Section: Biological Sciences

Dopaminergic neuromodulation of prefrontal cortex activity requires the NMDA receptor co-agonist D-serine

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Significance

Dopamine and glutamate in the prefrontal cortex are important substrates of higher cognitive functions, which are impaired in neuropsychiatric disorders. As regards glutamatergic pathways, a role for the NMDA receptor co-agonist D-serine has been highlighted yet its relationship to dopaminergic transmission remains unclear. In this study we reveal that D-serine plays a pivotal role in the modulation by dopamine of NMDA receptor activity and cognitive performance in the prefrontal cortex. Comprehensive evidence for this interaction is provided at the synaptic, neuronal, network, and behavioral level. These observations are of relevance to the pathophysiology and treatment of cognitive impairment in numerous disorders involving disruption of the frontocortical dialogue between dopamine and glutamate.

Abstract

Prefrontal control of cognitive functions critically depends upon glutamatergic transmission and NMDA receptors (NMDARs), the activity of which is regulated by dopamine. Yet, whether the NMDA receptor co-agonist D-serine is implicated in the dopamine-glutamate dialogue in prefrontal cortex (PFC) and other brain areas remains unexplored. Here, using electrophysiological recordings, we show that D-serine is required for the fine-tuning of glutamatergic neurotransmission, neuronal excitability and synaptic plasticity in the PFC through the actions of dopamine at D_1- and D_3- receptors. Using *in vivo* microdialysis, we show that D_1- and D_3- receptors exert a respective facilitatory and inhibitory influence on extracellular levels and activity of D-serine in the PFC, actions expressed primarily *via* the cAMP/PKA signalling cascade. Further, using fMRI imaging and behavioral assessment, we show that D-serine is required for the potentiation of cognition by D_3R blockade as revealed in a test of novel object recognition memory. Collectively, these results unveil a key role for D-serine in the dopaminergic neuromodulation of glutamatergic transmission and PFC activity, findings with clear relevance to the pathogenesis and treatment of diverse brain disorders involving alterations in dopamine-glutamate cross-talk.

Introduction

The prefrontal cortex (PFC) supports the higher-order and top-down coordination of complex behaviors, including executive function, working memory and social interactions (1). Dopaminergic (DAergic) inputs exert a marked influence on the activity of frontocortical circuits and the dynamic regulation of background dopamine (DA) levels allows for the optimization of PFC cognitive performances (2). Interestingly, DA exerts opposite effects on glutamatergic activity through activation of D_1-type (D_1- and D_5-) and D_2-type (D_2-, D_3-, and D_4-) receptors, respectively (3, 4). Specifically, D_1 receptor (D_1R) activation increases neuronal excitability and synaptic efficacy through modulation of *N*-methyl D-aspartate receptors (NMDARs), whilst D_2 receptor (D_2R) signaling produces opposite effects (4). Furthermore, activation of D_1R promotes Long-Term Potentiation (LTP) in the PFC (5, 6) whereas D_2R stimulation is required for inducing Long-Term Depression (LTD) (6). These observations support the current view that distinct cortical DA receptors differentially regulate cognitive functions through the modulation of NMDARs. However, the underlying mechanisms still await clarification. While D_1R activation in the PFC consistently facilitates working memory and executive function (7), the significance of PFC-localized D_2-type receptors is more complex inasmuch as D_2R and D_3R subtypes enact contrasting roles: activation of D_2R favors cognitive processes whereas D_3R stimulation exerts a negative impact on cognition (8). Accordingly, blockade of D_3Rs results in a broad-based positive influence upon cognition, including improvements in social novelty discrimination and novel object recognition (NOR) and a reduction in the cognitive deficits associated with a developmental model for schizophrenia (SCZ) (9, 10). Moreover, it has been proposed that DAR and NMDARs can form clusters at the surface of neurons (11) and that their reciprocal dialogue relies on intracellular signaling cascades involving protein kinases (12). Whether functional DA-NMDAR cross-talk involves others mechanisms is not known.

Activation of the canonical GluN1-GluN2 containing NMDARs requires binding of both glutamate and a co-agonist, either glycine or D- serine (13). We have previously shown that D-serine rather than glycine is the primary endogenous co-agonist of synaptic NMDARs at glutamatergic synapses connecting pyramidal neurons in the PFC of adult rats (14). This is important since genetic linkage and association studies show that the genes encoding the D-serine producing and degrading enzymes serine racemase (SR) and D-amino acid oxidase (DAAO), respectively, are risk genes for SCZ (15, 16). Accordingly, serum and cerebrospinal fluid levels of D-serine are reduced in SCZ patients (16-18). Moreover, clinical trials of D-serine supplementation or the administration of DAAO inhibitors have yielded encouraging

results (17, 18). An involvement of D-serine in SCZ is further supported by animal studies showing that multiple risk pathways for SCZ converge in SR deficient mice (19). Yet, our understanding of the significance of D-serine to the pathology and symptoms of SCZ remains limited, and the question of how it interacts with DA signaling remains to be resolved. This issue is, moreover, of broader pertinence in view of accumulating evidence that a malfunction of NMDAR is central to the pathophysiology of multiple brain disorders (20).

In the current study, we sought to determine whether and how D-serine and DA interact in the PFC. We show that D-serine is indeed required for the dopaminergic modulation of NMDARs in the PFC, as expressed in measures of neuronal excitability, synaptic plasticity and cognitive function, providing a novel framework for understanding PFC integrated DA-glutamate crosstalk under physiological and pathological conditions.

Results

Modulation of NMDAR and neuronal excitability by DA involves D-serine

To test the hypothesis that D-serine may be implicated in DA modulation of NMDAR functions, we first investigated whether DA modulations of isolated synaptic NMDAR-mediated excitatory postsynaptic currents (NMDA-EPSCs) recorded at +40 mV and -70 mV in adult rat prelimbic cortex (PrL) layer 5 pyramidal neurons (Fig.1 A-C) require D-serine. Low concentrations of DA (1 µM) potentiated NMDA-EPSCs by 73.9 ± 14.1% (Fig.1D, I, *SI appendix* Fig.S8) while higher concentrations (10 µM) decreased NMDA-EPSCs by 44.7 ± 6.7% (Fig.1D, I, *SI appendix* Fig.S8) confirming previous studies (21, 22). To determine if these bidirectional modulations of NMDA-EPSCs by DA imply changes in the occupancy of NMDARs by a co-agonist, we monitored the effects of DA after pre-saturation of the co-agonist binding site by exogenous D-serine (100 µM) (Fig.1E, I). This resulted in the occlusion of DA-effected NMDA-EPSCs modulation. We then directly addressed the implication of D-serine in the DA modulation of NMDA-EPSCs by treating the slices with *Rhodotorula gracilis* D-amino acid oxidase (*Rg*DAAO) (0.2 U/mL) to specifically deplete endogenous D-serine (14, 23) (Fig.1F, I). Akin to the effect of saturating the D-serine binding site, removal of D-serine with *Rg*DAAO markedly reduced the potentiating and inhibitory effect of 1 µM and 10 µM DA, respectively. As an important control we also confirmed, using enzymatic assays, that DA is not a substrate or an inhibitor for *Rg*DAAO (*SI appendix* Fig.S1). These data therefore indicate that positive and negative modulations of NMDAR by exogenous DA requires D-serine.

We next assessed whether D-serine is involved in the regulation of NMDA-EPSCs by endogenous DA. To this end, we first studied the effects of the DA reuptake inhibitor GBR12909 on NMDA-EPSCs. Bath applied GBR12909 (0.5 µM) potentiated NMDA-EPSCs (Fig.1G, I) similarly to exogenous low DA (1 µM). Remarkably, performing this experiment under D_1R blockade by addition of the antagonist SCH39166 (1 µM) not only abolished the potentiating effect of GBR12909, but also unmasked a depressant action on NMDA-EPSCs (Fig. 1G, I). To directly address whether the potentiating effect of GBR12909 requires D-serine acting on NMDARs, we next recorded NMDAR currents on PFC slices isolated from mice lacking the D-serine producing enzyme serine racemase ($SR^{-/-}$), in which D-serine content is ~10% of wild-type ($SR^{+/+}$) mice (24). Bath application of GBR12909 (0.5 µM) potentiated NMDA-EPSCs in $SR^{+/+}$ control slices and depressed these currents upon blockade of D_1Rs. In contrast, in slices from $SR^{-/-}$ mice, GBR12909 failed to induce any changes in NMDA-EPSCs (Fig.1H, I). Together, these results indicate that D-serine is required for appropriate DA modulation of NMDA-EPSCs.

As DA is known to regulate PFC activity through modulation of pyramidal neurons excitability (4) and modulation of NMDARs also influences PFC neuronal firing activity (25, 26), we next investigated whether D-serine is also involved in the DA modulation of layer 5 pyramidal neurons excitability. To this end, cell firing was induced by incremental square depolarizing current pulses during whole-cell current-clamp recordings. DA concentrations of 1 µM and 10 µM applied to rat PFC slices respectively increased and decreased neuronal excitability (*SI appendix* Fig.S2A,B) without altering membrane properties (*SI appendix* Table 1). Depletion of D-serine with *Rg*DAAO prevented these changes in excitability (*SI appendix* Fig.S2C,D). We also undertook these experiments in PFC slices from $SR^{-/-}$ mice and $SR^{+/+}$ mice. DA at 1 µM and 10 µM induced changes in $SR^{+/+}$ mice cell excitability similar to those observed in rats, without altering membrane properties (*SI appendix* Table 2; Fig.S2E,F). Absence of D-serine in $SR^{-/-}$ mice resulted in a complete loss of these DA modulations, mirroring the effect observed with *Rg*DAAO (*SI appendix* Fig.S2G,H). These results thus indicate that D-serine is required for DA-induced changes in NMDAR-dependent neurotransmission and excitability at PFC pyramidal neurons.

Opposite influence of D_1R and D_3R activation on NMDAR

Positive DA modulation on PFC pyramidal neurons is classically attributed to D_1-type receptor activation whereas negative modulation is thought to be mediated by the D_2-type receptor (3). We then tested whether modulation of NMDA-EPSCs by selective D_1-type and D_2-type agonists required D-serine. As D_3Rs were recently found to play an important

regulatory role in the PFC and in SCZ (27), we ensured that we could detect these receptors in patched PFC neurons using single cell RT-PCR (Fig.2A) and particularly focused on this subtype of receptor. Bath application of the preferential D_3R agonist PD128907 (1 µM) reduced NMDA-EPSCs whereas the D_1R agonist SKF81297 (10 µM) induced a reliable potentiation of NMDA-EPSCs (Fig.2C,E). As with DA, actions of these two agonists were strongly impaired by saturating the NMDARs co-agonist binding site with exogenous D-serine (100 µM, Fig.2D,E). Furthermore, application of S33084 (0.1 µM), a selective D_3R antagonist (10), fully abolished the inhibitory effect of PD128907 on NMDA-EPSCs but did not alter the potentiating effect of the D_1R agonist SKF81297 (*SI appendix* Fig.S3).

We next analyzed the effects of D_1- and D_3R agonists on NMDA-EPSCs in $SR^{-/-}$ mice. Mirroring rat PFC slices, application of SKF81297 and PD128907 respectively increased and decreased NMDAR-EPSCs in $SR^{+/+}$ slices (Fig.2F,H) and these effects were markedly compromised in $SR^{-/-}$ slices (Fig.2G,H). To further confirm that synaptic deficits in $SR^{-/-}$ mice were specifically attributable to a lack of D-serine and not to other putative modulators such as glycine or D-aspartate, we restored its levels in $SR^{-/-}$ mice by chronic subcutaneous administration ([D-serine]=150 mg/kg) for 21 days, as previously described (19). While levels of D-serine, D-aspartate and glycine in the medial PFC (mPFC) were respectively decreased by ~89%, ~35%, and ~69% in saline-treated $SR^{-/-}$ mice, treatment with D-serine selectively re-established levels of D-serine alone to near control values in mutant mice (*SI appendix* Fig.S4). Importantly, this resulted in the normalization of the modulatory actions of D_1R and D_3R agonists on NMDA-EPSCs in D-serine-treated but not in saline-treated $SR^{-/-}$ mice (Fig.2I-K). Thus, these rescue experiments confirm that in $SR^{-/-}$ mice, D-serine is the missing element affecting DAergic modulation of NMDARs. Collectively, these experiments demonstrate that D-serine is a critical factor for DA modulation of NMDARs *via* D_1- and D_3-Rs.

D-serine mediates DA modulations of synaptic plasticity

Activity-dependent changes of synaptic strength in the PFC rely on NMDARs (6, 14) and are strongly regulated by DA (3, 5, 6). In particular, D_1-type receptor stimulation enhances LTP whilst LTD requires the activation of both D_1- and D_2-type receptors (5, 6). To specifically determine the contribution of D-serine to DA modulation of NMDAR-dependent long-term changes in synaptic plasticity, we investigated the effect of DA receptor stimulation on excitatory post-synaptic field potential (fEPSP) plasticity in the PrL of $SR^{+/+}$ and $SR^{-/-}$ mice. Analyses of $SR^{-/-}$ mice synaptic function revealed that basal synaptic transmission was reduced by ~26% (Fig.3A), a decrease most likely attributable to post-synaptic mechanisms

as paired-pulse experiments did not show any changes in release probability (Fig.3B). These results are reminiscent of previously reported NMDAR hypofunction in $SR^{-/-}$ mice (19, 24). Although no difference in LTP magnitude between $SR^{+/+}$ and $SR^{-/-}$ mice was detectable (Fig.3C), D_1-type receptor activation by SKF81297 (10 µM) enhanced LTP in $SR^{+/+}$ (Fig.3D), consistent with previous investigations (5, 6), but not $SR^{-/-}$ mice (Fig.3E), thereby indicating that D-serine is required for DAergic modulation of PrL LTP.

We then investigated LTD. As previously reported (6), low-frequency stimulation (LFS) could only induce LTD when performed in conjunction with D_1- and D_2-type receptor activation (*SI appendix* Fig.S5, Fig.3F). Indeed, combining SKF81297 (10 µM) and quinpirole (1 µM) with LFS elicited LTD (*SI appendix* Fig.S5D). Strikingly the latter effect was abolished in D_3R knock-out ($D_3R^{-/-}$) mice (*SI appendix* Fig.S5D), indicating that D_3Rs play a critical role in the DA-dependent generation of LTD in the PrL. Accordingly, co-stimulation of D_1R and D_3R with SKF81297 and PD128907 combined with LFS reliably induced LTD in $SR^{+/+}$ mice (Fig.3F). Importantly, this DA-dependent LTD was markedly reduced by ~ 62% in $SR^{-/-}$ mice (Fig.3F). Collectively, these data indicate that D-serine is critical for the dopaminergic modulation of long-term synaptic plasticity in the PrL *via* both D_1R and D_3R.

Activation of D_1R and D_3R modulates PFC D-serine levels *in vivo*

To gain insights into how D-serine participates in the DAergic modulations of NMDAR function, we tested whether activation of D_1- and D_3Rs could regulate extracellular levels of this NMDAR co-agonist. To this end, we performed bilateral *in vivo* microdialysis in the mPFC of freely moving mice measuring basal extracellular levels of D- and L-serine for 1 h in response to perfusion of artificial cerebrospinal fluid (aCSF) as a control, the D_1R agonist SKF81297 (10 µM), the D_3R agonist PD128907 (1 µM) or a combination of both (Fig.4A-C). The basal levels of D-serine and L-serine in the mPFC were 812.80 ± 22.97 nM and 1514.00 ± 126.30 nM respectively, in the range of previous studies (28, 29). SKF81297 increased extracellular levels of D-serine level by ~17%, providing a mechanism for the D-serine dependent activation of NMDARs by D_1Rs reported herein. Although activation of D_3Rs with PD128907 alone did not induce any change in extracellular D-serine levels, it blunted the increase in D-serine levels elicited by D_1R activation (Fig.4C, left) indicating functional cross-talk between D_1R and D_3R. Interestingly, local infusion of SKF81297 also increased levels of L-serine, the precursor of D-serine, and this effect was also blunted by D_3Rs activation (Fig.4C, right). These data therefore indicate that D_1R and D_3R activation modulate extracellular levels of D-serine *via* direct release as well as increased biosynthesis by SR and/or decreased degradation by DAAO. Since we found that chronic supplementation of D-

serine was sufficient to restore D_1R and D_3R modulations of NMDARs in $SR^{-/-}$ mice, we conclude that release of D-serine is the primary process at play.

To ascertain whether the inhibitory influence of D_3R on D_1R is indeed functionally relevant at NMDARs, we recorded NMDA-EPSCs in the presence of PD128907 (1 µM) and under these conditions applied SKF81297 (10 µM). Instead of potentiating NMDA-EPSCs as occurs when applied alone (Fig.2F), SKF81297 in the presence of PD128907 induced no change (Fig.4D), thus corroborating the inhibitory action that D_3R activation exerts on D_1R function. These data suggest that D-serine function at synaptic NMDARs can be fine-tuned by DAergic input *via* the balanced activation of D_1- and D_3Rs (Fig.4D).

The balance of D_1 *vs* D_3R activation regulates D-serine functions *via* the cAMP/PKA pathway

We next sought to determine the signaling cascade underlying the D_1/D_3R cross-talk regulating D-serine function. To this end, we focused on the adenylyl cyclase (AC) – cAMP – protein kinase A (PKA) pathway because AC activity is differentially influenced by both D_1- and D_3R (3) and PKA is known to regulate exocytosis (30, 31). We posited that D_1R activation leads to an upregulation of AC activity to induce an increase in D-serine release whereas D_3Rs activation inhibits this process by suppression of AC. To test this hypothesis, we inhibited AC with SQ22536 (20 µM) (Fig.5A) or PKA with H89 (10 µM) (Fig.5B) in $SR^{+/+}$ PFC slices and under these conditions activated D_1Rs with SKF28197 (10 µM). Blocking AC or PKA yielded a similar blunting effect on D_1R-induced facilitation of NMDA-EPSCs (Fig.5A-B) as activation of D_3Rs (Fig.4D), thus corroborating our hypothesis. Accordingly, blockade of PKA also occluded the effect of D_3R activation (Fig.5C). To directly test whether this signaling pathway is indeed underlying DAergic modulation of D-serine functions, we assessed the effect of AC activation with forskolin (50µM) on $SR^{+/+}$ and $SR^{-/-}$ NMDA-EPSCs. While, akin to D_1R stimulation, AC activation induced a robust increase in NMDA-EPSCs amplitude in $SR^{+/+}$ mice (+57.60 ± 10.94%), this effect was abolished in $SR^{-/-}$ mice (Fig.5D). Together these data identify the AC-cAMP-PKA signaling cascade as a key intracellular pathway transducing the opposing actions of D_1R^- and D_3R on D-serine function.

Dopaminergic modulation of PFC cognitive functions involves D-serine

To evaluate the implication of this newly identified neuromodulatory interplay on cognition, we next investigated the role of D-serine in D_3R modulation of PFC-dependent cognitive processes. We previously showed that antagonizing D_3Rs with systemic administration of S33084 (0.63 mg/kg) enhances rat short-term memory in the novel object

recognition (NOR) task (10). Here, we further established that this effect was attributable to changes in PFC function by testing NOR memory in rats after intra-PFC administration of S33084 5 min prior to the acquisition phase (T1, *SI appendix* Fig.S6A). As with systemic administration, treatment resulted in the retention of the novel object 4h after T1, while in control vehicle-treated rats NOR memory was lost at this inter-trial interval (ITI) (10), *SI appendix* Fig.S6B), thus confirming the PFC origin of the pro-cognitive effect of D_3R blockade on recognition memory.

To test the involvement of NMDARs in this pro-cognitive effect, the non-competitive NMDAR channel blocker MK801 (0.05 mg/kg, i.p.) or the selective and competitive NMDAR antagonist CPP (10 mg/kg, i.p) were administered 15 min prior to S33084 (0.63 mg/kg, s.c). Both NMDAR blockers prevented the pro-cognitive effect of D_3R antagonism on NOR memory (Fig.6A). We also studied the contribution of the co-agonist modulatory binding site using the selective D-serine/glycine binding site antagonist, L701,324 (5 mg/kg, i.p.) (32), which also abolished the reversal of delay-induced impairment in NOR memory by S33084 (Fig.6A). These results indicate that the pro-cognitive action of S33084 depends on NMDAR function and activation of the co-agonist site, possibly *via* D-serine.

To further explore this possibility, we assessed NOR memory in $SR^{+/+}$ and $SR^{-/-}$ mice. The two genotypes displayed similar baseline NOR performances, being intact at 2 min and 1 h after the acquisition trial but lost after a 2 h ITI (*SI appendix* Fig.S6C*)*. Thus, a deficit in D-serine does not affect NOR performance *per se*. However, S33084 applied systemically (0.63 mg/kg, s.c) 30 min before T1 or by local intra-PFC injection (2.5 µg, 0.5 µL) 5 min prior to T1 reversed the 2 h delay-induced NOR impairment in $SR^{+/+}$ mice (Fig.6B, *SI appendix* Fig.S6D). This result indicates that, in mice, D_3R antagonism also yields a pro-cognitive action that relies on PFC neuromodulation. Strikingly, S33084 (0.63 mg/kg, s.c.) was ineffective in $SR^{-/-}$ mice (Fig.6B), thus demonstrating the pivotal role of D-serine in the DA modulation of PFC-dependent cognitive functions. We here further ensured that this impairment was indeed attributable to the lack of D-serine in $SR^{-/-}$ mice by performing rescue experiments in which D-serine supplementation of $SR^{-/-}$ mice reversed this deficit (Fig. 6B). Taken together, these data demonstrate that, in the PFC, modulation of D-serine function *via* D_3Rs impacts cognition.

Dopaminergic regulation of PFC global activity is impaired in SR knock-out mice*.*

We finally tested *in vivo* whether D_3R modulation of PFC activity is impaired in $SR^{-/-}$ mice by pharmacological magnetic resonance imaging (phMRI). Since antagonizing D_3Rs

potentiates the cerebral blood volume response (rCBV) to the DA-releasing compound D-amphetamine in cingulate cortex (33), we reasoned that if D-serine is required for optimal DAergic modulation of PFC activity, the rCBV change in response to D-amphetamine in the presence of S33084 (0.63 mg/kg, s.c.) should be impaired in $SR^{-/-}$ mice. As predicted, $SR^{-/-}$ mice displayed a blunted rCBV response to D-amphetamine (1 mg/kg, i.p.), as compared to control $SR^{+/+}$ mice (*SI appendix* Fig.S7A-C). These data therefore confirm, *in vivo*, that DA modulation of PFC activity occurs through regulation of D-serine.

Discussion

The present study reveals a novel role for D-serine in driving PFC dopamine-glutamate interactions at the cellular, network, and behavioral level. The underlying signaling pathway involves differential regulation of AC activity by D_1- and D_3-R, most likely resulting in PKA-mediated changes in D-serine release. The pharmacological tools employed in this study were in general used at a single dose which may perhaps be a limitation. Yet we selected doses selective for their respective targets and the use of several agents, together with other complementary approaches, reinforces our conclusions.

Activation of D_1R enhances NMDAR-dependent functions to influence neurotransmission, synaptic plasticity and cell excitability through D-serine. Conversely, activation of D_3R exerts an opposite and inhibitory action. Although contrasting effects of D_1- and D_2-type receptors on NMDAR currents have been reported to partly act *via* distinct excitatory and inhibitory microcircuits (34), such a regulatory mode can be ruled-out since in this study all electrophysiological recordings were performed in the presence of the $GABA_A$ receptor antagonist picrotoxin to block GABAergic inhibitory transmission. Still, the question remains of whether the DA modulations of D-serine functions unveiled herein are cell-autonomous. We found that activation of D_1Rs increased extracellular levels of D-serine and in consequence NMDAR-dependent functions in the PFC, an effect counteracted by activation of D_3Rs, indicating functional interactions between the two receptors. Our data further indicate that the underlying mechanism involves differential regulation of the AC-PKA pathway, consistent with a cell-autonomous effect. As previous studies showed that D_1- and D_3R positive pyramidal neurons are particularly abundant in L5 of mPFC and that the two receptors may reside on the same neurons (35), such a scenario is indeed conceivable.

Intriguingly, we found that modulation of L-serine levels by D_1- and D_3Rs mirrored their influence on D-serine. Although these observations might suggest intensification of the D-

serine biosynthesis pathway through activation of the glia-to-neuron serine shuttle (36), the fact that we could rescue DAergic control of synaptic and cognitive functions by D-serine supplementation in $SR^{-/-}$ mice indicates that modulation of D-serine biosynthesis is not the limiting step in DA regulation. Rather, DA-induced changes in neuronal D-serine release are most likely central to these processes, while L-serine shuttling and D-serine synthesis by SR would be secondarily upregulated as a homeostatic loop to support the metabolic demands imposed on neuronal circuitry.

Modulation of D-serine levels upon activation of D_1- or D_3R offers a multifaceted signaling system for DA fine-tuning of NMDAR function, thereby optimizing synaptic and network activity. Tonic, spontaneous DA release may increase NMDAR function through D_1-type receptors whilst the higher extracellular level of DA attained during phasic firing of VTA-derived mesocortical pathways would recruit D_3Rs. By inhibiting the AC-PKA pathway, D_3R would counteract D_1R activation to lower D-serine levels and as a consequence the operation of NMDAR. In line with this notion, enhancement and reduction of NMDAR activity by respectively low and high concentrations of DA has previously been reported (21, 22) and is coherent with the U-shaped function of the influence of DA receptors activation upon cognition (37, 38). In the case of PFC, such effects have been attributed to the contrasting level of expression of D_1- and D_2-type receptors (39) as well as the differential localization of these receptors (3, 39). In addition, there is compelling evidence for a differential facilitatory and suppressive influence upon cognitive function of D_1 and D_3R respectively (27, 38).

The striking defects in D_1- and D_3R-dependent modulation of neurotransmission and PFC function in $SR^{-/-}$ mice suggest that DA modulation specifically involves NMDAR co-agonism by D-serine but not glycine. It might then be enquired why $SR^{-/-}$ mice did not exhibit baseline deficits in the NOR procedure which requires functionally-intact NMDARs (40). We propose that, under conditions of a persistent lack of D-serine in the mPFC, glycine can, to some extent, assume a compensatory role at NMDARs. Possible reasons for the specific role of D-serine rather than glycine as a mediator of DA modulation of PFC functions include differential actions related to their spatial segregation. Indeed, we previously found that under physiological conditions synaptic pools of D-serine but not glycine gate synaptic NMDARs in the mPFC (14), whereas glycine preferentially activates extrasynaptic NMDARs (41).

As for potential pathophysiological relevance, by identifying D-serine as a novel substrate for the influence of DA upon glutamatergic transmission in PFC, the current observations are relevant to several neuropsychiatric disorders, such as SCZ where there is

a well-established link between a dysfunction of D-serine-mediated NMDAR signaling and both cognitive and positive symptoms (19). Our findings are also consistent with a previous report by Nomura and colleagues showing that neonatal D-serine supplementation of mice displaying a knockout for the SCZ susceptibility gene *pick1* rescues DA modulation of NMDAR-mediated control of pyramidal cell excitability (42). Interestingly, the atypical antipsychotic clozapine enhances D-serine release in rat frontal cortex (43) and elevates plasma levels of D-serine and L-serine in patients with SCZ (44). As clozapine notably acts on DA receptors, in light of our findings, one interpretation might be that clozapine actually changes the balance between D1- and D2-type receptors activation, thereby leading to an increase in D-serine levels. The present study may also offer a cellular explanation for previous studies showing that D-serine plays a major role in cocaine-induced sensitization (45, 46) and to studies suggesting that the novel antipsychotics cariprazine and blonaneserin strengthen cognitive functions by virtue of their attenuation of D_3R activity in the PFC (47,48). In addition, there is increasing interest in the potential utility for ligands at NMDAR in the management of other disorders from autism to Alzheimer's disease (49,50).

Finally, gender is clearly a factor related to the prevalence, clinical picture and treatment of many psychiatric disorders, so it should be pointed out that only males were used in the present study, representing a potential limitation to any clinical extrapolation. Nonetheless the present study provides a novel framework for an improved understanding and, ultimately, management of the diversity of brain disorders involving disruption of the frontocortical DA- glutamate interface (51,52).

Methods and Materials

A detailed description of all methods and materials can be found in the *SI Appendix*.

All experiments were conducted in accordance with European and French directives on animal experimentation and with local ethical committee approval. Electrophysiological recordings and behavioral analyses were performed on adult Wistar and Lister hooded rats as well as serine racemase knock-out mice ($SR^{-/-}$) and control littermates ($SR^{+/+}$) while *in vivo* microdialysis and phMRI experiments were performed only on mice. Only males were used. All quantitative data are expressed as mean ± SEM. Statistical analyses performed are detailed in the *SI Appendix and* in figure legends.

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Financial Disclosures

GD, XL, PL, NM, SS, RA, THA, BP, DJGW, GL, PF, AB, JTC, GC, LP, MG, KCFF, AMG, TF, AC, and JPM declare no conflict of interest. MJ Millan and JM Rivet respectively were and are full time employees of Institut de Recherche Servier and have no other interests to declare. S Schmidt and J Kehr are employees of Pronexus Analytical AB but declare no conflicts of interests.

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Figures legends

Fig.1. D-serine is required for dopamine modulations of NMDA-EPSCs. A: Diagram showing the slice and electrodes placements for recording NMDA-EPSCs at pyramidal neurons in layer 5 of the prelimbic (PrL) cortex. B-C: Pharmacologically isolated NMDA-EPSCs can be recorded in PrL neurons from rat slices at +40mV and at -70 mV in the presence of GABA-receptor blocker PTX (50 µM) and AMPA-receptor blocker NBQX (10 µM) and are blocked by D-AP5 (50 µM). D: Bath application of low (light green, 1 µM, n=6) or high (dark green, 10 µM, n = 7) concentrations of DA respectively up- (+73.9 ± 14.1%) or downregulate (-44.7 ± 6.7%) NMDA-EPSCs. E-F: Pre-incubation with 100 µM D-serine (triangle symbols) or treatment with *Rg*DAAO (square symbols) opposes occluding action on the positive ($DA_{1\ \mu M}$+D-Ser: +22.5 ± 4.6% n=7; $DA_{1\ \mu M}$+*Rg*DAAO: +5.4 ± 5.93%, n=7) and negative ($DA_{10\ \mu M}$+D-Ser: -18.7 ± 6.7%, n=6; $DA_{10\ \mu M}$+*Rg*DAAO: -16.8 ± 6.2%, n=5) regulations of NMDA-EPSCs by exogenous DA. G: Accumulation of endogenous DA by

application of the DA reuptake inhibitor GBR12909 (0.5 µM) increases NMDA-EPSCs in control rat slices (filled light green circles, +73.78 ± 21.35%, n=11) and decreases NMDA-EPSCs in the presence of the D1R antagonist SCH39166 (1 µM) (filled dark green circles, -35.68 ± 1.45%, n=13). H: GBR12909 (0.5 µM) increases NMDA-EPSCs in mouse $SR^{+/+}$ control slices (filled light green circles, +41.73 ± 12.12%, n=5) and decreases NMDA-EPSCs in the presence of the D1R antagonist SCH39166 (1 µM) (filled dark green circles, -22.84 ± 1.79%, n=5). GBR12909 (0.5 µM) induces no change in slices from $SR^{-/-}$ mice (empty green circles, +0.93 ± 4.22%, n=6). Top traces scale bars: 50 pA, 100 ms. Bottom traces scale bars: 50 pA, 500 ms. H: Histograms summarizing the DA modulations of NMDA-EPSCs and the opposing action of D-serine binding site occlusion, *Rg*DAAO treatment and serine racemase deletion. * p<0.05, ** p<0.01, ***p<0.001.

Fig.2. Modulations of NMDA-EPSCs by D_1- and D_3-receptors involves D-serine. A-B: Single-Cell multiplex RT-PCR showing the expression of D1 and D2 receptors subtypes in L5 excitatory neurons of the PrL area. C: Bath application of D_1R agonist SKF81297 10 µM increases NMDA-EPSCs (93.93 ± 14.05%, n=11) while the D_3R specific agonist PD128907 1 µM has an opposing action (-39.67 ± 8.1% n=8). D: Occluding the co-agonist binding site of NMDAR with 100 µM D-serine markedly impairs NMDAR-EPSCs modulations by DA receptors activation (SKF28197+D-ser: +17.04 ± 2.48%, n=6; PD128907+D-ser: -21.7 ± 5.6%, n=6). E: Histograms summarizing the effects of D_1R and D_3R agonists in the absence and presence of occluding D-serine. F: Modulations of NMDAR-EPSCs by D_1Rs and D_3Rs activation in $SR^{+/+}$ mice (SKF81297: +92.66 ± 23.5%, n=8; PD128907: -34.92 ± 4.95%, n=7). G: Modulations of NMDAR-EPSCs by D_1Rs and D_3Rs activation in $SR^{-/-}$ mice (SKF81297; +51.03 ± 10.91%, n=7; PD128907: -10.36 ± 11.75%, n=7). H: Histograms showing that modulations of NMDAR-EPSCs by D_1Rs and D_3Rs activation is impaired in $SR^{-/-}$ mice. I: Modulations of NMDAR-EPSCs by D_1Rs and D_3Rs activation in $SR^{-/-}$ mice chronically supplemented with D-serine (SKF81297; +86.86 ± 30.01%, n=7; PD128907: -26.46 ± 4.19%, n=14). J: Modulations of NMDAR-EPSCs by D_1Rs and D_3Rs activation in $SR^{-/-}$ mice chronically supplemented with control saline (SKF81297; +11.98 ± 10.32%, n=6; PD128907: -3.70 ± 4.56%, n=14). H: Histograms showing that chronic D-serine supplementation rescues modulations of NMDAR-EPSCs by D_1Rs and D_3Rs activation in $SR^{-/-}$ mice. * p<0.05; ** p<0.01. Top traces scale bars: 50 pA, 100 ms. Bottom traces scale bars: 50 pA, 500 ms.

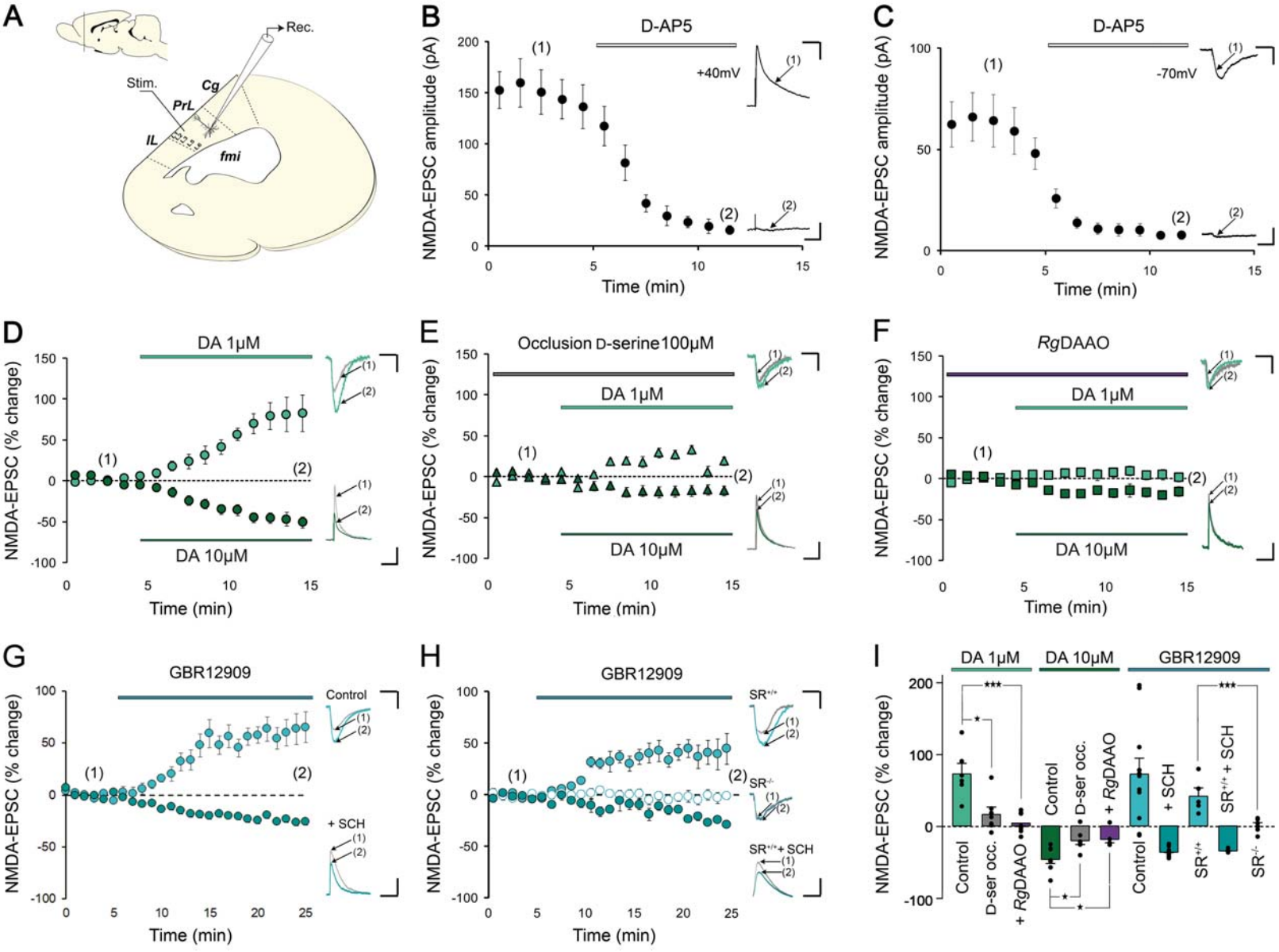
Fig.3. D-serine mediates DA regulations of synaptic plasticity. A: Input/output relationship in wild-type *vs* SR deficient mice ($SR^{+/+}$ n=44; $SR^{-/-}$ n=21). B. Paired-pulse ratio profiles in wild-type *vs* SR deficient mice ($SR^{+/+}$ n=44; $SR^{-/-}$ n=21). C: LTP of the field

potential in wild-type *vs* SR deficient mice ($SR^{+/+}$: +48.88 ± 9.20%, n=9; $SR^{-/-}$: +48.13 ± 14.6%, n=8). D: Effect of the D_1R-type agonist SKF81297 (10 µM) on LTP in $SR^{+/+}$mice (+83.66 ± 10.72%, n=10) E: Effect of SKF81297 (10 µM) on LTP in $SR^{-/-}$ mice (+61.64 ± 14.66%, n=9). F. LTD induced by concomitant application of SKF81297, PD128907 and LFS in wild-type *vs* SR deficient mice ($SR^{+/+}$: -32.39 ± 7.58%, n=6; $SR^{-/-}$: -12.20 ± 5.44%, n=6). * p<0.05. Scale bars: 0.1 mV, 2 ms.

Fig.4. DA receptors activation regulates extracellular mPFC D-serine levels in freely moving mice. A: Mice were implanted with two microdialysis probes placed in the left and right mPFC. Microdialysates were collected every 20 min and D- and L-serine were quantified using UHPLC-MS/MS. B: Left chromatogram shows separation of a standard mixture of D-serine at LLOQ (20 nM) and L-serine in AAS18 in aCSF (black line) and D,L-serine-d3 (230 nM, internal standard, gray line). Right chromatogram shows a typical microdialysis sample in basal conditions (vehicle, black line) and D,L-serine-d3 (gray line). C: Changes in extracellular D-serine and L-serine levels from 20 to 60 min during drug infusion in the mPFC. Histograms represent values for area under the curve (AUC). Left, the D_1R agonist SKF81297 increased the extracellular levels of D-serine by 17.40 ± 4.73% (n=8 samples, 6 mice) while D_3R agonist PD128907 on its own had no effect (vehicle: -0.25 ± 0.49% change, n=10 samples from 6 mice *vs* PD128907: -1.90 ± 2.4% change, n=10 samples from 6 mice) but blocked the potentiating effect of SKF81297 (-0.08 ± 3.13% change, n=13 samples from 7 mice). Right, SKF81297 also increased the extracellular levels of L-serine by 33.10 ± 15.05% while D_3R agonist PD128907 had no effect on its own (vehicle: -1.31 ± 1.15% change *vs* PD128907: 0.00 ± 8.90% change) but blocked the potentiating effect of SKF81297 (-9.15 ± 6.32% change). * p<0.05, **p<0.01, ***p<0.001. D: Recordings of NMDA-EPSCs in the mPFC of mice showing that in the presence of PD128907, SKF81297 fails to potentiate NMDA responses (-3.15 ± 6.16% change, n=6).

Fig.5: DA modulations of D-serine NMDAR co-agonist functions occurs through cAMP/PKA signaling. A: The facilitating effect of D_1Rs activation on NMDA-EPSCs is abolished by blocking production of cAMP by the adenylate cyclase enzyme (AC) (-4.52± 3.21%, n=12). B: Similarly, blocking downstream the PKA enzyme prevents the facilitating effect of D_1Rs activation on NMDA-EPSC (+6.18 ± 3.27%, n=12). C: Downregulation of NMDAR-EPSCs by D_3Rs activation is also abolished by H89 blockade, which is indicative of an occlusion. D: Activating AC increased NMDA-EPSCs in $SR^{+/+}$ mice (+51.61 ± 11.62%, n=10) but not in $SR^{-/-}$ mice (-0.63 ± 2.01%, n=9; p<0.001). Scale bars: 20 pA, 100 ms.

Fig.6. Pro-cognitive action of D_3R antagonist S33084 relies on D-serine modulation of NMDARs. A: Administration of the D_3R antagonist S33084 (0.63 mg/kg, s.c.) reverses the impairment in NOR memory induced by a 4 hours inter-trial interval (ITI) in rats (n=12). Systemic administration of the NMDAR antagonists, MK801 (B, n=12) and CPP (C, n=12) as well as the glycine/D-serine binding site blocker L701,324 (C, n=12) all prevented the enhancement of NOR memory induced by S33084. B: Administration of the D_3R antagonist S33084 (0.63 mg/kg, s.c.) reversed the impairment in novel object recognition (NOR) memory induced by a 2 hours ITI in $SR^{+/+}$ mice (vehicle, n=14; S33084, n=18) but not in $SR^{-/-}$ mice (vehicle: n=9; S33084, n=10). The pro-cognitive effect of S33084 is rescued in $SR^{-/-}$ mice by chronic supplementation of D-serine ($SR^{+/+}$+saline, n=7; $SR^{-/-}$+D-ser, n=8) performed by s.c. administration with 300 mg/kg D-serine (or control saline) on day 1 and then 150 mg/kg D-serine (or control saline) daily for 20 days. **p<0.01, ***p<0.001 compared to control $SR^{+/+}$ mice treated with vehicle. $^{++}$p<0.01, $^{+++}$p<0.001 compared to $SR^{+/+}$ mice treated with S33084.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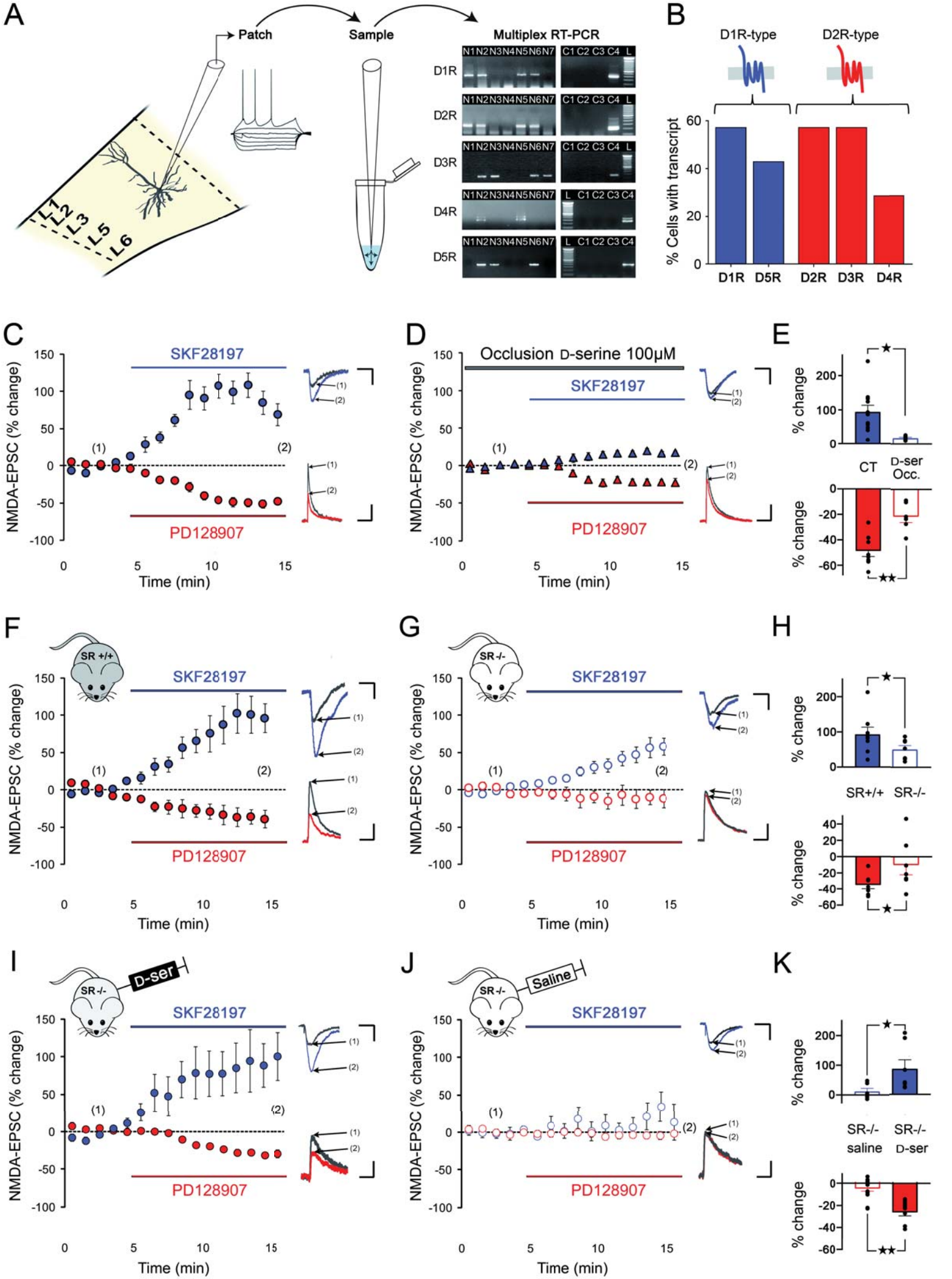
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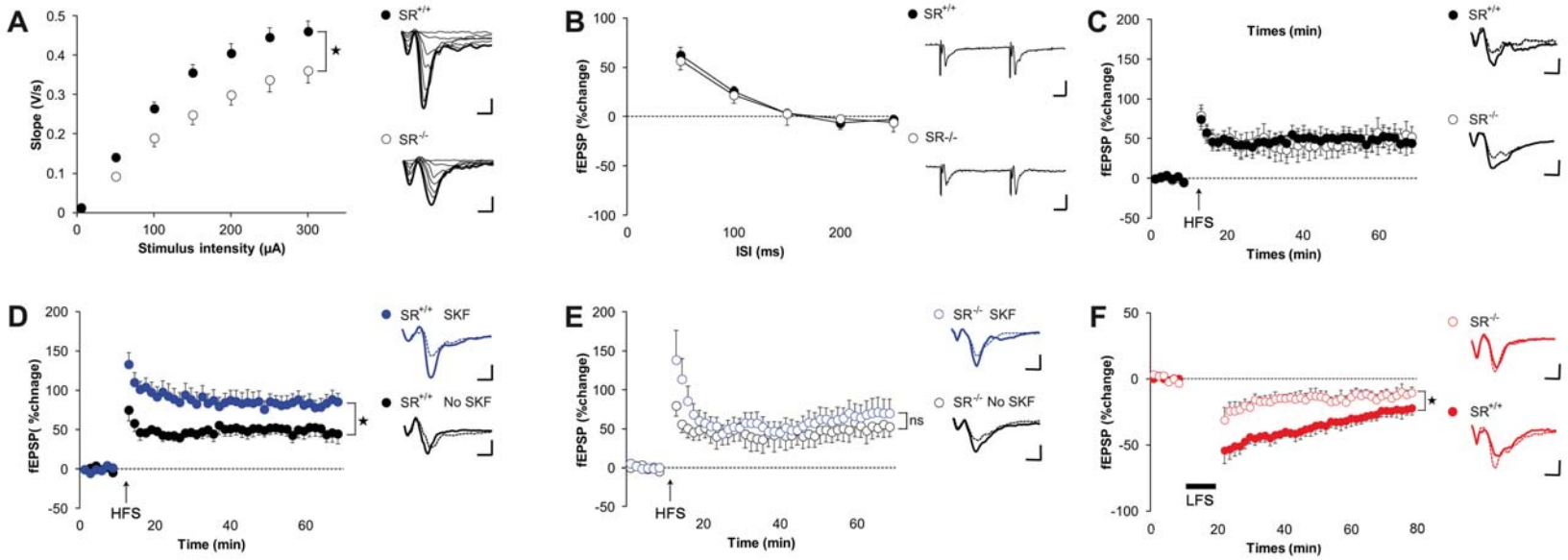
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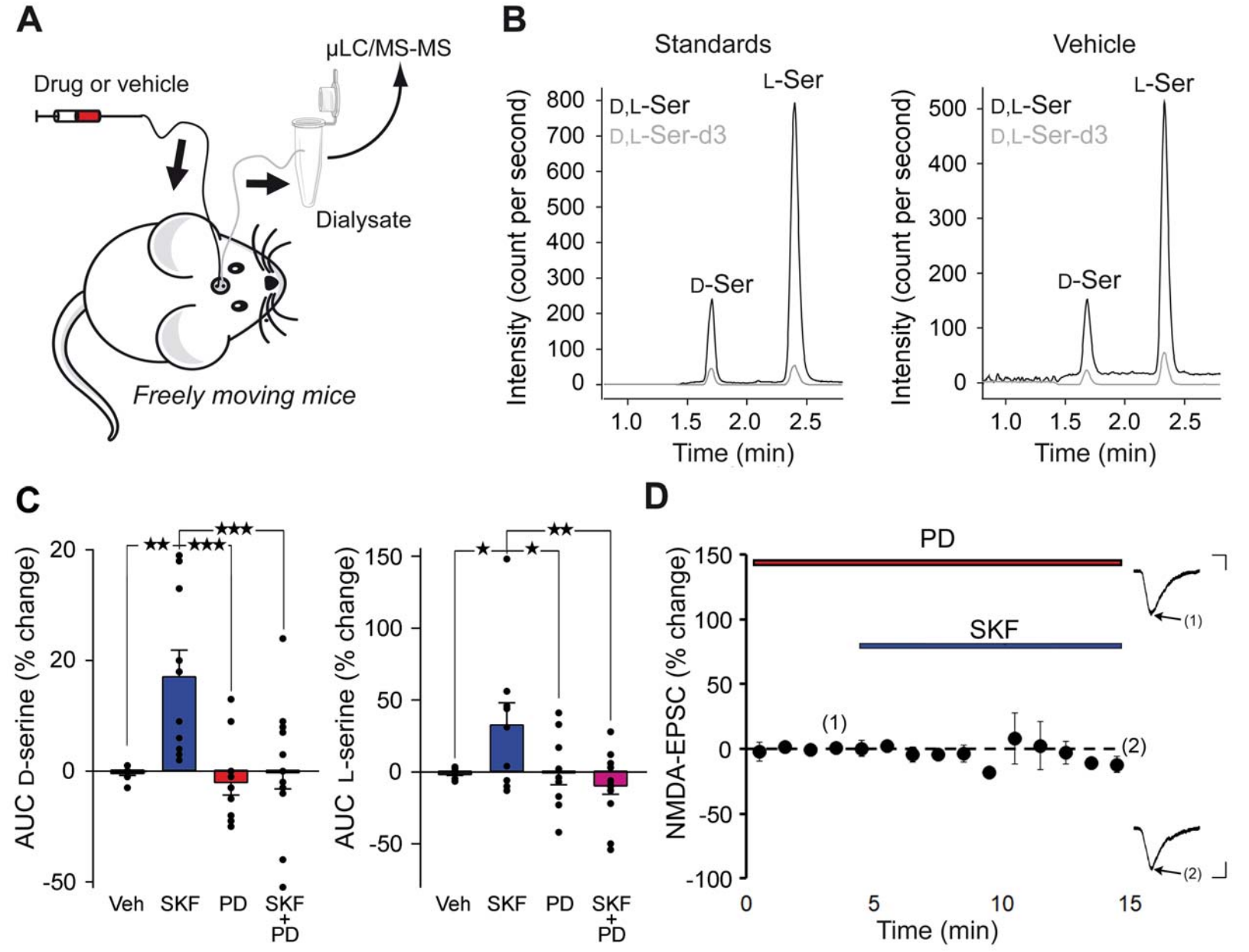
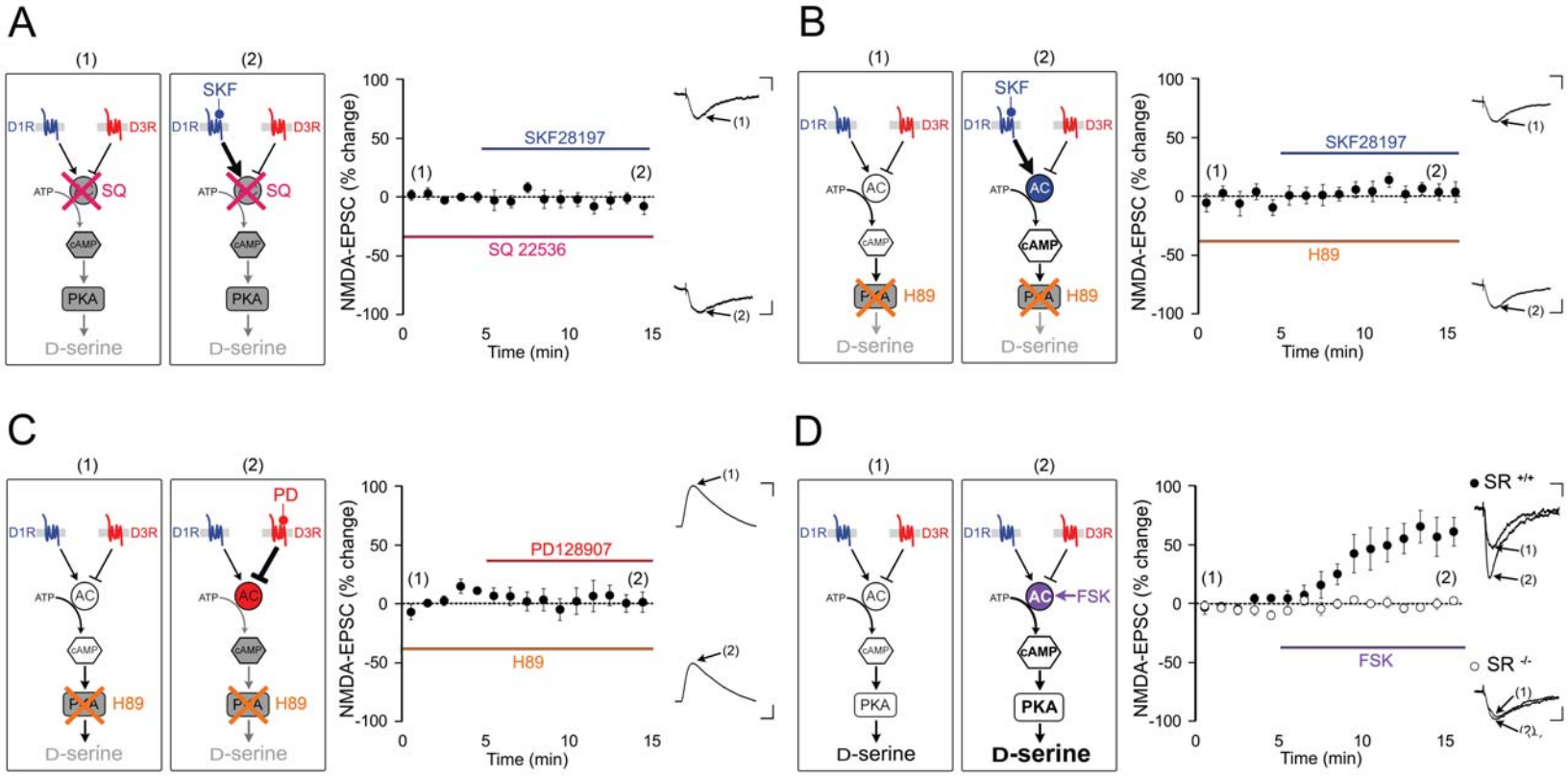
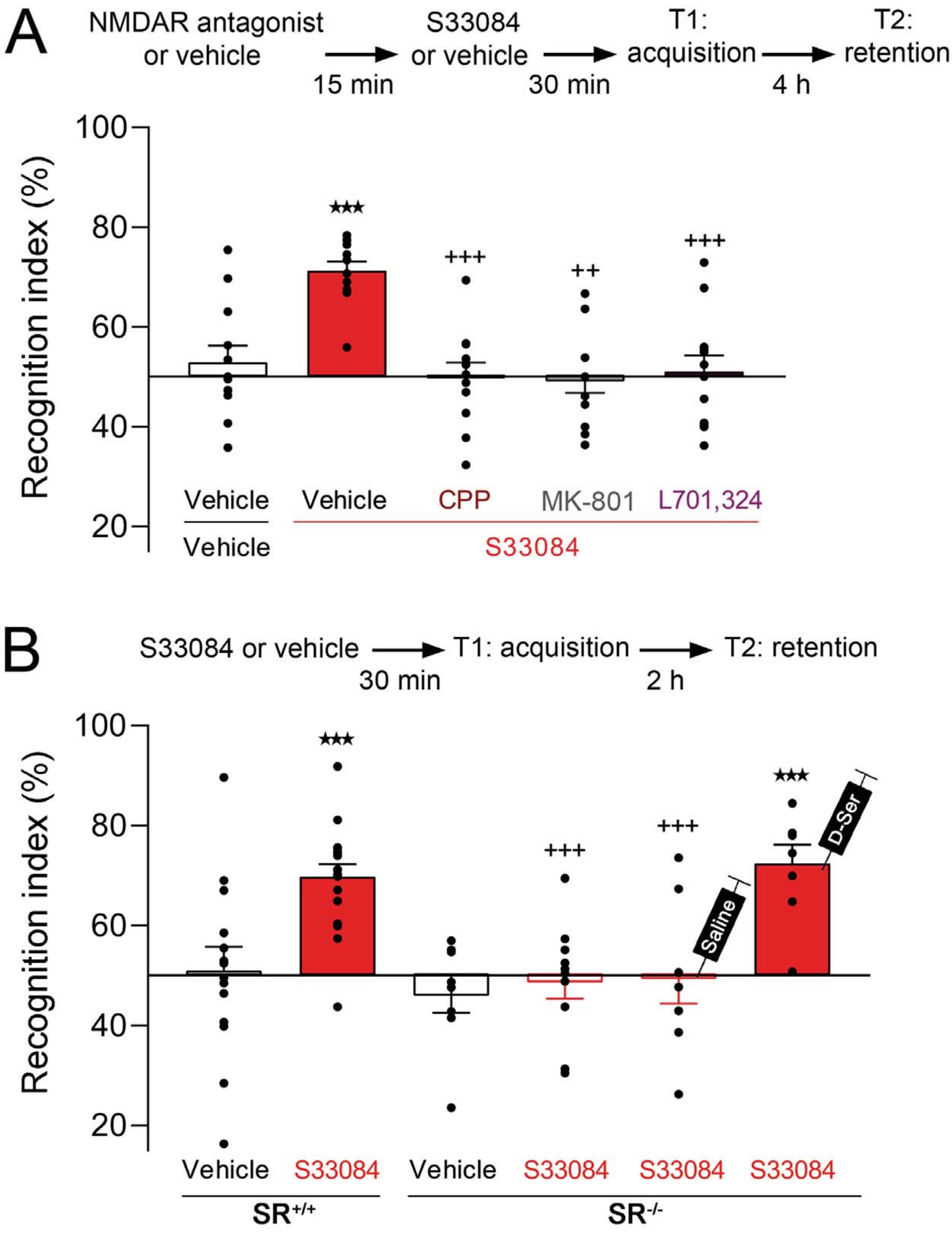
HFS
Time (min)
SR-/- SKF
SR-/- No SKF
ns
F
fEPSP (%change)
LFS
Times (min)
SR-/-
SR+/+


A
Drug or vehicle
μLC/MS-MS
Dialysate
Freely moving mice
B
Standards
Vehicle
D,L-Ser
D,L-Ser-d3
D-Ser
L-Ser
Intensity (count per second)
Time (min)
C
AUC D-serine (% change)
AUC L-serine (% change)
Veh
SKF
PD
SKF + PD
D
PD
SKF
NMDA-EPSC (% change)
Time (min)
(1)
(2)


A
(1)
(2)
SKF
D1R
D3R
ATP
AC
SQ
cAMP
PKA
D-serine
NMDA-EPSC (% change)
SKF28197
SQ 22536
Time (min)
B
H89
C
PD
PD128907
D
FSK
SR +/+
SR -/-


A
NMDAR antagonist or vehicle
15 min
S33084 or vehicle
30 min
T1: acquisition
4 h
T2: retention
Recognition index (%)
100
80
60
40
20
★★★
+++
++
+++
Vehicle
Vehicle
CPP
MK-801
L701,324
Vehicle
S33084
B
S33084 or vehicle
30 min
T1: acquisition
2 h
T2: retention
Recognition index (%)
100
80
60
40
20
★★★
+++
+++
★★★
Saline
D-Ser
Vehicle
S33084
Vehicle
S33084
S33084
S33084
SR+/+
SR-/-