Disruption of hippocampal–prefrontal cortex activity by dopamine D2R-dependent LTD of NMDAR transmission

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Functional connectivity between the hippocampus and prefrontal cortex (PFC) is essential for associative recognition memory and working memory. Disruption of hippocampal–PFC synchrony occurs in schizophrenia, which is characterized by hypofunction of NMDA receptor (NMDAR)-mediated transmission. We demonstrate that activity of dopamine D2-like receptors (D2Rs) leads selectively to long-term depression (LTD) of hippocampal–PFC NMDAR-mediated synaptic transmission. We show that dopamine-dependent LTD of NMDAR-mediated transmission profoundly disrupts normal synaptic transmission between hippocampus and PFC. These results show how dopaminergic activation induces long-term hypofunction of NMDARs, which can contribute to disordered functional connectivity, a characteristic that is a hallmark of psychiatric disorders such as schizophrenia.

medial prefrontal cortex | long-term depression | NMDA receptor hypofunction | D2R | schizophrenia

The hippocampus to medial prefrontal cortex (PFC) projection is important for executive function and working and long-term memory (1, 2). Glutamatergic neurons of the ventral hippocampal cornu ammonis 1 (CA1) region project directly to layers 2–6 of ipsilateral PFC, and this connection synchronizes PFC and hippocampal activity during particular behavioral conditions (3–5). Disruption of hippocampal–PFC synchrony is associated with cognitive deficits that occur in disorders such as schizophrenia (6). Hippocampal–PFC uncoupling can be achieved by NMDA receptor (NMDAR) antagonism (7), and NMDAR hypofunction is a recognized feature of schizophrenia (8). However, it is unclear, first, how changes in NMDAR function at this synapse may arise, and second, how NMDAR hypofunction affects hippocampal–PFC synaptic transmission.

Canonically, NMDARs are considered to contribute little to single synaptic events, but the slow kinetics of NMDARs contribute to maintaining depolarization, leading to the generation of bursts of action potentials (9–13). Furthermore, NMDARs coordinate spike timing relative to the phase of field potential oscillations (14, 15). NMDAR transmission itself undergoes synaptic plasticity (16, 17), and this can have a profound effect on sustained depolarization, burst firing, synaptic integration, and metaplasticity (9, 11, 18, 19). In PFC, NMDARs are oppositely regulated by dopamine receptors; D1-like receptors (D1Rs) potentiate and D2-like receptors (D2Rs) depress NMDAR currents (20). Interestingly, NMDAR hypofunction (8, 21) and dopamine D2 receptor activity (22) are potentially converging mechanisms contributing to schizophrenia (23).

We now examine the contribution of NMDARs to transmission at the hippocampal–PFC synapse. We show that NMDAR activity provides sustained depolarization that can trigger action potentials during bursts of hippocampal input to PFC. We next demonstrate that dopamine D2 receptor-dependent long-term depression (LTD) of NMDAR transmission profoundly attenuates summation of synaptic transmission and neuronal firing at the hippocampal–

PFC input. These findings allow for a mechanistic understanding of how alterations in dopamine and NMDAR function can lead to the disruption of hippocampal–PFC functional connectivity, which characterizes certain psychiatric disorders.

Results

Role of NMDARs in Hippocampal–PFC Synaptic Transmission. In this study, we focus on the contribution of NMDARs to synaptic transmission at the hippocampal–PFC synaptic input. Experiments were conducted in slices of rat prefrontal cortex in which hippocampal–PFC fibers are preserved (see refs. 24, 25; Fig. S1 and Table S1). Layer 5 pyramidal neurons were current clamped at −70 mV, and single stimuli were applied to the hippocampal fiber tract [evoked excitatory postsynaptic potential (EPSP) mean amplitude, 3.2 ± 0.9 mV; $n = 10$]. The application of the NMDAR antagonist (D)-2-amino-5-phosphonopentanoic acid (AP5) did not affect peak EPSP amplitude ($108 \pm 16\%$; Fig. 1A; $P = 0.6$), but significantly reduced the 90–10% decay time of single EPSPs (from 67 ± 11 to 34 ± 4 ms, reduction to $56 \pm 6\%$ of control; Fig. 1A; $P = 0.003$), demonstrating NMDAR contribution to the duration of depolarization during a single EPSP. To examine the contribution of NMDAR activation to the summation of bursts of synaptic events, we delivered trains of 10 stimuli at different frequencies to the hippocampal afferents and measured the area under the summed EPSPs. AP5 significantly attenuated the synaptic response to 20 Hz ($36 \pm 6\%$ of control;

Significance

Synaptic transmission between the hippocampus and prefrontal cortex is required for many executive cognitive functions. It is believed that disruption of this communication contributes to symptoms observed in psychiatric disorders including schizophrenia. Hyperdopaminergic tone and hypofunction of NMDA receptor-mediated glutamate transmission are distinctive elements of schizophrenia. Here we demonstrate that activation of low-affinity D2-like dopamine receptors leads to a lasting depression of NMDA receptors at the hippocampal–prefrontal projection of juvenile rats, leading to a marked disruption of synaptic transmission. These data demonstrate a link between dopamine and hypofunction of NMDA receptor-mediated transmission with potential implications for psychiatric disease.
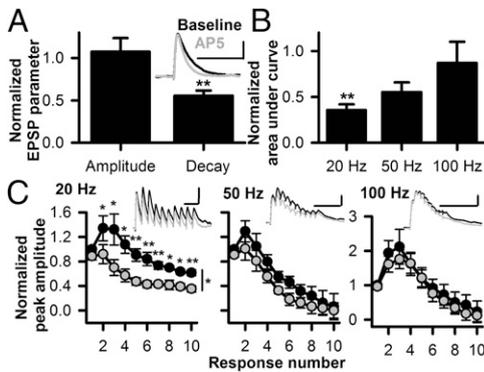
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Fig. 1. NMDAR inhibition reduces temporal summation of hippocampal–PFC transmission. (A) AP5 (50 μM) reduces 90–10% decay time of single EPSPs [$n =$ 10; paired t test $t_{(9)} = 3.96$; $P = 0.003$] without affecting peak amplitude [$t_{(9)} = 0.50$; $P = 0.63$]. (*Inset*) Before and after AP5 application. (B) Effect of AP5 on the area under the curve of EPSP bursts at different frequencies ($n = 10$). AP5 attenuates area under the curve at 20 Hz [paired t test $t_{(9)} = 3.39$; $P = 0.008$], but not at 50 Hz [$t_{(9)} = 1.63$; $P = 0.14$] or 100 Hz [$t_{(9)} = 1.36$; $P = 0.21$]. (C) Analysis of peak amplitude of each response during 20-, 50-, and 100-Hz bursts (nonspiking cells only, $n = 7$). Consistent with a lack of effect on single EPSP amplitude, response 1 was not affected by AP5 at 20 Hz [paired t test, $t_{(6)} = 1.98$; $P = 0.095$], 50 Hz [$t_{(6)} = 1.84$; $P = 0.12$], or 100 Hz [$t_{(6)} = 0.29$; $P = 0.78$]. Two-way repeated measures ANOVA (factors: drug, response number; Greenhouse-Geisser correction applied) of responses 2–10 showed a main effect of response number at 20 Hz [$F_{(1.13,6.81)} = 8.92$; $P = 0.019$], a main effect of drug [$F_{(1,6)} = 20.73$; $P = 0.004$], and crucially, a drug × response number interaction [$F_{(8,14.66)} = 3.72$; $P = 0.042$]. At 50 Hz, a main effect was shown for both factors [response number $F_{(1.06,6.37)} = 16.36$ ($P = 0.019$); drug $F_{(1,6)} = 11.05$ ($P = 0.016$)], but no interaction was observed [$F_{(1.632,9.791)} = 0.753$; $P = 0.471$]. At 100 Hz, a main effect was shown for response number [$F_{(1.36,8.14)} = 22.16$; $P = 0.001$], but not for drug [$F_{(1,6)} = 0.778$; $P = 0.412$], and there was no interaction [$F_{(1.66,9.94)} = 0.99$; $P = 0.389$]. (*Insets*) Example traces from same cell shown in A. (Scale bars, 100 ms/1 mV.) */** = $P < 0.05/0.01$, respectively.

Fig. 1 B and C; $P = 0.008$, paired t test), but not to 50 Hz (55 ± 11% of control; Fig. 1 B and C; $P = 0.14$) or 100 Hz (87 ± 23%; Fig. 1 B and C; $P = 0.2$; $n = 10$) stimulation.

As an alternative measure of summation of synaptic transmission, we determined the effect of AP5 on the amplitude of each EPSP during the bursts. This analysis was only performed on those cells that did not fire action potentials during the stimulation ($n = 7/10$). At 20 Hz stimulation, but not at 50 or 100 Hz, there was an overall decrease in temporal summation of EPSPs in the presence of AP5 compared with control conditions.

In some cells (3/10), burst stimulation of hippocampal afferents produced sufficient depolarization to elicit action potential firing. The presence of AP5 reduced the number of action potentials per stimulus train (Fig. S2A).

Thus, NMDAR-mediated transmission makes a significant frequency-dependent contribution to the synaptic activity of PFC neurons during hippocampal burst firing. In addition, NMDAR-mediated transmission makes a significant contribution to PFC neuronal spiking in response to hippocampal bursts.

LTD of NMDAR-Mediated Synaptic Transmission. Given the importance of NMDAR-mediated transmission to synaptic transmission during hippocampal burst activity demonstrated earlier, we examined how LTD of NMDAR-mediated transmission would affect hippocampal–PFC transmission. To first determine that plasticity of NMDAR transmission can occur at the hippocampal–PFC synapse, NMDAR-mediated EPSCs (EPSC$_{NMDA}$) were isolated by voltage-clamping cells at −40 mV and blocking AMPAR- and GABA$_A$R-mediated synaptic transmission with 5 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) and 50 μM picrotoxin, respectively.

Theta-frequency stimulation (TFS; 300 stimuli, 5 Hz, test intensity) resulted in lasting LTD of EPSC$_{NMDA}$ (to 58.5 ± 5.3% of baseline; Fig. 2A), which was not associated with a significant change in EPSC decay [baseline weighted tau = 164 ± 21 ms; post-TFS Tw = 204 ± 39 ms; paired t test $t_{(9)} = -0.95$; $P = 0.4$], indicating that LTD was unlikely to be associated with a switch in NMDAR subunits.

Importantly, TFS had no effect on EPSC$_{AMPA}$ (95.6 ± 6.4%; Fig. 2B). A two-way ANOVA (factors: receptor, stimulation) revealed a statistically significant interaction [$F_{(1,36)} = 15.9$; $P < 0.001$], indicating that TFS induces LTD specifically of NMDAR-mediated transmission. Furthermore, the paired pulse ratio of EPSC$_{NMDA}$ was not altered by TFS [baseline = 1.61 ± 0.1; post-TFS = 1.85 ± 0.18; $n = 6$; paired t test $t_{(5)} = -1.4$; $P = 0.22$]. This also suggests it is unlikely that LTD of EPSC$_{NMDA}$ is a result of a decrease in transmitter release.

We next examined which transmitters/receptors are responsible for LTD of EPSC$_{NMDA}$. Bath application of D2-like dopamine receptor antagonist sulpiride (10 μM) completely blocked the induction of LTD of EPSC$_{NMDA}$ (Fig. 2 C and F) but had no effect on basal transmission or maintenance of LTD (Fig. S3 A and B). In contrast, D1R-like antagonist SCH23390 (10 μM) did not block LTD (Fig. 2 C and F). Furthermore, the D1R-like agonist SKF81297 (0.5 μM) had no effect on EPSC$_{NMDA}$ (Fig. S4A), suggesting a differential role for D1 and D2Rs in regulation of NMDA receptors. Furthermore, bath application of the D2R agonist quinpirole (10 μM) also depressed EPSC$_{NMDA}$ (55.4 ± 2.8% of baseline; Fig. 2D). Delivery of TFS after quinpirole LTD had no significant effect on EPSC$_{NMDA}$ amplitude (60.6 ± 3.9%; Fig. 2D), suggesting quinpirole and TFS-LTD share common mechanisms. Together, these results suggest dopamine D2 receptors are critical for activity-dependent LTD of EPSC$_{NMDA}$.

To examine whether LTD of EPSC$_{NMDA}$ relied on NMDAR activation, neurons were voltage clamped at −100 mV during TFS, at which holding potential no EPSC$_{NMDA}$ was observed (Fig. S3C, *Inset*). Under these conditions, the induction of LTD still occurred (Fig. 2E, TFS at −100 mV; Fig. S3C). As a control, we show that hyperpolarization to −100 mV in the absence of TFS had no lasting effect on synaptic transmission (Fig. 2E, −100 mV control; Fig. S3C). Finally, delivering TFS in the presence of 50 μM AP5 did not prevent induction of LTD (Fig. 2E; AP5 + TBS; Fig. S3D). Together, these results suggest that LTD of EPSC$_{NMDA}$ does not require NMDAR activation.

We further investigated involvement of other G protein-coupled receptors in induction of NMDAR-LTD. Neither of the muscarinic receptor antagonists atropine (1 μM; Fig. 2F and Fig. S4B) and scopolamine (10 μM; Fig. 2F and Fig. S4C) prevented the induction of activity-dependent LTD. In addition, the broad-spectrum mGluR antagonist LY341495 (100 μM) did not prevent the induction of LTD (Fig. 2F and Fig. S4D).

D2-like dopamine receptors are coupled with inhibition of the adenylyl cyclase-cAMP-protein kinase A (PKA) pathway (20, 22, 26). Consistent with a key role of D2-like receptors in the generation of NMDAR-LTD, inhibition of PKA by bath application of H89 (10 μM) or KT5720 (200 nM) resulted in a slowly developing LTD of EPSC$_{NMDA}$ (Fig. 3A). Furthermore, prior activation of D2-like receptors with quinpirole markedly reduced subsequent depression by H89 (Fig. 3B), implying that D2 activation induces LTD via inhibition of PKA activity. Consistent with the lack of effect of the D1-like agonist SKF81297 upon EPSC$_{NMDA}$, the PKA agonist forskolin (10 μM) did not produce lasting effects on basal EPSC$_{NMDA}$ amplitude (Fig. 3C). Application of forskolin resulted in de-depression of EPSC$_{NMDA}$ when applied after prior TFS (Fig. S4E), implicating PKA inhibition in activity-dependent LTD of EPSC$_{NMDA}$. Previous studies have indicated that activation of glycogen synthase kinase3-beta (GSK3β) can result in depression of NMDAR transmission (27). However, we found that LTD of EPSC$_{NMDA}$ was unaffected by
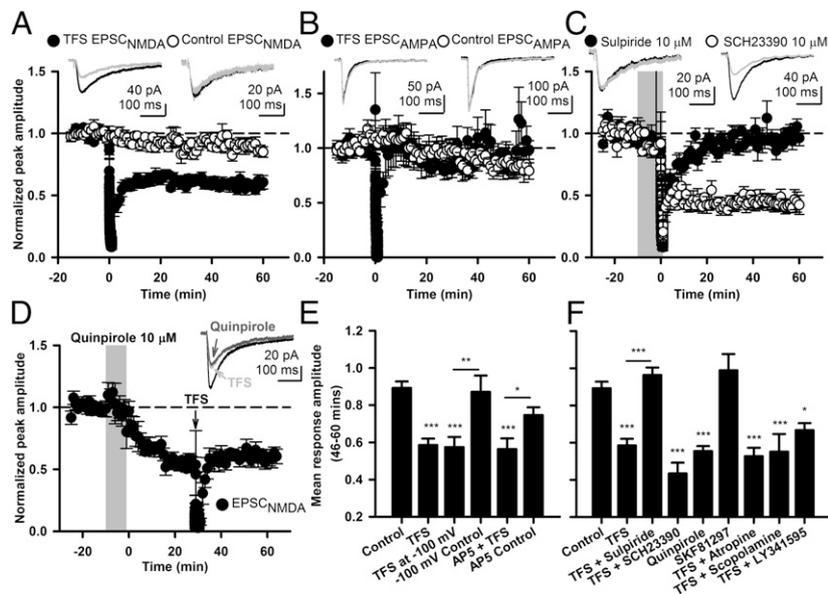
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Fig. 2. TFS induces D2R-dependent LTD of EPSC$_{NMDA}$, but not of EPSC$_{AMPA}$. (*A*) Three hundred stimuli delivered at 5 Hz (TFS; delivered at time = 0 min, filled circles) induced LTD of hippocampal–PFC EPSC$_{NMDA}$ (depressed to 58.5 ± 5.3% of baseline; *n* = 10). Open circles: time-matched control NMDA EPSCs (89.3 ± 3.5%; *n* = 13). (*B*) TFS does not depress EPSC$_{AMPA}$ (filled circles; 95.6 ± 6.4%; *n* = 10) compared with time-matched control EPSC$_{AMPA}$ (open circles; 85.3 ± 8.2%; *n* = 7). (*C*) Induction of NMDAR-LTD was prevented by prior bath application (shaded region) of the D2-like dopamine receptor antagonist sulpiride (10 μM; filled circles; 96.3 ± 4.0%; *n* = 5; one-way ANOVA, Bonferroni post hoc vs. TFS alone; *P* < 0.001), whereas D1-like dopamine receptor antagonist SCH23390 (10 μM) failed to block induction of NMDAR-LTD (open circles, 43.4 ± 6.2%; *n* = 5; *P* = 1.0). (*D*) Bath application of D2-like dopamine receptor agonist quinpirole (10 μM) significantly depressed EPSC$_{NMDA}$ (55.4 ± 2.8%; *n* = 5; Bonferroni vs. control *P* = 0.001). Subsequent TFS failed to further depress EPSC$_{NMDA}$ [60.6 ± 3.9%; paired *t* test t$_{(4)}$ = −1.14; *P* = 0.316]. (*E*) Summary of data in *A* and Fig. S3. LTD of EPSC$_{NMDA}$ by TFS does not require NMDAR activation. Hyperpo-larization of cell to −100 mV during TFS does not prevent induction of NMDAR-LTD, and hyperpolarization to −100 mV alone does not alter EPSC$_{NMDA}$ amplitude. Blockade of NMDARs with AP5 (50 μM) before TFS does not prevent induction of NMDAR-LTD compared with AP5 applied without TFS. [One-way ANOVA, main effect $F_{(5,42)}$ = 14.62; *P* < 0.001.] (*F*) Summary of data in *C* and *D* and Fig. S4. Bath application of D2R antagonist sulpiride prevents induction of NMDAR-LTD by TFS, whereas inhibition of D1Rs (SCH23390) does not. Pharmacological activation of D2Rs (quinpirole) induces LTD, but activation of D1Rs (SKF81297) has no effect. Muscarinic acetylcholine receptors (atropine, scopolamine) and mGluRs (LY341495) are not required for TFS-induced plasticity. One-way ANOVA main effect [$F_{(8,50)}$ = 14.76; *P* < 0.001]. */**/*** = *P* < 0.05/0.01/0.001, respectively, Bonferroni post hoc compared against control group unless otherwise indicated. (*Insets*) Representative traces at baseline (black) and 46–60 min (gray).

either of the GSK3β inhibitors 2-methyl-4-(phenylmethyl)-1,2,4-thiadiazolidine-3,5-dione (TDZD-8) or SB216763 (Fig. S3*F*). In addition, inclusion of 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA) in the patch pipette did not prevent the induction of LTD (10 mM; Fig. 3*D*). Thus, brief, 5-Hz stimulation results in LTD of EPSC$_{NMDA}$ that relies on dopamine D2 receptor activation and inhibition of cAMP/PKA.

Disruption of Hippocampal–PFC Transmission by LTD of NMDARs. We next examined the effects of TFS-induced LTD of NMDARs on single EPSPs and temporal summation of EPSPs at 20, 50, and 100 Hz at the hippocampal–PFC synapse 30–40 min after TFS. No change in peak amplitude of single EPSPs was detected after TFS (Fig. 4*A*). However, the decay time of single EPSPs was signifi-cantly decreased after TFS (Fig. 4*A*). The effects of TFS on peak amplitude and EPSP decay are consistent with the effects of AP5 on single EPSPs (Fig. 1*A*). Therefore, these data suggest that, as with the voltage-clamp experiments, TFS also induces robust LTD of NMDAR-mediated transmission under the current clamp con-ditions of the present experiments. In addition, these data also show that TFS is without effect on AMPAR-mediated transmission, which is consistent with the experiments showing that TFS delivered, under voltage-clamp conditions, results in LTD of EPSC$_{NMDA}$, but not EPSC$_{AMPA}$. (Fig. 2 *A* and *B*)

We then investigated the effects of NMDAR-LTD on sum-mation of EPSPs (Fig. 4 *B* and *C*). Summation of synaptic transmission during 20-Hz stimulation was significantly reduced after TFS (area under the curve, 51.4 ± 4.6%; Fig. 4 *B* and *C*). Peak-by-peak analysis of EPSP trains evoked at 20 Hz also

showed that amplitudes of responses 2–10 were reduced after TFS (Fig. 4*C*). Furthermore, TFS altered the short-term plas-ticity of the 20-Hz train (response × TFS interaction *P* = 0.042).

TFS significantly reduced synaptic summation at 50 Hz, as measured by the area under the curve (67 ± 19%; Fig. 4*B*); however, no significant effect was observed when analyzing peak amplitudes of burst responses (Fig. 4*C*). Summation of 100-Hz synaptic stimuli was not significantly affected by NMDAR-LTD (Fig. 4 *B* and *C*).

In four of 11 experiments, burst stimulation of hippocampal afferents produced sufficient depolarization to elicit spiking under control conditions (Fig. S2*B*). After TFS induced LTD of EPSC$_{NMDA}$, there was a reduction in the average number of action potentials per train of EPSPs (Fig. S2*B*).

Together, these results demonstrate that LTD of NMDAR transmission can disrupt, in a frequency-dependent manner, sum-mation of hippocampal–PFC synaptic transmission and can atten-uate subsequent action potential firing.

Disruption of Hippocampal–PFC Transmission by LTD of NMDARs Is Dependent on Activation of Dopamine D2Rs. Finally, we examined whether the disruption of synaptic transmission that occurred after TFS stimulation could be prevented by blocking LTD of EPSC$_{NMDA}$. Thus, the experiments described earlier were re-peated in the presence of 10 μM sulpiride to block D2R-dependent induction of NMDAR-LTD. Under these conditions, TFS had no effect on EPSP decay (94 ± 7% of baseline; Fig. 5*A*). Summation of EPSPs in response to bursts of synaptic stimuli was not significantly reduced at any firing frequency, as measured
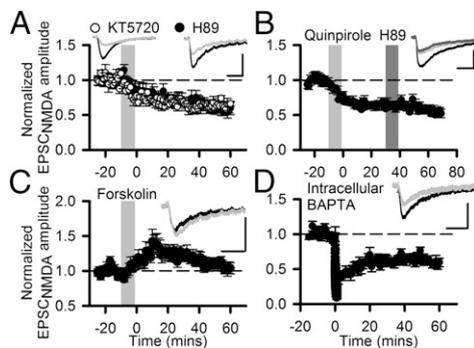
Fig. 3. NMDAR-LTD is mediated by PKA. (*A*) Inhibition of PKA mimicked the effect of D2-like dopamine receptor agonist quinpirole, producing a slowly developing LTD of EPSC$_{NMDA}$ in response to either H89 [10 μM; 58.7 ± 4.7%; $n = 5$; one-way ANOVA main effect $F_{(7,53)} = 13.76$; $P < 0.001$; Bonferroni vs. control, $P = 0.01$] or KT5720 (200 nM; 63.6 ± 5.3%; $n = 5$; Bonferroni vs. control, $P = 0.028$). (*B*) D2-like dopamine agonist quinpirole (10 μM, light gray shading and trace) depressed EPSC$_{NMDA}$ to 64.2 ± 3.8% ($n = 5$). Subsequent application of PKA inhibitor H89 (10 μM, dark gray shading and trace) resulted in a small further depression to 55.7 ± 2.5% of baseline [paired t test $t_{(4)} = 6.06$; $P = 0.004$]. The effect of H89 after quinpirole (8.6 ± 1.4% depression) was significantly smaller than that of H89 applied alone [38.8 ± 5.1% depression; t test $t_{(8)} = 5.67$; $P < 0.001$]. (*C*) PKA agonist forskolin induced a transient potentiation of EPSC$_{NMDA}$ (10 μM; amplitude 6–20 min postdrug = 128.4%; $P = 0.001$), which returned back to baseline levels within an hour of washout (107.3 ± 5.5%; $n = 8$; $P = 0.26$). (*D*) Inclusion of calcium chelator BAPTA in the intracellular recording solution did not prevent induction of NMDAR-LTD by TFS (10 mM; 61.7 ± 5.6%; $n = 5$; Bonferroni vs. TFS: $P = 1.0$). (*Insets*) Representative traces at baseline (black) and 46–60 min (gray). (Scale bars, 40 pA/100 ms.)

by area under curve (Fig. 5*B*) or by EPSP peak amplitudes (Fig. 5*C*). Finally, action potential firing was not altered after TFS in the presence of sulpiride (Fig. S2*C*).

These data show that activation of D2Rs results in LTD of NMDAR transmission that disrupts hippocampal–PFC synaptic activity. These results provide an insight into potential mechanisms by which dopamine receptor hyperfunction and NMDAR hypofunction might underlie the disruption of hippocampal–PFC synchrony that occurs in schizophrenia.

Discussion

The results of this study show that synaptic transmission from hippocampus to PFC is critically dependent on the activation of NMDARs in a frequency-dependent manner. Disruption of hippocampal–PFC transmission occurs after attenuation of NMDAR activity after application of AP5 or LTD of NMDAR-mediated synaptic transmission that relies critically on the activation of dopamine D2Rs. These results show that alterations in NMDAR function brought about by D2Rs contribute to disordered functional connectivity between hippocampus and PFC, a characteristic that is a hallmark of disorders such as schizophrenia.

NMDARs and Transmission Between Hippocampus and PFC. The hippocampal–PFC connection is critical in associative learning (2), and synchronization of PFC–hippocampal activity is important for working memory (5, 28, 29). We found that bursts of EPSPs at lower (20 Hz), but not higher (50 or 100 Hz), frequency are highly sensitive to NMDAR antagonism. Thus, NMDARs play a crucial role in determining the synaptic activity profile between hippocampus and PFC, maintaining the postsynaptic cell at depolarized membrane potential longer during 20 Hz transmission. Frequencies of ∼20 Hz occur in the hippocampus during spatial navigation (30), so NMDARs control synaptic transmission at physiologically relevant frequencies. In addition, NMDAR activation also contributed to spike firing in layer 5

PFC pyramidal neurons during bursting activity. Therefore, NMDAR activity controls hippocampal–PFC synaptic transmission and regulates the output of PFC pyramidal cells. Hence, NMDAR-dependent regulation of transmission between hippocampus and PFC may play an important role in associative and working memory.

LTD of EPSC$_{NMDA}$. Our data show that activity-dependent LTD of EPSC$_{NMDA}$ at the hippocampal–PFC synapse relies on endogenous activation of D2Rs. This significantly extends the physiological relevance of previous studies demonstrating that activation of D2Rs by exogenous agonists can depress NMDA agonist currents or NMDA EPSCs (27, 31–33). In contrast to the report that in 9–10-mo-old mice only a subpopulation of PFC pyramidal neurons express D2Rs (34), we found that in every case tested, LTD was D2R-dependent. Therefore, under the conditions of our experiments, it is unlikely that a large proportion of pyramidal neurons lack D2Rs. In contrast to previous reports of D1R-mediated enhancement of NMDAR transmission (20, 35), the application of a D1 agonist had no effect on the hippocampal–PFC EPSC$_{NMDA}$ in the present study. Although changes in NMDAR function in the hippocampus have been demonstrated to be dependent on muscarinic (36) and group I mGluR activation (37) the activity-dependent LTD in our study did not rely on activation of either of these receptor types.

Different mechanisms for D2R-dependent depression of NMDA responses have been suggested previously (32, 38). Although we were not able to confirm a role for GSK3β (27) in the D2R-dependent LTD of EPSC$_{NMDA}$, our results showing that the PKA inhibitor H89 depresses EPSC$_{NMDA}$ are consistent with a D2R-dependent inhibition of cAMP/PKA signaling being critical for
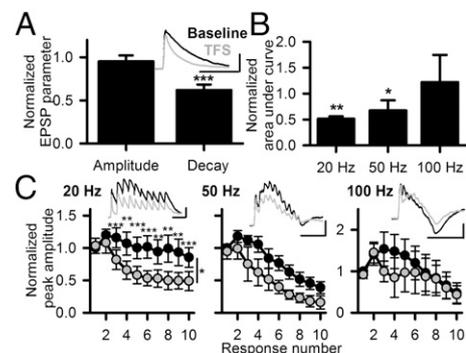

Fig. 4. TFS-induced NMDAR-LTD impairs temporal summation during burst stimulation. (*A*) TFS reduces the decay time of single EPSPs [57.1 ± 7.3% of baseline; $n = 11$; paired t test, $t_{(10)} = 5.49$; $P < 0.001$] without affecting peak amplitude [99 ± 7%; $t_{(10)} = 0.99$; $P = 0.345$]. (*B*) Thirty minutes after TFS, the area under the curve of 20 Hz [paired t test, $t_{(10)} = 4.64$; $P = 0.001$] and 50 Hz [$t_{(10)} = 3.10$; $P = 0.011$] synaptic bursts was reduced, whereas at 100 Hz, no effect was observed [$t_{(10)} = 0.313$; $P = 0.761$]. (*C*) Analysis of peak amplitude of each response during different frequency bursts (nonspiking cells only, $n = 8$ at 20 Hz; $n = 7$ at 50 and 100 Hz). Statistical analysis showed that the amplitude of response 1 was not affected by TFS at any frequency [repeated measures ANOVA 20 Hz: $F_{(2,16)} = 0.8$ ($P = 0.5$); 50 Hz: $F_{(2,12)} = 1.1$ ($P = 0.4$); 100 Hz: $F_{(1.170,7.020)} = 2.6$ ($P = 0.15$)]. Two-way repeated-measures ANOVA (factors: TFS, response number; Greenhouse-Geisser correction applied) of responses 2–10 in the 20-Hz burst showed a main effect of response number [$F_{(1.895,13.262)} = 15.7$; $P < 0.001$] and of TFS [$F_{(1,7)} = 73.0$; $P < 0.001$] and a response × TFS interaction [$F_{(2.030,14.210)} = 3.3$; $P = 0.042$]. At 50 Hz, a main effect of response number was found [$F_{(2.275,13.653)} = 12.1$; $P = 0.001$]; however, no significant main effect of TFS [$F_{(1,6)} = 5.3$; $P = 0.061$] or interaction [$F_{(2.221,13.325)} = 1.1$; $P = 0.4$] was observed. At 100 Hz, effects were not significant for response number [$F_{(1.762,10.57)} = 3.6$; $P = 0.07$], TFS [$F_{(1,6)} = 0.8$; $P = 0.4$] or their interaction [$F_{(2,11.997)} = 2.4$; $P = 0.13$]. */**/*** = $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. (*Insets*) Representative traces at baseline (black) and after TFS (gray). (Scale bars, 2 mV/100 ms.)
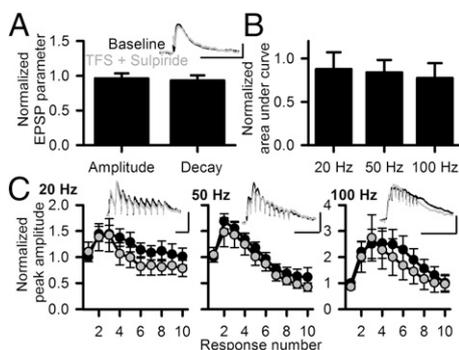
Fig. 5. D2R antagonism blocks TFS-induced effects on NMDAR transmission and TFS reduction in temporal summation. (*A*) In the presence of D2-like dopamine receptor antagonist sulpiride, TFS affects neither the peak amplitude [$n = 7$; paired t test, $t_{(6)} = 0.33$; $P = 0.75$] nor the decay time of individual EPSPs [$t_{(6)} = 0.419$; $P = 0.69$]. (*Inset*) Example EPSPs. (*B*) When delivered in the presence of sulpiride (10 µM), TFS had no effect on area under the curve of synaptic bursts at 20 [paired t test, $t_{(6)} = 1.36$; $P = 0.22$], 50 [$t_{(6)} = 1.42$; $P = 0.21$], or 100 [$t_{(6)} = 1.55$; $P = 0.17$] Hz. (*C*) Response by response analysis of temporal summation (nonspiking cells only) showed that peak amplitude changed as a function of response number at 20 Hz [two-way repeated-measures ANOVA, main effect of response number $F_{(2.381,16.667)} = 16.0$; $P < 0.001$], 50 Hz [$F_{(1.625,9.751)} = 14.1$; $P = 0.002$], and 100 Hz [$F_{(1.274,6.371)} = 8.3$; $P = 0.023$]; however, in the presence of sulpiride, no main effect of TFS was observed at any frequency [20 Hz: $n = 7$; $F_{(1,7)} = 4.7$; $P = 0.067$; 50 Hz: $n = 6$, $F_{(1,6)} = 1.8$, $P = 0.2$; 100 Hz: $n = 6$, $F_{(1,6)} = 1.8$, $P = 0.2$], nor were response × TFS interactions seen [20 Hz: $F_{(1.723,12.063)} = 1.7$ ($P = 0.2$); 50 Hz: $F_{(2.482,14.892)} = 0.3$ ($P = 0.8$); 100 Hz: $F_{(1.492,7.460)} = 2.9$ ($P = 0.12$)]. (*Insets*) Representative traces at baseline (black) and after TFS (gray). (Scale bars, 2 mV/100 ms.)

LTD (38). This finding for layer 5 PFC pyramidal cells is in contrast to a previous report suggesting a lack of cAMP/PKA signaling in D2R inhibition of NMDA currents in CA1 pyramidal neurons (31), but is in keeping with the mechanisms proposed to underlie the D2R-dependent decrease in NMDAR-dependent calcium influx in striatopallidal neurons (39).

That TFS resulted in LTD selectively of EPSC$_{NMDA}$, but not of EPSC$_{AMPA}$, indicates that LTD of NMDA transmission is not likely to rely on a decrease in transmitter release (39). Several mechanisms have been postulated for LTD of NMDARs, including internalization of receptors (37), diffusion of receptors to extrasynaptic sites (40), or a switch in NMDAR subunits (41). A recent report has suggested that prolonged D2R activation can decrease surface expression of NR2B-containing NMDARs in the CA1 region of hippocampus (42), and evidence suggests NR2B subunits have greater lateral mobility than NR2A (43). However, we found that LTD was not associated with a change in decay kinetics of EPSC$_{NMDA}$, suggesting no change in NMDAR subunit composition in our experiments. PKA activity has been shown to enhance NMDAR function via phosphorylation of NR1, NR2A, and NR2B subunits (44–46); thus, a reduction in PKA activity and subsequent dephosphorylation of NMDARs may provide a potential mechanism underlying the decrease in NMDAR currents (44).

Consequences of LTD of NMDAR for Synaptic Transmission. Activity-dependent plasticity of NMDAR transmission occurs at many synapses and can lead to metaplasticity (11, 18, 19). NMDAR plasticity may also play a homeostatic role. Plasticity of NMDAR transmission can accompany that of AMPA receptor (AMPAR) transmission, thereby preserving the AMPAR:NMDAR ratio (47). However, as TFS did not induce plasticity of AMPAR-mediated transmission, it is unlikely that the activity-dependent LTD of NMDARs fulfils a homeostatic function at the hippocampal–PFC input.

Aside from metaplasticity, a consequence of the long-term plasticity of NMDA transmission is the effect on synaptic transmission. We demonstrate that the normal EPSP summation and spike firing in layer 5 pyramidal neurons, driven by bursts of activity in hippocampal afferents, is significantly attenuated after activity-dependent LTD of NMDAR transmission. Previous studies have underlined the importance of NMDAR transmission in temporal summation between pairs of PFC pyramidal neurons, where they may play a key role in recurrent excitation (48) and persistent firing (49). Such effects may contribute to working memory processes in PFC. NMDA receptors also play a key role in integration of synaptic input in dendritic branches (10) and in the generation of plateau potentials, which can result in somatic spiking (50). Although temporal summation did not result in spiking in the majority of cases in this study, probably because of the relatively modest EPSP amplitude (~2–3 mV), NMDAR-mediated transmission may facilitate spatiotemporal integration of separate inputs in hippocampal neurons (51). Correspondingly, NMDAR LTD is likely to profoundly affect the input–output characteristics of PFC neurons receiving direct input from the hippocampus.

Implications of D2R-Induced LTD of NMDA. Dysfunction of the PFC is a widely reported phenomena in schizophrenia, in which the role of PFC in executive function and working memory is thought to be disrupted. Traditionally, hypotheses concerning mechanisms of schizophrenia have focused on dysfunction of the dopaminergic and glutamatergic systems, with D2R-like receptor hyperactivity (52) and NMDAR hypofunction (8, 53) being key features. Recently, it has been shown that cotreatment of patients with schizophrenia with both D2R-like antagonists and agents that increase NMDAR coagonist activity, such as D-serine or glycine transporter antagonists, are more effective than either treatment alone (54–56), supporting hypotheses of schizophrenia that include interaction of dopamine and NMDAR systems (21, 57, 58).

Here we present evidence that NMDAR transmission is a key driver of normal EPSP summation and action potential firing at the hippocampal–PFC projection where glutamatergic and dopaminergic projections converge on layer 5 pyramidal neurons (59, 60). D2R activation leads to a long-lasting reduction of NMDAR function in the hippocampal–PFC pathway that critically disrupts temporal summation at the hippocampal–PFC synapse and can lead to decreased PFC spiking in response to bursts of hippocampal activity. As there is evidence showing the importance of hippocampal–PFC signaling in working memory (5, 28, 29), associative memory (2), and executive function (61), cognitive faculties negatively affected in schizophrenia, we hypothesize that D2R-induced NMDAR hypofunction at the hippocampal–PFC pyramidal cells is a key dysfunction in the pathology of schizophrenia.

Materials and Methods

Animals. Experiments were conducted in male pigmented rats (Lister Hooded strain) postnatal day 30–32. All experiments were performed in accordance with the U.K. Animals (Scientific Procedures) Act 1986 and associated guidelines and had approval from the University of Bristol Ethics Committee. All efforts were made to minimize suffering and the number of animals used.

Electrophysiology. Rats were decapitated under isoflurane anesthesia, and the brain was removed and rapidly submerged in ice-cold (2–4 °C) oxygenated (95% O$_2$–5% CO$_2$) artificial cerebrospinal fluid (aCSF) containing (in mM): 124 NaCl, 3 KCl, 26 NaHCO$_3$, 1.25 NaH$_2$PO$_4$, 1 MgSO$_4$, 10 D-glucose, and 2 CaCl$_2$. The brain was cut at an 11° modified coronal angle using a custom brain matrix (Zivic Instruments), and 400-µm slices were made using a vibratome before storing in room temperature aCSF for ≥1 h before use. Slices equivalent to +2.7 to +2.2 mm from bregma were perfused with aCSF at 2 mL/min. Whole-cell patch clamp recordings were made from layer 5 neurons using 2–6-MΩ glass pipettes filled with either cesium

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methylsulfonate-based solution (voltage clamp) or potassium gluconate-based solution (current clamp). Hippocampal–PFC responses were evoked by applying 0.1-ms constant current pulses to the hippocampal fiber tract, using a concentric bipolar stimulating electrode (24). Where applicable, NMDAR-mediated currents were isolated by bath application of picrotoxin (50 μM) and NBQX (5 μM) and voltage clamp of cells at −40 mV.

Detailed information on electrophysiology and analysis can be found in *SI Materials and Methods*.

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