



Published in final edited form as:

Mol Psychiatry. 2016 March ; 21(3): 386–393. doi:10.1038/mp.2015.61.

Role for neonatal D-serine signaling: prevention of physiological and behavioral deficits in adult *Pick1* knockout mice

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Abstract

NMDA glutamate receptors play key roles in brain development, function, and dysfunction. Regulatory roles of D-serine in NMDA receptor-mediated synaptic plasticity have been reported. Nonetheless, it is unclear whether and how neonatal deficits in NMDA-receptor-mediated neurotransmission affect adult brain functions and behavior. Likewise, the role of D-serine during development remains elusive. Here we report behavioral and electrophysiological deficits associated with the frontal cortex in *Pick1* knockout mice, which show D-serine deficits in a neonatal and forebrain specific manner. The pathological manifestations observed in adult *Pick1* mice are rescued by transient neonatal supplementation of D-serine, but not by a similar treatment in adulthood. These results indicate a role for D-serine in neurodevelopment and provide novel insights on how we interpret data of psychiatric genetics, indicating the involvement of genes associated with D-serine synthesis and degradation, as well as how we consider animal models with neonatal application of NMDA receptor antagonists.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary information is available at *Molecular Psychiatry's* website.

Keywords

PICK1; D-serine; NMDA; developmental trajectory; prefrontal cortex; prepulse inhibition

Introduction

NMDA glutamate receptors mediate excitatory neurotransmission and synaptic plasticity, and their modulation by endogenous factors has a critical role in these phenomena. Specifically, NMDA receptors are required for long-term potentiation (LTP) and long-term depression (LTD), cellular processes of neural plasticity that underlie learning and memory.^{1,2} NMDA receptors are activated by glutamate and require several co-agonists, including D-serine.³ A crucial role of D-serine in neural plasticity has also been demonstrated.⁴⁻⁶ On the other hand, excess NMDA receptor mediated signaling can lead to excitotoxicity, which may underlie many brain disorders, including stroke, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS).⁷⁻¹⁰ D-serine is also known to play a role in this toxic cascade in a context-dependent manner, including in some cases of stroke and ALS.¹¹⁻¹³

During brain development, NMDA receptors are crucial for the generation of spontaneous synchronous network activity in the neonatal brain.¹⁴⁻¹⁷ NMDA receptors are also essential for the conversion of silent to active synapses by recruiting AMPA receptors.¹⁸ However, it is unclear whether and how neonatal deficits in NMDA-receptor-mediated neurotransmission affect adult brain functions and behavior. Likewise, the role of D-serine during development remains elusive. Given that NMDA receptors are modulated by D-serine, animal models with transient deficits in D-serine during neonatal stages may provide a good tool to address this question.

PICK1 is a multifunctional scaffold protein that interacts with many proteins, including several types of glutamate receptors and serine racemase, the D-serine synthesizing enzyme.^{19,20} PICK1 has been extensively studied in the context of neural plasticity,²¹⁻³² and mice deficient in *Pick1* are also available.²² Nonetheless, the physiological role of PICK1-serine racemase protein interactions remains elusive. In cell cultures, PICK1 can augment the enzymatic activity of serine racemase and D-serine synthesis.²¹ *Pick1* knockout mice display a significant reduction in D-serine levels, which is specific to the forebrain during the neonatal stage: the level of D-serine is normal in adult *Pick1* mice.²¹

Here we aimed to address the significance of D-serine in brain development on adult brain function and behavior, by using *Pick1* knockout mice. As the first step, we examined behavioral and electrophysiological characteristics associated with the frontal cortex of the *Pick1* mice. We then tested whether the representative deficits in adulthood are normalized by transiently supplying D-serine only during the neonatal stage.

Materials and Methods

Animals

We used C57BL/6J male *Pick1* knockout mice,²¹ which have been previously characterized in the context of hippocampal and cerebellar neural plasticity.^{23,33} For all groups, pups were weaned on postnatal day 21, genotyped and housed in sex-matched groups of five in standard mouse cages in accordance with protocols approved by the Johns Hopkins University, the University of Maryland, and RIKEN Animal Care and Use Committees.

Behavioral Tests

Behavioral tests were conducted on male *Pick1* knockout mice and control littermates in adulthood (over 2 months of age), with a one-week interval between tests. We conducted the assays based on our published protocols with minor modifications.³⁴⁻⁴⁰

Open field test—Horizontal and vertical locomotor activity were assessed for 2 h using activity chambers equipped with infrared beams (San Diego Instruments). Horizontal and vertical movements, stereotypies, and time spent in the center or along the walls of the chamber (thigmotaxis) were automatically recorded.

Prepulse inhibition (PPI) of the acoustic startle—The PPI test was performed as previously described.^{35,40} Two identical startle chambers (San Diego Instruments) were used for measuring startle reactivity. During each PPI session, a mouse was exposed to the following types of trials: pulse alone (a 120 dB, 100 ms, broadband burst); the omission of stimuli (no-stimulus trial); and four prepulse–pulse combinations (prepulse–pulse trials) consisting of a 20 ms broadband burst used as a prepulse and presented 80 ms before the pulse using one of five prepulse intensities (74, 78, 82, 86, and 90 dB). Each session consisted of six presentations of each type of trial presented in a pseudorandom order. The background noise presented throughout the entire session was 70 dB.

Forced Swim Test (FST)—The FST was employed to evaluate depression-associated responses. Latency to immobility and total immobility were analyzed during the last 4 min of the 6 min test using the SMART program (San Diego Instruments).

Elevated plus maze (EPM)—The EPM was used to assess anxiety as described previously.³⁵ Anxiety was measured by the percent of time spent in the open arms [calculated as $100 \times (\text{time spent in the open arms} / (\text{time in the open arms} + \text{time in the closed arms}))$].

Y-maze—Spontaneous alternation as a measure of spatial working memory was assessed in the Y-maze. The spontaneous alternation behavior was calculated as the number of triads (visiting three different arms on consecutive choices, e.g., ABC, but not ABB or ACC) divided by the total number of arms entered minus two.⁴¹

Quantitative real time PCR

Quantitative real-time PCR was performed by the ABI Prism 7900HT sequence detection system (Applied Biosystems). Note, we performed this molecular study with frontal cortex homogenates from mice that had been injected with saline for another assay. The primers used for the study were as follows:

Stargazin (forward): 5'-ccagcaagaagaacgaggaa-3'
Stargazin (reverse): 5'-ttgcttcacagaccttga-3'
Parvalbumin (forward): 5'-ttctggacaaagacaaaagtgg-3'
Parvalbumin (reverse): 5'-tgaggagaagcccttcagaat-3'
Gad67 (forward): 5'-tggagcagatcctggtgact-3'
Gad67 (reverse): 5'-gccattcaccagctaaaccaa-3'
Actin (forward): 5'-gatgacgatcgcctgcgtgctcg-3'
Actin (reverse): 5'-gcctgtggtacgaccagaggcatacag-3'

Electrophysiology

Adult mice were anesthetized with chloral hydrate (400 mg/kg i.p.) and perfused with ice cold artificial cerebrospinal fluid (ACSF, in mM: 125 NaCl, 25 NaHCO₃, 10 glucose, 3.5 KCl, 1.25 NaH₂PO₄, 0.5 CaCl₂, 3 MgCl₂, 0.075 Na₂S₂O₅, pH 7.4). Coronal brain slices (300 μm) containing the medial prefrontal cortex were made with a Vibratome and incubated for at least 1 h at 35°C. Recordings were made using 35°C O₂-saturated ACSF, but with CaCl₂ adjusted to 2 mM and MgCl₂ to 1 mM. Recording electrodes were made from glass pipettes filled with (in mM) 115 K-gluconate, 10 HEPES, 2 MgCl₂, 20 KCl, 2 Mg-ATP, 2 Na₂-ATP, 0.3 GTP (pH 7.3; 280 mOsm) and Neurobiotin (0.125%). Prefrontal cortical pyramidal neurons were identified under visual guidance using infrared-differential interference contrast (IR-DIC) video microscopy (Olympus BX50-WI). Whole-cell current-clamp signals were amplified (10x) with a Multiclamp 700B (Axon Instruments), digitized with an A/D converter (Digidata, Axon Instruments) and sampled with Axoscope 9.0 (Axon Instruments) at 20 KHz.⁴²

Neonatal or adult D-serine treatment (Fig. 3)

D-serine (500 mg/kg; Sigma-Aldrich) was dissolved in sterile physiological saline and administered intraperitoneally (i.p.), using a 30-gauge needle, every day for 2 weeks beginning on postnatal day 3 (P3). The dose was chosen according to a representative publication, which showed that 300–900 mg/kg of D-serine ameliorated a PPI deficit induced by dizocilpine in adult mice.⁴³ The effects of D-serine were evaluated in adulthood using the PPI and Y-maze tests and electrophysiological recordings assessing NMDA modulation of pyramidal cell firing in prefrontal cortical slices.

Since it was a control experiment, the conditions of the adult D-serine treatment were the same as the neonatal treatment (500 mg/kg i.p daily for 2 weeks) except for the timing: it started at the age of 8 weeks. After two weeks of treatment we waited one week to avoid

acute effects of the D-serine and then did either electrophysiology or behavioral testing (Y-maze followed by PPI after another one week interval).

Statistical Analysis

Statistical analysis was performed by ANOVA. A student's t-test was used to compare two sets of data. Values in graphs are expressed as the mean plus the standard error of the mean (SEM). Significance levels are marked as follows: * $P < 0.05$.

Results

Behavioral and molecular alterations in adult *Pick1* knockout mice

We analyzed the behavioral consequences of deleting the *Pick1* gene by performing behavioral tests relevant to diverse aspects of mental conditions. *Pick1* knockout mice did not differ from wild-type littermates in open field activity over 2 h (Supplementary Fig. 1), but when we divided the 2 h into four bins of 30 min, we found them to be significantly hyperactive during the 60–90 min interval (Fig. 1A, Supplementary Fig. 1). The hyperactivity of *Pick1* knockout mice was unlikely related to altered emotionality, as no significant group differences were seen in the elevated plus maze and the forced swim test (Fig. 1B, C, Supplementary Fig. 2). We also assessed prepulse inhibition (PPI) of the acoustic startle response, a measure of sensorimotor gating, which is reportedly impaired in patients with several neuropsychiatric disorders, including schizophrenia (Fig. 1D).⁴⁴ Two-way repeated measures ANOVA showed a significant group effect, $F(1,89)=6.04$, $p=0.026$ and significant group by prepulse interaction, $F(4,89)=6.21$, $p<0.001$. Post-hoc tests demonstrated that, compared to controls, knockouts had significantly decreased PPI at the prepulse intensities of 74, 78 and 82 dB. Notably, *Pick1* knockout mice showed an increase in startle response, indicating that impaired PPI was not due to insufficient startle response (Supplementary Fig. 3). In addition, *Pick1* knockout mice also exhibited significantly fewer spontaneous alternations in the Y-maze compared to wild-type mice (Fig. 1E), which is a sign of working memory deficit.^{44,45} Abnormalities in PPI and working memory could result from deficits of the prefrontal cortex.⁴⁶ We also conducted an exploratory expression study for candidate molecules (AMPA receptor regulator *Stargazin* and interneuron markers of *Parvalbumin* and *Gad67*): real-time PCR indicated an increase in the expression of *Stargazin* and *Parvalbumin*, but not that of *Gad67*, in adult *Pick1* knockout mice (Supplementary Fig. 4).

Alterations in NMDA-elicited firing of prefrontal cortical pyramidal neurons in adult *Pick1* knockout mice

As behavioral deficits in *Pick1* mice suggested altered prefrontal cortical function, we hypothesized that pyramidal neurons in the prefrontal cortex of *Pick1* knockout mice may display altered physiological properties. To address this question, we tested the excitability of pyramidal neurons in brain slices containing the prefrontal cortex of adult *Pick1* knockout mice and wild-type littermates. The number of action potentials evoked with intracellular current injections before and after bath application of NMDA (4 μ M) was examined as a readout of cell excitability (Fig. 2, Supplementary Table 1). We also tested whether the

modulation of NMDA responses by D1 dopamine receptors is affected in *Pick1* knockout mice by adding the selective D1 agonist SKF38393 (2 μ M).⁴²

The resting membrane potential was not different between wild-type and *Pick1* knockout mice, and was not affected by the addition of NMDA and SKF38393 (wild-type: -63.3 ± 1.5 mV at baseline, -62.0 ± 2.2 mV with NMDA, -60.3 ± 3.6 mV with NMDA and SKF38393; *Pick1* knockout mice: -65.8 ± 3.1 mV at baseline, -63.5 ± 3.1 mV with NMDA, -62.0 ± 5.7 mV with NMDA and SKF38393). Thus, basic membrane properties of prefrontal pyramidal neurons were not affected in *Pick1* knockout mice.

We then tested whether *Pick1* knockout mice exhibited altered modulation of excitability by NMDA in pyramidal neurons. The number of action potentials evoked by a constant-amplitude current pulse was markedly increased by application of NMDA in wild-type mice (from 6.6 ± 0.5 to 11.4 ± 2.8 spikes; a 73% increase from baseline) (Fig. 2, Supplementary Table 1). Although the D1 agonist SKF38393 alone did not change the number of action potentials, the combined addition of NMDA and SKF38393 synergistically augmented the number of action potentials (to 13.1 ± 4.8 ; a 101% increase from baseline) in wild-type mice. In *Pick1* knockout mice, however, the increase in the number of action potentials by NMDA was significantly attenuated (from 5.8 ± 1.7 to 7.8 ± 2.3 ; a 33% increase from baseline), and the addition of SKF38393 to NMDA only slightly elevated the response (to 8.5 ± 3.4 ; a 45% increase from baseline). A two-way ANOVA with genotype and drug treatment as factors revealed genotype differences (ANOVA $F_{1,22}=5.82$, $p=0.024$), indicating that NMDA responses are impaired in *Pick1* knockout mice. There was no significant interaction between genotype and D1 agonist treatment ($p=0.914$), indicating that D1 modulation of NMDA responses was not affected in *Pick1* knockout mice.

Influence of neonatal D-serine on behavioral defects in adult *Pick1* knockout mice

Here we show that *Pick1* knockout mice have prefrontal cortex-associated behavioral deficits in adulthood. Which mechanism(s) underlie these deficits? One possibility is that down-regulation of D-serine during development, at the neonatal stage, may contribute to these adult deficits in *Pick1* knockout mice. To address this question, we administered D-serine (500 mg/kg, i.p.) daily to pups beginning on postnatal day 3 (P3) for 2 weeks, and then maintained the animals until adulthood without any further supplementation of D-serine (Fig. 3). We did not observe any adverse effects of neonatal injections (data not shown). We evaluated the effects of this neonatal treatment with D-serine by monitoring possible improvement in PPI and spontaneous alternations in the Y-maze in adult mice. Compared to saline treatment, D-serine treatment in the neonatal stage significantly increased the PPI in *Pick1* knockout, but not in wild-type mice (Fig. 4). We assessed the PPI effects separately for *Pick1* knockout mice and wild-type mice, as our pilot data had not revealed effects of D-serine on wild-types; this design increases the power of analysis. Two-way repeated measures ANOVA of the data for knockout mice revealed a significant effect of treatment, $F(1,99)=4.8$, $p=0.041$, but not for wild-type mice, $F(1,64)=0.6$, $p=0.47$. A trend of beneficial effect of neonatal D-serine treatment was also seen in the adult behaviors in Y-maze (Supplementary Fig. 5). Note, due to unexpected baseline changes elicited by neonatal injection itself, this statement on Y-maze is not conclusive.

We also tested the impact of adult D-serine administration (500 mg/kg, i.p.; daily beginning at the age of 8 weeks for 2 weeks) on these behaviors. In regard to PPI, we cannot make any meaningful statements because repeated injections in adulthood significantly altered the baseline response in both wild-type and knockout mice (Supplementary Fig. 6A).

Meanwhile, we observed conclusive evidence that adult D-serine treatment did not rescue the deficit in spontaneous alternations in *Pick1* knockout mice (Supplementary Fig. 6B).

Influence of neonatal D-serine on electrophysiological defects in adult *Pick1* knockout mice

We next addressed whether neonatal D-serine treatment of *Pick1* knockout mice may normalize the electrophysiological deficits observed in adult mice. We prepared prefrontal cortical slices from D-serine-treated *Pick1* knockout mice, and conducted electrophysiological assessments similar to those presented in Fig. 2. The resting membrane potentials of *Pick1* knockout mice treated with D-serine during the neonatal period and adulthood were -66.6 ± 4.7 and -67.9 ± 3.1 mV, respectively. Unlike untreated *Pick1* knockout mice, NMDA (4 μ M) elicited strong increases in evoked action potential firing in slices from D-serine-treated *Pick1* knockout mice. The number of action potentials evoked by current injection rose from 3.9 ± 1.2 to 7.4 ± 3.2 with NMDA administration (89% increase from baseline) (Fig. 5A, Supplementary Table 1). The addition of SKF38393 to NMDA slightly increased the number of action potentials to 8.9 ± 3.6 . A two-way ANOVA revealed differences between treated and untreated *Pick1* knockout mice ($F_{1,22}=7.91$, $p=0.010$). These results indicate that D-serine treatment during development rescued adult NMDA function in *Pick1* knockout mice.

In contrast, when we conducted D-serine treatment in adulthood, responses of neurons were noticeably blunted in *Pick1* knockout mice: to NMDA (5.7 ± 1.7 at baseline to 8.2 ± 3.2 spikes for a 46% increase in action potential firing) or NMDA plus SKF38393 (9.7 ± 3.6 , a 70% increase from baseline) (Fig. 5B, Supplementary Table 1). To statistically confirm the contrastive responses between neonatal and adult D-serine treatment to *Pick1* mice, we employed a two-way ANOVA. First, a significant effect of D-serine treatment ($F_{2,42}=7.502$, $p=0.002$) was revealed in *Pick1* mice. Second, post hoc comparisons using Tukey's HSD indicate that neonatal treatment rescued the electrophysiological deficits in untreated *Pick1* mice ($p=0.002$), whereas this rescue was absent upon the treatment in adulthood ($p=0.52$). Thus, we conclude that neonatal, but not adult, D-serine treatment rescues NMDA function in adult *Pick1* knockout mice.

Discussion

It has been an unanswered question whether D-serine during brain development plays a role in adult brain function and behavior, although its physiological and pathological role in adult animals have been well studied.^{6,43,47-50} To address this question, here we utilized *Pick1* knockout mice in which the D-serine level is selectively reduced in the forebrain only during the neonatal stage.²¹ Adult *Pick1* knockout mice showed several behavioral abnormalities and electrophysiological deficits in the prefrontal cortex. Importantly, neonatal, but not adult, D-serine supplement rescued such adult deficits. These observations suggest a novel

role for D-serine during brain development, with significant influence on adult brain function and behavior.

How does altered D-serine during development affect adult performance? The most probable scenario is that neonatal D-serine influences NMDA receptors, which in turn affects brain network formation. In *Pick1* knockout mice, a neonatal-specific deficit in D-serine may lead to transient hypo-NMDA function during a critical period for proper brain development. Alternatively, D-serine may influence brain development by an unknown mechanism independent of its action on NMDA receptors. The next question, although it is beyond the scope of the present study, is to characterize how neonatal D-serine administration to *Pick1* knockout mice may change (possibly normalize) neural network formation during the developmental trajectory, in comparison with non-treated *Pick1* knockout and wild-type mice. By comparing these data with those from mice with direct genetic modulation of NMDA receptors, we may be able to clarify whether the effects of neonatal D-serine supply are via NMDA receptors.

Acute or sub-chronic administration of phencyclidine (PCP), a NMDA receptor antagonist, to rodents can produce behaviors resembling phenomena observed in schizophrenia.⁵¹ Clinical observation of PCP-induced psychosis and schizophrenia-like manifestation in normal subjects with non-competing NMDA receptor antagonists indicate a possible role of adult NMDA neurotransmission in the disease.^{52,53,54} The current view on the mechanisms by which NMDA antagonists may produce psychosis relates to their ability to selectively suppress local inhibitory processes yielding cortical disinhibition and these effects are evident in adolescents and adults.⁵⁵ Thus, the mechanism leading to behavioral deficits in *Pick1* knockout mice is different from those elicited by adult and adolescent administration of NMDA receptor antagonists that functionally impair glutamatergic neurotransmission.⁵⁶ Instead, as discussed above, neonatal D-serine and NMDA receptors are likely to influence neural circuit formation during neurodevelopment, secondarily affecting adult brain function and behavior in neonatal manipulations that reduce NMDA receptor activity. Thus, neonatal administration of PCP may be a useful model for adult mental illnesses,⁵⁷⁻⁶⁰ but is justified in the context of neurodevelopment, distinct from models with adult and adolescent administration of NMDA receptor antagonists.

As PICK1 is a multifunctional protein, the phenotypes of *Pick1* knockout mice reflect not only D-serine associated abnormalities but also deficits elicited by many other mechanisms. Thus, in the present study, we conducted neonatal supplement of D-serine to address the primary cause of D-serine. Nonetheless, one may wonder why we did not use conditional knockout mice lacking *serine racemase*,⁶¹ the synthesizing enzyme for D-serine, for this study. This is in part because we currently do not have access to the conditional mice. Furthermore, as Serine racemase generates not only D-serine but also pyruvate, keto-acid, and ammonia,⁶² its knockout may not be a tool to purely address the role for D-serine. Thus, both conditional *serine racemase* knockout and *Pick1* knockout mice are complementary towards investigating the neonatal role for D-serine.

Genetic studies have suggested that genes encoding key molecules of D-serine synthesis, degradation, and regulation (e.g., *D-amino acid oxidase*, *serine racemase*, and possibly *G72*)

are frequently associated with bipolar disorder and schizophrenia.^{63–65} A recent genome wide association study has indicated 108 chromosomal loci that associate with schizophrenia, which include the locus of the *serine racemase* gene.⁶⁶ In addition, a chromosomal abnormality associated with schizophrenia is reportedly linked to down-regulation of *phosphoserine aminotransferase 1*, *PSAT1*, a key enzyme for the synthesis of L-serine, the precursor of D-serine.⁷⁰ Some studies have found reduced D-serine in the cerebrospinal fluid of patients with schizophrenia,^{67,68} whereas, other studies including a meta-analysis found no differences.⁶⁹ Accordingly, the *Pick1* knockout mice with D-serine deficit specific to neonatal stage may model schizophrenia in which adult D-serine level is normal.

Thus far, NMDA hypofunction has been modeled primarily using experimental animals, which include the *serine racemase* knockout mice in which D-serine deficiencies are present throughout life. The *Pick1* knockout model offers a unique opportunity to investigate the role of D-serine deficiency limited to the neonatal stage for behavior during adulthood. Our findings demonstrate that neonatal D-serine deficiency, on its own, is sufficient to induce some behavioral phenotypes, such as impaired prepulse inhibition and spontaneous alternation that may serve as pathophysiological markers for schizophrenia or other major mental disorders, and that such effects are rescued by neonatal D-serine treatment. Future studies are needed to determine the relative importance of neonatal vs. adult D-serine deficiency in the pathology of major mental illness.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Ms. Yukiko Lema for organizing the figures and manuscript. This work was supported by USPHS grants MH-084018 (A.S.), MH-094268 Silvo O. Conte center (A.S.), MH-069853 (A.S.), MH-085226 (A.S.), MH-088753 (A.S.), MH-092443 (A.S.), MH-091387 (A.S.), MH-057683 (P.O.), MH-083728 (M.V.P.), Stanley (A.S.), RUSK (A.S.), S-R foundations (A.S.), NARSAD (A.S., P.O., M.V.P.), Maryland Stem Cell Research Fund (A.S.), MEXT KAKENHI (T.T.), JST CREST (T.T.), and Naito and Uehara Memorial Foundations (J.N.).

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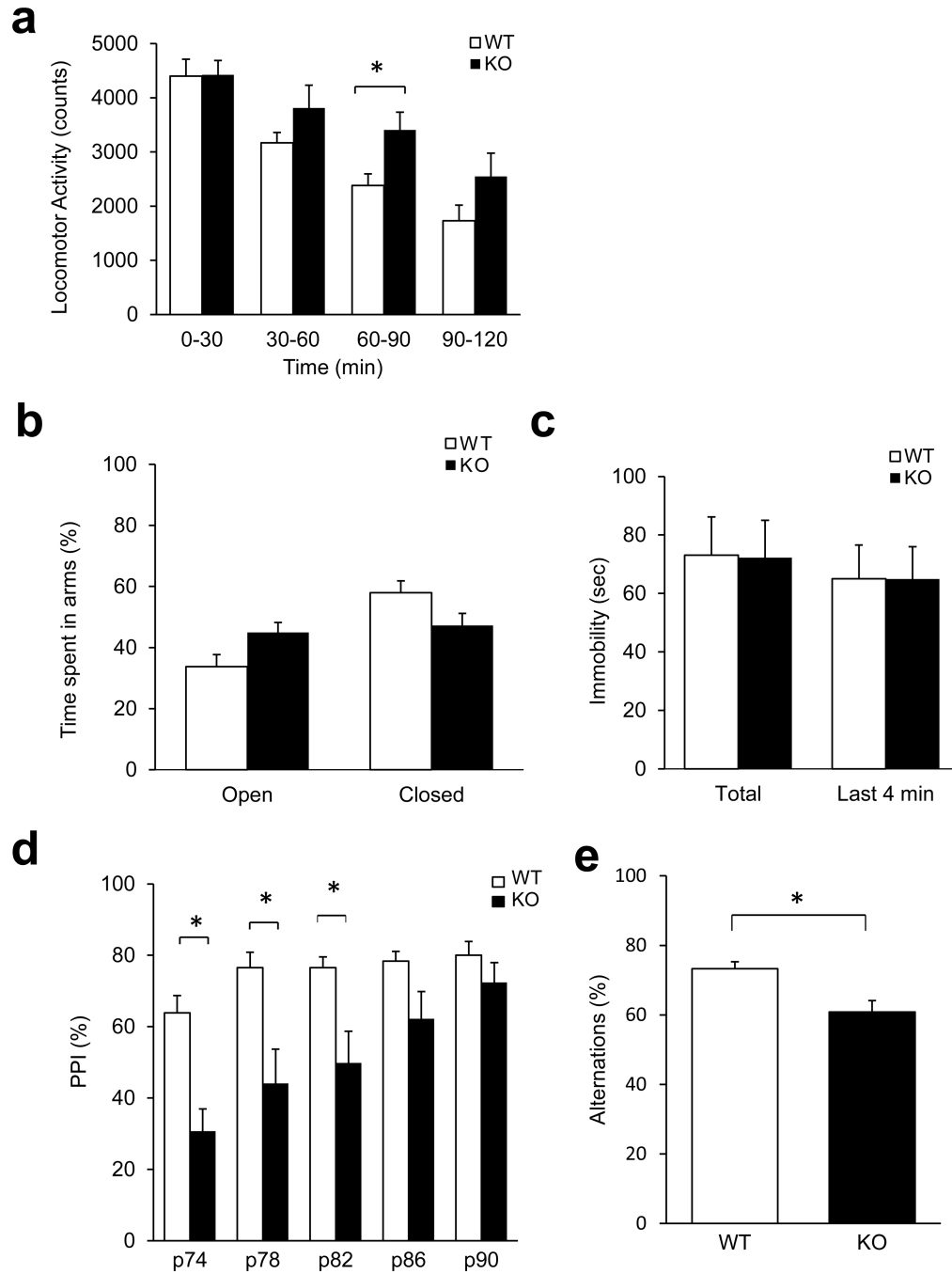


Figure 1. Behavioral changes in adult male *Pick1* knockout mice in adulthood

A) Horizontal locomotor activity. There was a significant group by interval interaction [F(23, 383)=1.83, p=0.012]. Pairwise multiple comparison procedures (Holm-Sidak method) showed significantly more ambulatory activity in *Pick1* knockout mice at the 60–90 min interval (*p<0.05), p=0.054 for the 90–120 interval, n=8 per group.

B) Behavior in the elevated plus maze. No difference between wild-type and *Pick1* knockout and mice was observed. n=7 per group.

C) Time immobile in the forced swim test. No differences between wild-type and *Pick1* knockout mice was observed. n=8 per group.

D) Prepulse inhibition (PPI) of the acoustic startle. *Pick1* knockout mice demonstrated significantly lower PPI at the prepulse intensities of 74, 78 and 82 dB. *p<0.05 vs. wild-type at the same prepulse intensity. n=11 (knockout), n=8 (wild-type).

E) Spontaneous alternations in the Y maze. *Pick1* knockout mice showed significantly fewer alternations in the Y maze compared to wild-type mice, *p<0.05, n=8 per group.

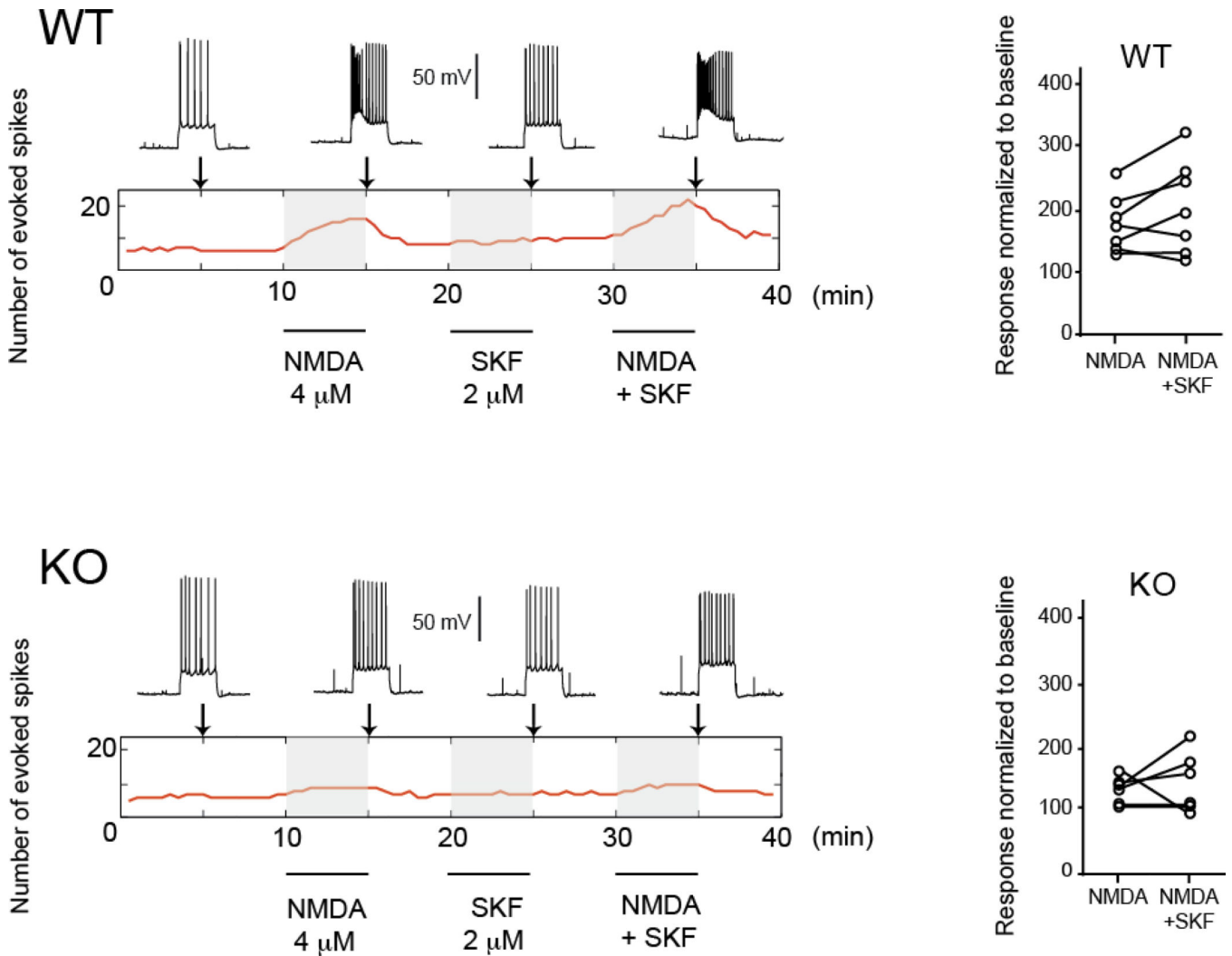


Figure 2. NMDA effects on prefrontal cortex pyramidal cell excitability in adult *Pick1* knockout mice

Top: Examples of traces used to assess excitability 5 min before 4 μ M NMDA application (left), after 5 min of NMDA perfusion, after 5 min of the D1 agonist SKF38393 (SKF, 2 μ M), and during the combined perfusion of 4 μ M NMDA and 2 μ M SKF in a prefrontal cortical slice from a wild-type mouse. The rectangular area with the red line indicates the changes in the number of evoked action potentials over time with the three treatments (NMDA alone, SKF alone, NMDA+SKF, shaded areas). The vertical arrows indicate the time at which the traces on top were obtained. Right, population data indicating normalized responses to baseline after NMDA alone and NMDA + SKF. **Bottom:** Similar display for a representative prefrontal cortex pyramidal neuron in a *Pick1* knockout mouse (left) and population data for NMDA and NMDA + SKF for all knockout mice recorded, showing a reduced NMDA effect in slices from *Pick1* knockout mice.

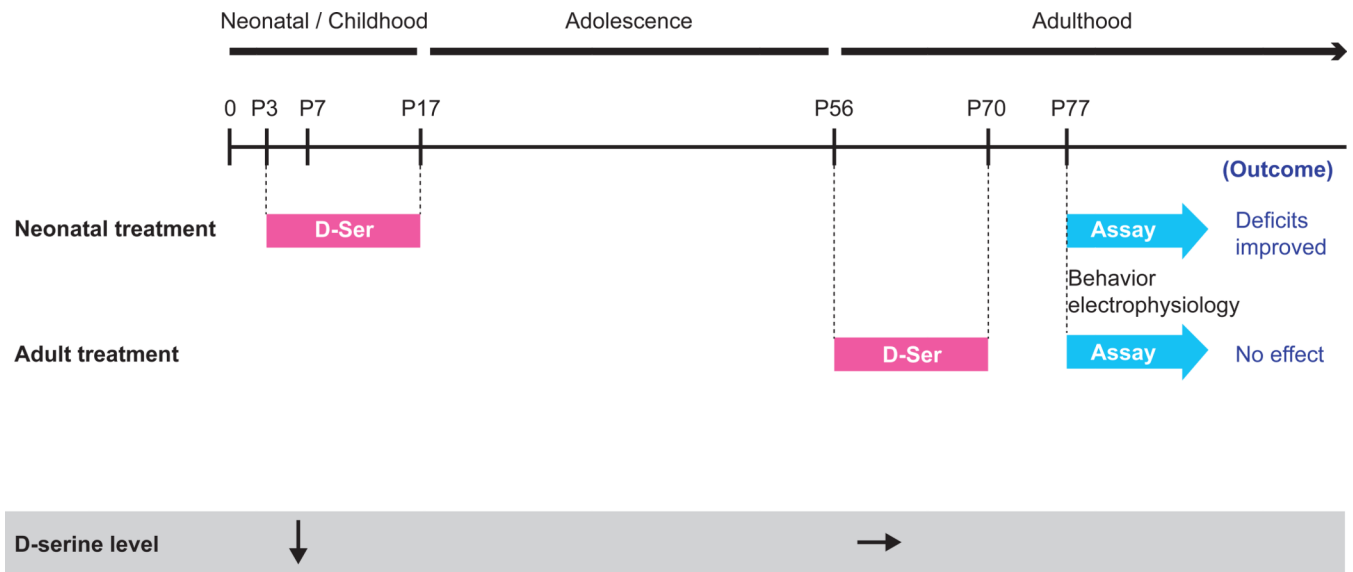


Figure 3. Time line for D-serine treatment and summary of results

We have previously published that *Pick1* knockout mice have reduced D-serine levels at P7 (neonatal stage), but not in adulthood (P56) (Hikida 2008). Based on those results, here we tested whether supplementation of D-serine during the neonatal period (P3–P17) would improve behavioral and electrophysiological deficits seen in adulthood and indeed it did. As a control experiment we also treated the mice with D-serine in adulthood (P56–P70), followed by a washout period of 1 week, and found no effect on behavior or electrophysiology.

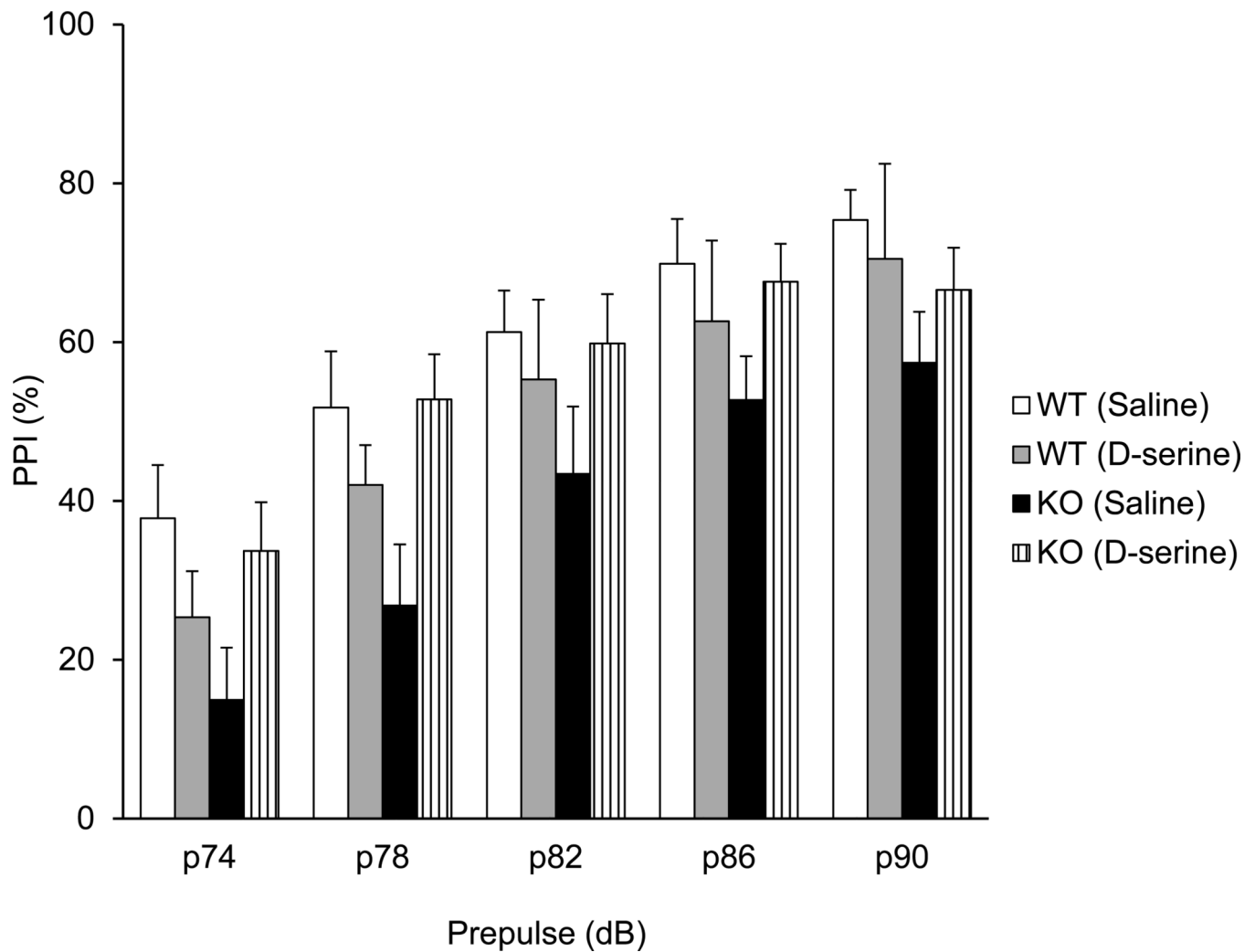


Figure 4. Effect of neonatal D-serine treatment on PPI in adulthood

D-serine 500 (mg/kg) was injected daily for 2 weeks starting on postnatal day 3. The PPI deficit was significantly rescued in adult *Pick1* knockout mice treated with D-serine in the neonatal stage. Two-way repeated measures ANOVA of the data for knockout mice revealed a significant effect of treatment, $F(1,99)=4.8$, $p=0.041$. $n=8$, wild-type with saline treatment; $n=8$, wild-type with D-serine treatment; $n=5$, *Pick1* knockout mice with saline treatment; $n=13$, *Pick1* knockout mice with D-serine treatment.

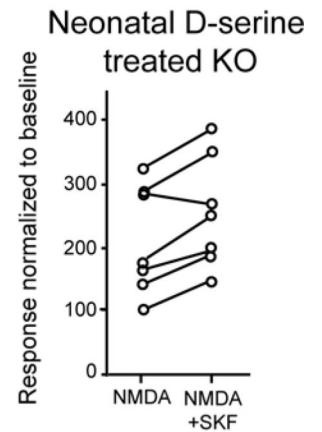
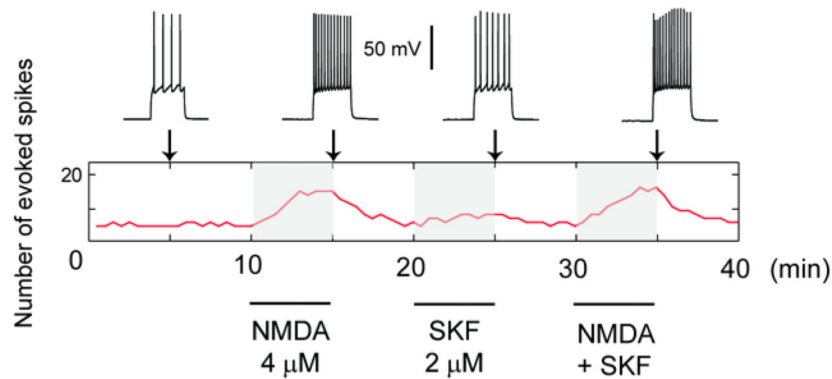
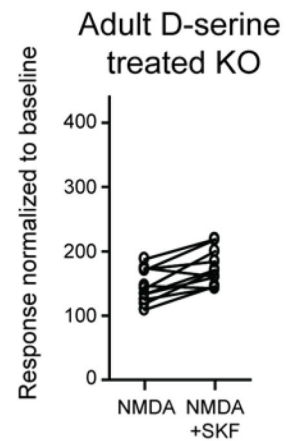
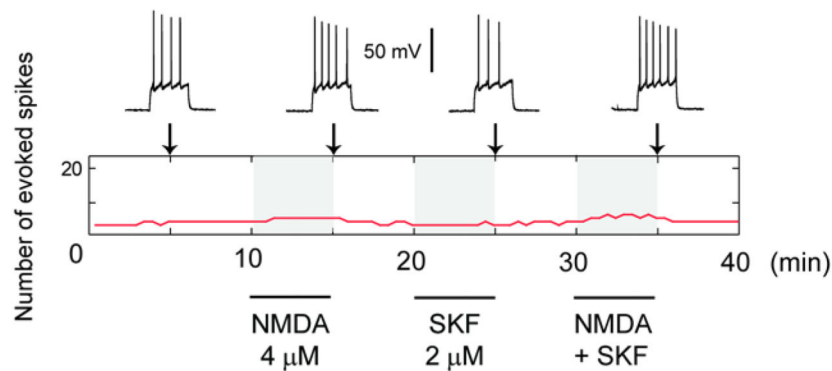
a**Neonatal D-serine treated KO****b****Adult D-serine treated KO**

Figure 5. Rescue of electrophysiological deficits in adulthood by neonatal D-serine treatment, but not by adult D-serine treatment

Responses to NMDA application in prefrontal cortical brain slices from adult *Pick1* knockout mice that had been treated with D-serine. Data are presented in the same format of those in Fig. 2.

A) Neonatal D-serine treatment (from postnatal day 3 to day 16) rescued the electrophysiological changes in *Pick1* knockout mice.

B) Adult D-serine treatment did not rescue the electrophysiological changes in *Pick1* knockout mice.