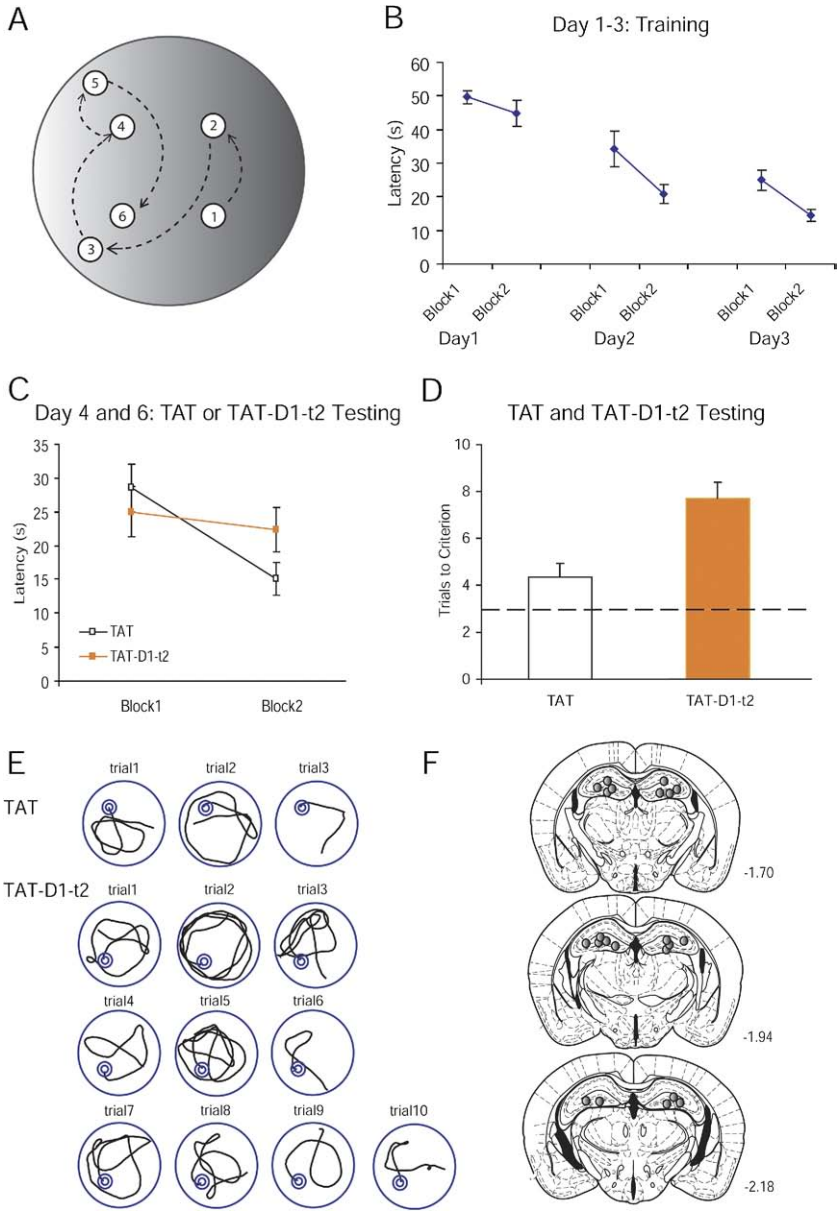


**Figure 5.** Intrahippocampal infusion of TAT-D1-t2 disrupts delayed match-to-place memory in the water maze. **(A)** Varied platform locations in delayed match-to-place task in the water maze. The number in each circle represents the day of training or testing. **(B)** Swimming latencies to reach the platform during Block 1 and Block 2 on Days 1–3 of training. **(C)** Swimming latency in Block 1 and Block 2 on Days 4 and 6 of TAT and TAT-D1-t2 testing. Latencies declined from Block 1 to Block 2 in mice treated with the control peptide ($p < .005$) but not TAT-D1-t2 ($p = .47$), indicating that blocking the D1R-NR1 interaction disrupts spatial working memory. **(D)** The number of trials in Block 2 for the animal to reach the criterion of three consecutive trials within 20 sec to reach the platform. TAT-D1-t2-treated mice required significantly more trials to reach criterion ($p < .005$). The dashed line represents the minimal baseline. **(E)** Representative swim paths in Block 2 on testing day for mice treated with the control and TAT-D1-t2 peptides. **(F)** schematic brain sections to show the placement of the tip of injectors. The number under each section denotes its position relative to bregma (in millimeters), according to the atlas of Paxinos and Franklin published in *The Mouse Brain Stereotaxic Coordinates, 2nd ed.*, Copyright Elsevier 2001 (79). All statistical details in results.

surprising as latency has been shown to be a less sensitive measure to reveal memory deficit in DMP task in mice (27).

A more sensitive measure in mouse DMP task is "trials to criterion" (27). Therefore, to explore further the working memory deficits, we continued training the mice in the DMP task and asked how many trials were required for mice to reach criterion performance (i.e., the number of trials needed to reach three consecutive trials within 20-sec latency). We found that TAT-D1-t2-treated mice required significantly more trials to reach criterion (7.7 ± .7) compared with control-treated mice (4.4 ± .5, paired $t_{10} = 4.61$, $p < .005$), confirming that disruption of D1-NR1 interaction impairs spatial working memory (Figure 5D).

Importantly, this disruption was not due to nonspecific effects on motivation, motor function, or search strategy because escape latencies (paired TAT vs. t2 difference = 3.51 ± 4.38, $t_{10} = .53$, $p = .48$), swimming speed (paired TAT vs. t2 difference = .97 ± .91, $t_{10} = 1.15$, $p = .31$), and thigmotaxic behaviors (i.e., percentage of time swimming near the walls: paired TAT vs. t2 difference= −.5 ± .85, $t_{10} = .40$, $p = .54$) were similar in the first block of training in TAT-D1-t2-treated group versus TAT group. Moreover, the drug effect in the "trials to criterion" measure is not due to differential performance in Trials 4 through 6 in Block 2 in swimming speed (paired TAT vs. t2 difference = .69 ± 1.14, $t_{10} = .61$, $p = .56$) or thigmotaxic behaviors (paired TAT vs. t2 difference= −1.4 ± 1.51, $t_{10} = -.9$, $p = .37$).

## Discussion

Consistent with previous studies demonstrating that D1R agonists stimulation increases LTP of field EPSPs (11,35,36), we have demonstrated that D1R stimulation can augment LTP of mEPSC in dissociated primary cultured rat hippocampal neurons in vitro. Moreover, we have shown that the D1R-induced increase in LTP is mediated by the D1R–NR1 interaction. Previous studies have suggested the involvement of both glutamate and dopamine in both LTP and in spatial working memory in the hippocampus (37,38). By studying the effects of the interfering TAT-D1-t2 peptide, our study provides the first evidence for the

involvement of the D1R: NMDAR complex in facilitating both dopamine-glutamate dependent LTP and spatial working memory, as indexed by a DMP water maze task. Although the interfering TAT-D1-t2 peptide couples to the NR1 subunit within its C1 region of carboxyl tail (17,30), we have previously shown that application of the TAT-D1-t2 peptide in hippocampal neurons by itself does not affect NMDAR channel activity (12). Furthermore, the C1 region of the NR1 subunit can interact with CaMKII, and therefore the peptide can also specifically disrupt the D1R–NR1 interaction and prevent the activation of CaMKII. This suggests that the effects of the disrupting D1–NR1 interaction on LTP and spatial working memory may be mediated by CaMKII-dependent processes (31,34). Several studies have shown that CaMKII activity can affect spatial memory (39–47) through phosphorylation events that ultimately lead to increased α-amino-3-hydroxy-5-methylisoxazole-4-isoxazolepropionic acid receptor at the synapse, an event that is involved in both LTP and spatial memory (48,49). Although NMDARs are considered mediators of LTP, dopamine receptors are largely considered modulators of LTP. However, there is evidence that DA may play a larger role in LTP (11,35–37), presumably by the activation of PKA and subsequent downstream effectors such as cyclic adenosine monophosphate–responsive element binding protein (5,50,51). Although this may be the case, we have now shown that the dissociation of D1R: NMDAR complex upon D1R activation facilitates the NR1a–CaMKII interaction and enhances CaMKII activity, an affect blocked by the TAT-D1-t2 peptide. Interestingly, when this same peptide was administered in vivo, affected mice exhibited working memory deficits, which we cautiously attribute to disrupted CaMKII activity in the hippocampus. Therefore, we speculate that the D1R:NMDAR complex may play a role as a synaptic tag to facilitate LTP in the CA1 region of the hippocampus.

We are aware that the intracellular application of the D1-t2 peptide failed to block fully the SKF 81297-induced increase in the frequency and amplitude of mEPSC. This may be because of poor diffusion of the peptide to the relevant receptors or additional D1-NR1 independent pathways such as PKA-dependent pathway and Src family tyrosine kinase pathway (10,52,53). Indeed, we have obtained data (unpublished) indicating that the cAMP inhibitor Rp-cAMP (10 μmol/L, 5 min) is able to reduce the SKF 81297-induced upregulation of mEPSC significantly, which is consistent with previous studies suggesting that activation of D1R upregulated NMDA-mediated LTP through a PKA-dependent pathway (10,52,53). Thus, activation of D1R may regulate NMDA-mediated mEPSC through two pathways; the PKA-dependent pathway and the D1-NR1 protein–protein interaction. The relationship between these two pathways remains to be investigated in the future.

In this study, we looked at hippocampal involvement in DMP water maze paradigm that has been used in many other rodent studies (1,27,33). This may not be identical to working memory assessed by certain delayed visuospatial tasks used in humans or primates that mainly focus on the frontal cortex involvement (54,55). Future studies are necessary to investigate whether our findings extend to frontal cortical areas in other types of working memory paradigms. The prefrontal cortex, which along with other limbic cortical structures including the cingulate cortex has received considerable attention and been implicated as forming part of the neural network underlying schizophrenia (18,19,56). Dopaminergic *hypofunctionality* in these regions is thought to contribute significantly not only to the observed impairment of "working memory," cognitive function, and negative symptomatology (57,58) associated with schizophrenia but to hallucinations as well (59,60). Although the functional consequence of prefrontal dopaminergic neurotransmission is not fully known, recent work suggests that pyramidal neurons within the cortex/hippocampus underlie the cellular basis for working memory and that dopamine acting through D1-like receptors can modulate this process (61–63). The role of D1-like receptors in the maintenance and expression of prefrontal hypodopaminergic states of schizophrenia is further supported by observations that 1) D1-like receptor stimulation can alleviate some of the "negative" symptomatology of schizophrenia (63,64) and 2) that prefrontal cortical D1 receptors are reduced in schizophrenics, the magnitude of which is related to the severity of negative symptoms (64–66). Furthermore, cortical glutamatergic activity has also been postulated to play a key role in the pathophysiology of schizophrenia (67–72). Reductions of NMDA receptor neurotransmission in the prefrontal cortex mimic most of the behavioral symptomatology associated with cognitive deficits in schizophrenia (72,73). Postmortem studies have identified a relative decrease of the NR1 subunit in the hippocampus of schizophrenia brains (74). In therapeutic trials, agents that enhance NMDA receptor activity have selectively improved the persistent negative symptoms in schizophrenia patients (75,76). Furthermore, NMDA blocking agents such as phencyclidine (PCP) induce a cluster of symptoms that is often indistinguishable from schizophrenia (77). These findings, together with our previous study indicating that NMDAR enhances D1 function through the D1–NR1a interaction (17), suggest that a hypofunction of D1R and NMDAR may be implicated in the pathology of schizophrenia. Indeed, the hypodopaminergic neurotransmission may be secondary to the reduced NMDAR function. A recent study demonstrated that that repeated PCP treatment impairs latent learning through a prefrontal cortical dysfunction of NMDA–CaMKII signaling, which can be rescued by the D1R agonist (78); this is in line with our result that uncoupling D1–NR1a interaction led to impaired working memory and decreased NR1–CaMKII association and CaMKII activity.

In summary, our study provides additional evidence for the importance of dopamine-glutamate interactions in learning and memory. Moreover, the D1-NMDA receptor interaction may provide the molecular framework by which dopamine is involved in glutamate-dependent LTP and memory in the hippocampus.

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1. Niewoehner B, Single FN, Hvalby Ø, Jensen V, Meyer zum Alten Borgloh S, Seeburg PH, *et al.* (2007): Impaired spatial working memory but spared spatial reference memory following functional loss of NMDA receptors in the dentate gyrus. *Eur J Neurosci* 25:837–846.
2. Granado N, Ortiz O, Suárez LM, Martín ED, Ceña V, Solís JM, Moratalla R (2008): D1 but not D5 dopamine receptors are critical for LTP, spatial learning, and LTP-Induced arc and zif268 expression in the hippocampus. *Cereb Cortex* 18:1–12.

3. Lewis DA, Gonzalez-Burgos G (2006): Pathophysiologically based treatment interventions in schizophrenia. *Nat Med* 12:1016–1022.
4. Kelley AE (2004): Memory and addiction: Shared neural circuitry and molecular mechanisms. *Neuron* 44:161–179.
5. Wolf ME, Mangiavacchi S, Sun X (2003): Mechanisms by which dopamine receptors may influence synaptic plasticity. *Ann N Y Acad Sci* 1003: 241–249.
6. Missale C, Fiorentini C, Busi C, Collo G, Spano PF (2006): The NMDA/D1 receptor complex as a new target in drug development. *Curr Top Med Chem* 6:801–808.
7. Snyder GL, Fienberg AA, Huganir RL, Greengard P (1998): A dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (Mr 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. *J Neurosci* 18:10297–10303.
8. Cepeda C, Colwell CS, Itri JN, Chandler SH, Levine MS (1998): Dopaminergic modulation of NMDA-induced whole cell currents in neostriatal neurons in slices: Contribution of calcium conductances. *J Neurophysiol* 79:82–94.
9. Zheng P, Zhang XX, Bunney BS, Shi WX (1999): Opposite modulation of cortical N-methyl-D-aspartate receptor-mediated responses by low and high concentrations of dopamine. *Neuroscience* 91:527–535.
10. Gurden H, Takita M, Jay TM (2000): Essential role of D1 but not D2 receptors in the NMDA receptor-dependent long-term potentiation at hippocampal-prefrontal cortex synapses in vivo. *J Neurosci* 20:RC106: 1–5.
11. Huang YY, Kandel ER (1995): D1/D5 receptor agonists induce a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus. *Proc Natl Acad Sci U S A* 92:2446–2450.
12. Lee FJ, Xue S, Pei L, Vukusic B, Chery N, Wang Y, *et al.* (2002): Dual regulation of NMDA receptor functions by direct protein–protein interactions with the dopamine D1 receptor. *Cell* 111:219–230.
13. Huang KX, Bergstrom DA, Ruskin DN, Walters JR (1998): N-methyl-D-aspartate receptor blockade attenuates D1 dopamine receptor modulation of neuronal activity in rat substantia nigra. *Synapse* 30:18–29.
14. Konradi C, Leveque JC, Hyman SE (1996): Amphetamine and dopamine-induced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. *J Neurosci* 16:4231–4239.
15. Keefe KA, Ganguly A (1998): Effects of NMDA receptor antagonists on D1 dopamine receptor-mediated changes in striatal immediate early gene expression: Evidence for involvement of pharmacologically distinct NMDA receptors? *Dev Neurosci* 20:216–228.
16. Scott L, Kruse MS, Forssberg H, Brismar H, Greengard P, Aperia A (2002): Selective upregulation of dopamine D1 receptors in dendritic spines by NMDA receptor activation. *Proc Natl Acad Sci U S A* 99:1661–1664.
17. Pei L, Lee FJ, Moszczynska A, Vukusic B, Liu F (2004): Regulation of dopamine D1 receptor function by physical interaction with the NMDA receptors. *J Neurosci* 24:1149–1158.
18. Fletcher P (1998): The missing link: A failure of fronto-hippocampal integration in schizophrenia. *Nat Neurosci* 1:266–267.
19. Kotrla KJ, Weinberger DR (1995): Brain imaging in schizophrenia. *Ann Rev Med* 46:113–122.
20. Castner SA, Williams GV (2007): Tuning the engine of cognition: A focus on NMDA/D1 receptor interactions in prefrontal cortex. *Brain Cogn* 63: 94–122.
21. Jay TM (2003): Dopamine: A potential substrate for synaptic plasticity and memory mechanisms. *Prog Neurobiol* 69:375–390.
22. Leonard AS, Lim IA, Hemsworth DE, Horne MC, Hell JW (1999): Calcium/calmodulin-dependent protein kinase II is associated with the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci U S A* 96:3239–3244.
23. Man HY, Wang Q, Lu WY, Ju W, Ahmadian G, Liu L, *et al.* (2003): Activation of PI3-kinase is required for AMPA receptor insertion during LTP of mEPSCs in cultured hippocampal neurons. *Neuron* 38:611–624.
24. Xia Z, Storm DR (2005): The role of calmodulin as a signal integrator for synaptic plasticity. *Nat Rev Neurosci* 6:267–276.
25. Nai Q, McIntosh JM, Margiotta JF (2003): Relating neuronal nicotinic acetylcholine receptor subtypes defined by subunit composition and channel function. *Mol Pharmacol* 63:311–324.
26. Lu W, Man H, Ju W, Trimble WS, MacDonald JF, Wang YT (2001): Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron* 29:243–254.
27. Chen G, Chen KS, Knox J, Inglis J, Bernard A, Martin SJ, *et al.* (2000): A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature* 408:975–979.
28. Liao D, Scannevin RH, Huganir R (2001): Activation of silent synapses by rapid activity-dependent synaptic recruitment of AMPA receptors. *J Neurosci* 21:6008–6017.
29. Navakkode S, Sajikumar S, Frey JU (2007): Synergistic requirements for the induction of dopaminergic D1/D5-receptor-mediated LTP in hippocampal slices of rat CA1 in vitro. *Neuropharmacology* 52:1547–1554.
30. Ehlers MD, Zhang S, Bernhadt JP, Huganir RL (1996): Inactivation of NMDA receptors by direct interaction of calmodulin with the NR1 subunit. *Cell* 84:745–755.
31. Malenka RC, Nicoll RA (1999): Long-term potentiation—A decade of progress? *Science* 285:1870–1874.
32. Opazo P, Watabe AM, Grant SG, O'Dell TJ (2003): Phosphatidylinositol 3-kinase regulates the induction of long-term potentiation through extracellular signal-related kinase-independent mechanisms. *J Neurosci* 23:3679–3688.
33. O'Carroll CM, Martin SJ, Sandin J, Frenguelli B, Morris RG (2006): Dopaminergic modulation of the persistence of one-trial hippocampus-dependent memory. *Learn Mem* 13:760–769.
34. Dash PK, Moore AN, Kobori N, Runyan JD (2007): Molecular activity underlying working memory. *Learn Mem* 14:554–563.
35. Otmakhova NA, Lisman JE (1996): D1/D5 dopamine receptor activation increases the magnitude of early long-term potentiation at CA1 hippocampal synapses. *J Neurosci* 16:7478–7486.
36. Sajikumar S, Frey JU (2004): Late-associativity, synaptic tagging, and the role of dopamine during LTP and LTD. *Neurobiol Learn Mem* 82:12–25.
37. Lemon N, Manahan-Vaughan D (2006): Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. *J Neurosci* 26:7723–7729.
38. Lisman JE: Grace AA (2005): The hippocampal-VTA loop: Controlling the entry of information into long-term memory. *Neuron* 46:703–713.
39. Silva AJ, Paylor R, Wehner JM, Tonegawa S (1992): Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. *Science* 257:206–211.
40. Bach ME, Hawkins RD, Osman M, Kandel ER, Mayford M (1995): Impairment of spatial but not contextual memory in CaMKII mutant mice with a selective loss of hippocampal LTP in the range of the theta frequency. *Cell* 81:905–915.
41. Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD, Kandel ER (1996): Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274:1678–1683.
42. Rotenberg A, Mayford M, Hawkins RD, Kandel ER, Muller RU (1996): Mice expressing activated CaMKII lack low frequency LTP and do not form stable place cells in the CA1 region of the hippocampus. *Cell* 87:1351–1361.
43. Miller S, Yasuda M, Coats JK, Jones Y, Martone ME, Mayford M (2002): Disruption of dendritic translation of CaMKIIalpha impairs stabilization of synaptic plasticity and memory consolidation. *Neuron* 36:507–519.
44. Wood MA, Kaplan MP, Park A, Blanchard EJ, Oliveira AM, Lombardi TL, Abel T (2005): Transgenic mice expressing a truncated form of CREB-binding protein (CBP) exhibit deficits in hippocampal synaptic plasticity and memory storage. *Learn Mem* 12:111–119.
45. Yasuda M, Mayford MR (2006): CaMKII activation in the entorhinal cortex disrupts previously encoded spatial memory. *Neuron* 50:309–318.
46. Cacucci F, Wills TJ, Lever C, Giese KP, O'Keefe J (2007): Experience-dependent increase in CA1 place cell spatial information, but not spatial reproducibility, is dependent on the autophosphorylation of the alpha-isoform of the calcium/calmodulin-dependent protein kinase II. *J Neurosci* 27:7854–7859.
47. Poulsen DJ, Standing D, Bullshields K, Spencer K, Micevych PE, Babcock AM (2007): Overexpression of hippocampal Ca2+/calmodulin-dependent protein kinase II improves spatial memory. *J Neurosci Res* 85:735–739.
48. Lisman J, Schulman H, Cline H (2002): The molecular basis of CaMKII function in synaptic and behavioural memory. *Nat Rev Neurosci* 3:175–190.
49. Schmitt WB, Sprengel R, Mack V, Draft RW, Seeburg PH, Deacon RM, Rawlins JN, Bannerman DM (2005): Restoration of spatial working memory by genetic rescue of GluR-A-deficient mice. *Nat Neurosci* 8:270–272.

50. Athos J, Impey S, Pineda VV, Chen X, Storm DR (2002): Hippocampal CRE-mediated gene expression is required for contextual memory formation. *Nat Neurosci* 5:1119–1120.
51. Pittenger C, Huang YY, Paletzki RF, Bourtchouladze R, Scanlin H, Vronskaya S, Kandel ER (2002): Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. *Neuron* 34:447–462.
52. Frey U, Huang YY, Kandel ER (1993): Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science* 260:1661–1664.
53. Stramiello M, Wagner JJ (2008): D1/5 receptor-mediated enhancement of LTP requires PKA, Src family kinases, and NR2B-containing NMDARs. *Neuropharmacology* 55:871–877.
54. Williams GV, Goldman Rakic PS (1995): Blockade of dopamine D1 receptors enhances memory fields of prefrontal neurons in primate cerebral cortex. *Nature* 376:572–575.
55. Muller U, Yves von Cramon D, Pollmann S (1998): D1 versus D2 receptor modulation of visuospatial working memory in humans. *J Neurosci* 18: 2720–2728.
56. Cannon TD (1996): Abnormalities of brain structure and function in schizophrenia: Implications for etiology and path-physiology. *Ann Med* 28:533–539.
57. Travis MJ, Kerwin R (1997): Neuroimaging. *Curr Opin Psychiatry* 10:16–25.
58. McPhillips MA, Barnes TRE (1997): Negative symptoms. *Curr Opin Psychiatry* 10:30–36.
59. Rossell SL, David AS (1997): The neuropsychology of schizophrenia: Recent trends. *Curr Opin Psychiatry* 10:26–29.
60. Silbersweig DA, Stern E, Frith C, Cahill C, Holmes A, Grootoonk S, *et al.* (1995): A functional neuroanatomy of hallucinations in schizophrenia. *Nature* 378:176–179.
61. Dolan RJ, Fletcher P, Frith CD, Friston KJ, Frackwiak RSJ, Grasby PM (1995): Dopaminergic modulation of impaired cognitive activation in the anterior cingulate cortex in schizophrenia. *Nature* 378:180–182.
62. Goldman-Rakic PS, Selemon LD (1997): Functional and anatomical aspects of prefrontal pathology in schizophrenia. *Schizophr Bull* 23:437–458.
63. Davidson M, Harvey PD, Bergman RL, Pwchik P, Kaminsky R, Losonczy MF, Davis KL (1990): Effects of D1 agonist SKF-38393 combined with haloperidol in schizophrenic patients. *Arch Gen Psychiatry* 47:190–191.
64. Lidow MS, Williams GV, Goldman Rakic PS (1998): The cerebral cortex: A case for a common site of action of anti-psychotics. *Tips* 19:136–140.
65. Okubo Y, Suhara T, Suzuki K, Kobayashi K, Inoue O, Terasaki O, *et al.* (1997): Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature* 385:634–636.
66. Okubo Y, Suhara T, Sudo Y, Toro M (1997): Possible role of dopamine D1 receptors in schizophrenia. *Mol Psychiatry* 2:292–292.
67. Dean B, Scarr E, Bradbury R, Copolov D (1999): Decreased hippocampal NMDA receptors in schizophrenia. *Synapse* 32:67–69.
68. Iverson SD (1995): Interactions between excitatory amino acids and dopamine systems in the forebrain: Implications for schizophrenia and Parkinson's disease. *Behav Pharmacol* 6:478–491.
69. Olney JW, Farber NB (1995): Glutamate receptor dysfunction in schizophrenia. *Arch Gen Psychiatry* 52:998–1007.
70. Thornberg SA, Saklad SR (1996): A review of NMDA receptors and the phencyclidine model of schizophrenia. *Pharmacol Ther* 16:82–93.
71. Tamminga CA (1998): Schizophrenia and glutamatergic transmission. *Crit Rev Neurobiol* 12:21–36.
72. Mohn AR, Gainetdinovw RR, Caron MG, Koller B (1999): Mice with reduced NMDA receptor expression display behaviors related schizophrenia. *Cell* 98:427–436.
73. Jentsch JD, Redmond DE, Elsworth JD, Taylor JR, Youngren KD, Roth RH (1997): Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long term administration of phencyclidine. *Science* 277:953–955.
74. Gao XM, Sakai K, Roberts RC, Conley RR, Dean B, Tamminga CA (2000): Ionotropic glutamate receptors and expression of N-methyl-D-aspartate receptor subunits in subregions of human hippocampus: Effects of schizophrenia. *Am J Psychiatry* 157:1141–1149.
75. Goff DC, Coyle JT (2001): The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *Am J Psychiatry* 158:1367–1377.
76. Javitt DC (2001): Management of negative symptoms of schizophrenia. *Curr Psychiatry Rep* 3:413–417.
77. Carlsson A, Hansson LO, Waters N, Carlsson ML (1997): Neurotransmitter aberrations in schizophrenia; new perspectives and therapeutic implications. *Life Sci* 61:75–94.
78. Mouri A, Noda Y, Noda A, Nakamura T, Tokura T, Yura Y, *et al.* (2007): Involvement of a dysfunctional dopamine-D1/N-methyl-d-aspartate-NR1 and Ca2+/calmodulin-dependent protein kinase II pathway in the impairment of latent learning in a model of schizophrenia induced by phencyclidine. *Mol Pharmacol* 71:1598–1609.
79. Paxinos G, Franklin KBJ (2001): *The Mouse Brain in Stereotaxic Coordinates*, *2nd ed*. New York: Elsevier.