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Glutamate receptor composition of the post-synaptic density is altered in genetic mouse models of NMDA receptor hypo- and hyperfunction

Darrick T. Balu^{a,b} and Joseph T. Coyle^{a,b,*}

^a Department of Psychiatry, Harvard Medical School, Belmont, MA USA

^b Laboratory for Psychiatric and Molecular Neuroscience, McLean Hospital, Belmont, MA, USA

Abstract

The *N*-methyl-D-aspartate receptor (NMDAR) and α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor (AMPA) are ionotropic glutamate receptors responsible for excitatory neurotransmission in the brain. These excitatory synapses are found on dendritic spines, with the abundance of receptors concentrated at the postsynaptic density (PSD). We utilized two genetic mouse models, the serine racemase knockout (SR^{−/−}) and the glycine transporter subtype 1 heterozygote mutant (GlyT1^{+/-}), to determine how constitutive NMDAR hypo- and hyperfunction, respectively, affect the glutamate receptor composition of the PSD in the hippocampus and prefrontal cortex (PFC). Using cellular fractionation, we found that SR^{−/−} mice had elevated protein levels of NR1 and NR2A NMDAR subunits specifically in the PSD-enriched fraction from the hippocampus, but not from the PFC. There were no changes in the amounts of AMPAR subunits (GluR1, GluR2), or PSD protein of 95kDa (PSD95) in either brain region. GlyT1^{+/-} mice also had elevated protein expression of NR1 and NR2A subunits in the PSD, as well as an increase in total protein. Moreover, GlyT1^{+/-} mice had elevated amounts of GluR1 and GluR2 in the PSD, and higher total amounts of GluR1. Similar to SR^{−/−} mice, there was no protein changes observed in the PFC. These findings illustrate the complexity of synaptic adaptation to altered NMDAR function.

Keywords

serine racemase; glycine transporter; postsynaptic density; NMDA receptor; AMPA receptor; schizophrenia

1. Introduction

Ionotropic glutamate receptors (AMPA, kainate, and NMDA subtypes) serve as the mediators of excitatory glutamatergic signaling in the brain. AMPARs are the principal transducers of fast excitatory neurotransmission that regulate the strength of glutamatergic excitatory synapses. Most AMPARs exist as tetramers comprised of four glutamate receptor

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*Corresponding author: Joseph T. Coyle, M.D., Harvard Medical School, McLean Hospital, 115 Mill Street Room 122, Belmont, MA 02478, USA., joseph_coyle@hms.harvard.edu, Tel.: 1-617-855-2101, Fax: 1-617-855-2705.

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subunits, GluR1-4 (Derkach et al., 2007). Receptor subunit composition is brain region dependent and determines the functional properties of the channel, including Ca^{2+} permeability. While AMPARs are involved in rapid excitatory synaptic transmission and determine synaptic strength, NMDARs mediate a slower component of excitatory transmission and are critical postsynaptic mediators of activity-dependent synaptic plasticity (Lau and Zukin, 2007). Throughout most of the brain, the heterotetrameric receptor is composed of two NR1 subunits and two NR2 subunits, all of which contribute transmembrane domains to form the pore of the ion channel that is characterized by high Ca^{2+} permeability. The NR1 subunit has eight different splice variants, which may affect channel function. NR2 subunits may be expressed in four different forms (NR2A-D), each of which confers different biophysical and pharmacologic properties to the channel (Lynch and Guttman, 2001). NMDARs are considered “molecular coincidence detectors” because activation requires postsynaptic depolarization (removal of Mg^{2+} block from the channel pore at resting membrane potential), and the binding of two agonists, glutamate, and either glycine or D-serine at the glycine modulatory site (GMS) (Tsien, 2000). The influx of Ca^{2+} through the NMDAR triggers a cascade of intracellular events that regulate many types of neuroplasticity (Greer and Greenberg, 2008; Wayman et al., 2008), including long-term potentiation (LTP), dendritic patterning, spine elaboration and synaptogenesis, of which the latter three are perturbed in schizophrenia and animal models of the disease (Ross et al., 2006).

Our laboratory has previously generated genetic models of both NMDAR hypofunction (serine racemase knockout (SR^{-/-}) mouse) and hyperfunction (glycine transporter 1 heterozygote (GlyT1 ^{+/-}) mouse). The SR enzyme converts L-serine to D-serine. As a result, SR^{-/-} mice have >80% reduction in brain D-serine, exhibit reduced global NMDAR-mediated neurotransmission, and impaired LTP at the Schaffer collateral-CA1 pyramidal neuron synapse (Basu et al., 2009). Pyramidal neurons in the medial prefrontal cortex (mPFC) of these mutants also have reduced dendritic arborization and spine density (Devito et al., 2010). GlyT1^{+/-} mice have a 50% reduction in the expression and activity of the glycine high-affinity sodium-dependent transporter, GlyT1, which is expressed in brain with a distribution that is complementary to SR (Tsai et al., 2005). In the hippocampus of GlyT1 ^{+/-} mice, the GMS is virtually fully occupied. In addition, whole-cell patch-clamp recordings in the CA1 region of the hippocampus demonstrate that NMDARs in GlyT1^{+/-} mice display faster decay kinetics and suggest an increase in the contribution of NR2A, as compared to NR2B subunits, to synaptic glutamatergic neurotransmission (Martina et al., 2005).

In the brain, axonal processes may form synapses on neuronal cell bodies or other axons, but in most cases they form on dendrites. More than 90% of all dendritic excitatory synapses in the central nervous system are on dendritic spines (Fiala et al., 2002). The distal tip of these spine heads contains a PSD, an electron dense structure specialized for postsynaptic signaling and plasticity that consists of glutamate receptors, signaling molecules, and scaffolding proteins (Sheng and Hoogenraad, 2007). The PSD dynamically changes its structure and composition in response to synaptic activity (Sheng and Hoogenraad, 2007). Regulation of glutamate receptor composition in the PSD is complex and modulated at many levels. The number of NMDARs at the surface is determined by trafficking, as well as clathrin-mediated internalization and insertion, all of which can be influenced by alternate splicing and the phosphorylation state of receptor subunits (Lau and Zukin, 2007). Under basal conditions, AMPARs undergo constitutive recycling between synapses and the cytosol, but during LTP induction they are more actively recycled through an endosome pathway to enhance exocytosis. AMPAR subunit composition, phosphorylation state, PSD proteins (protein interacting kinase 1 (PICK1), glutamate receptor interacting protein

(GRIP)), and actin cytoskeletal dynamics all regulate receptor trafficking (Derkach et al., 2007).

The purpose of this study was to examine how constitutive NMDAR hypofunction and hyperfunction in SR^{-/-} and Glyt1^{+/-} mice, respectively, affect the glutamate receptor composition of the PSD in brain areas rich in glutamatergic signaling, the hippocampus and PFC. We found that both negative and positive modulation of NMDAR function results in the altered protein expression of particular ionotropic glutamate receptor subunits.

2. Results

2.1. SR^{-/-} mice

The hippocampus was examined for changes in NMDAR levels in both PSD-enriched and whole cell lysate (WCL) fractions. In the PSD (Fig. 1A–B), SR^{-/-} mice had significantly more NR1 ($t(14) = 3.20$; $p = 0.006$) and NR2A ($t(22) = 4.22$, $p = 0.0004$) protein than WT mice, but no difference in the amount of NR2B ($t(24) = 1.38$, $p = 0.18$) and PSD95 ($t(19) = 1.15$, $p = 0.27$). The amounts of the AMPAR subunits GluR1 ($t(17) = 0.09$, $p = 0.93$) and GluR2 ($t(18) = 0.00$, $p = 1.0$) were not significantly different between genotypes. In contrast to the PSD fraction, there were no genotype differences observed in the WCL fraction (Fig. 1C–D) in the protein amounts of NR1 ($t(17) = 0.17$, $p = 0.95$), NR2A ($t(21) = 0.05$, $p = 0.96$), NR2B ($t(19) = 0.88$, $p = 0.39$), and PSD95 ($t(22) = 0.893$, $p = 0.36$). Likewise, there was no difference in the expression of GluR1 ($t(10) = 0.09$, $p = 0.92$) and GluR2 ($t(10) = 0.80$, $p = 0.26$) in the WCL fraction. Given that our previous electrophysiological experiments were performed in 3–4 week old mice (Basu et al., 2009), we also measured NMDAR levels in the hippocampi of juvenile animals (Table 1). Unlike adult mice, there were no significant changes in levels of NMDAR subunits in the PSD fraction. However, there was a significant increase in the total amount of NR2B protein in the SR^{-/-} mice.

The same biochemical parameters were examined for the PFC (Table 2). However, unlike in the hippocampal PSD fraction, no significant differences were observed in any of the NMDAR subunits (NR1: $t(22) = 0.04$, NR2A: $t(21) = 0.14$, NR2B: $t(22) = 0.20$) and PSD95 ($t(20) = 0.89$). In addition, no changes were observed in AMPAR subunits (GluR1: $t(10) = 0.83$, GluR2: $t(10) = 0.27$). In the WCL fraction, no genotypic differences were observed in the protein amounts of NR1 ($t(10) = 0.10$), NR2A ($t(10) = 0.19$), NR2B ($t(10) = 0.34$), PSD95 ($t(22) = 0.64$), GluR1 ($t(9) = 0.21$), and GluR2 ($t(9) = 0.63$).

2.2. GlyT1^{+/-} mice

In the hippocampal PSD fraction (Fig. 2A–B), GlyT1^{+/-} mice had significantly higher protein levels of NR1 ($t(19) = 3.12$; $p = 0.006$) and NR2A ($t(18) = 3.44$, $p = 0.003$) protein than WT mice, but no difference in the amount of NR2B ($t(18) = 1.37$, $p = 0.19$) and PSD95 ($t(12) = 0.04$, $p = 0.97$). Moreover, GlyT1^{+/-} mice had significantly higher levels of both GluR1 ($t(21) = 3.58$, $p = 0.002$) and GluR2 ($t(21) = 2.95$, $p = 0.008$) than WT mice. In the WCL fraction (Fig. 2C–D), GlyT1^{+/-} mice had significantly increased levels of NR1 ($t(18) = 2.32$; $p = 0.03$) and NR2A ($t(17) = 2.87$, $p = 0.01$), but not NR2B ($t(16) = 1.92$, $p = 0.08$) and PSD95 ($t(13) = 0.50$, $p = 0.62$). With respect to AMPAR subunits, GlyT1^{+/-} mice had elevated levels of GluR1 ($t(10) = 3.75$, $p = 0.004$), but not GluR2 ($t(10) = 0.78$, $p = 0.45$), as compared to WT mice.

However, in the PFC (Table 3), there were no significant differences between genotypes in the amounts of NR1 ($t(19) = 0.96$), NR2A ($t(20) = 1.08$), NR2B ($t(18) = 0.65$), PSD95 ($t(8) = 0.03$), GluR1 ($t(8) = 0.16$), and GluR2 ($t(8) = 0.22$) in the PSD fraction. In the WCL fraction, there were also no differences in the amounts of NR1 ($t(10) = 0.36$), NR2A ($t(19) =$

0.22), NR2B ($t(19) = 0.28$), PSD95 ($t(14) = 0.95$), GluR1 ($t(21) = 0.21$), and GluR2 ($t(20) = 0.46$).

3. Discussion

Ionotropic glutamate receptors, specifically NMDARs and AMPARs, are the key mediators of excitatory neurotransmission in the brain and play critical roles in synaptic plasticity. Most of this neurotransmission occurs at the PSD of dendritic spines that are enriched with glutamate receptors. In this study, we used two complementary genetic mouse models to examine how constitutive changes in NMDAR function via modulation of GMS occupancy affect the molecular composition of the PSD. We found both overlapping and divergent effects of synaptic D-serine deficiency and glycine excess on NMDAR and AMPAR expression and localization. SR^{-/-} mice displayed elevated NR1 and NR2A levels specifically in the hippocampal PSD fraction. Interestingly, not only did GlyT1^{+/-} mice also have elevated levels of NR1 and NR2A in the hippocampal PSD fraction, but also an increase in the total amounts of these proteins. Moreover, in the hippocampus, GlyT1^{+/-} mice had elevated synaptic protein levels of GluR1 and GluR2, with only an increased total amount of GluR1 protein.

One major mechanism for regulating NMDAR function is altering subunit composition. NR2 subunits are key determinants of NMDAR channel properties and signaling. NR2A-containing receptors have higher peak open probability upon activation and possess shorter rise and decay times than receptors containing the NR2B subunit (Lau and Zukin, 2007). The subunit composition and accumulation of NMDARs at synapses is regulated by neuronal activity, and changes in NMDAR subunit composition have been shown to modify synaptic plasticity (Lau and Zukin, 2007). Previous hippocampal electrophysiologic studies from our laboratory demonstrated that the decay kinetics and pharmacology of hippocampal NMDARs differed between mouse models and suggested a greater synaptic contribution of NR2B receptors in SR^{-/-} mice (Basu et al., 2009) and NR2A receptors in GlyT1^{+/-} mice (Martina et al., 2005; Tsai et al., 2004). Our current biochemical findings are in agreement with the electrophysiological results obtained from the SR^{-/-} and GlyT1^{+/-} studies. In 3–4 week old SR^{-/-} mice, the age at which the electrophysiology was performed, there was an increase in the total protein amount of NR2B, without a change in the PSD. Although both NR2A and NR2B subunits are prevalent in the adult forebrain, NR2B subunits are reported to be more abundant extra synaptically (Groc et al., 2006; Tovar and Westbrook, 1999). Therefore, this population of NR2B-containing NMDARs would not be quantified in our PSD-enriched fractions. Interestingly, the pattern of NMDAR expression is different in adult SR^{-/-} mice. This could be due to the changes in the NR2A/NR2B ratio that occurs with postnatal development (Williams et al., 1993). In the GlyT1^{+/-} mice, upregulation of synaptic NR2A subunits might serve as a compensatory homeostatic mechanism because glycine has an approximately 10-fold higher affinity for NR2B-containing NMDARs (Danysz and Parsons, 1998).

Activation of NMDARs is required for the induction of many types of synaptic plasticity, including LTP. Even though both NR2A and NR2B-containing NMDARs contribute to LTP induction, the precise contribution of different populations of NMDARs to synaptic plasticity remains unclear (Lee et al., 2010). We have previously demonstrated that LTP in the hippocampus was impaired, but not absent, in juvenile SR^{-/-} mice (Basu et al., 2009), and was accompanied behaviorally by only subtle deficiencies in hippocampal-dependent memory of adult mice (Basu et al., 2009). Our findings of increased NR1 and NR2A protein levels in the hippocampal PSD fraction of adult SR^{-/-} mice suggests a compensatory mechanism, whereby the number of NMDARs at the synapse is increased to overcome the NMDAR hypofunction caused by a reduction in D-serine. However, unlike glycine, D-

serine has a similar affinity for NR2 subtypes (Danysz and Parsons, 1998). Given that the total amounts of NR1 and NR2A protein were not elevated in the hippocampus of SR^{-/-} mice, our results suggests that the trafficking of these NMDAR subunits to the synapse is increased, rather than a general increase in total protein synthesis.

Whereas NMDARs mediate a slower component of excitatory transmission, AMPARs mediate rapid excitatory synaptic transmission and thus determine synaptic strength (Lee et al., 2010). Most AMPARs in the hippocampus (CA3-CA1 synapse) are heterotetramers, consisting mainly of GluR2 plus GluR1 or GluR3 subunits, with subunit composition having dramatic effects on channel properties. GluR2 containing AMPARs exhibit a lower Ca²⁺ permeability, open probability, and channel conductance than GluR2 lacking AMPARs (Derkach et al., 2007). The insertion and stabilization of AMPARs into the PSD, which is a very dynamic process, is also dependent upon AMPAR subunit composition, cytoskeletal organization, and neuronal activity. In particular, NMDAR activation and the downstream signaling events triggered by Ca²⁺ influx lead to the phosphorylation of GluR1-containing AMPARs and actin polymerization that in turn regulates AMPAR insertion, stabilization, and electrical properties. The concomitant increase observed in both GluR1/2 and NR1/2A subunits in the PSD of GlyT1^{+/-} mice, suggests that the increase in NMDARs could be responsible for the elevation of synaptic AMPARs. Interestingly, even though SR^{-/-} mice had increased hippocampal protein levels of NR1 and NR2A in the PSD, they did not have increased levels of AMPARs in the PSD. This suggests that even though there is an increase in the total number of NMDARs in the PSD, there might not be a sufficient augmentation of NMDAR activity that would lead to AMPAR incorporation and/or stabilization.

There was a clear divergence in the effects of genetic GMS modulation on glutamatergic receptors in the PSD of hippocampal and PFC neurons. The lack of effect in the PFC across both genetic models could be due to numerous factors. First, since GlyT1 expression is higher in the adult rodent hippocampus than in the cortex (Jursky and Nelson, 1996; Zafra et al., 1995), a reduction in the amount of transporter would likely have a greater effect on glutamatergic transmission in the hippocampus. On the other hand, SR and D-serine levels are comparable across these brain regions (unpublished data). Hence, the selective effects in the hippocampus of SR^{-/-} mice could relate to the greater importance, relative to the PFC, of D-serine as a co-agonist for NMDAR function.

Finally, the lack of changes found in the total protein levels of NMDAR and AMPAR subunits are in agreement with previous reports from other genetic mouse models of SR deficiency (Inoue et al., 2008; Labrie et al., 2009) and GlyT1^{+/-} mice (Imamura et al., 2008). The present work extends these prior studies because they did not investigate receptor subunit levels in the PSD. It is the amount of receptors present in the PSD, which arguably gives a more functional representation of glutamatergic signaling, as it these receptors that are positioned to respond to synaptic glutamate. Future research should delineate the mechanisms underlying the changes in the profiles of synaptic glutamate receptors across both models.

4. Conclusions

In summary, we have shown that constitutive, genetic perturbation in the availability of the NMDAR co-agonists, D-serine and glycine, changes the molecular landscape of ionotropic glutamate receptors in the PSD of hippocampal neurons. Moreover, our findings demonstrate the complexity of synaptic adaptation to altered NMDAR function. This research highlights the GMS as a potential therapeutic target for diseases in which a dysregulation of NMDAR function is thought to contribute to the pathology, as is the case with schizophrenia (Balu and Coyle, 2010).

5. Experimental Procedures

5.1. Animals

SR^{-/-} (Basu et al., 2009) and GlyT1^{+/-} (Tsai et al., 2004) mice were generated as previously described. The serine racemasenull mutation to the first coding exon and the glycine transporter 1 null mutation to exons 2–3 have been backcrossed for over 10 generations onto a C57BL/6J background. SR^{+/-} and GlyT1^{+/-} parents were bred to produce wild-type (WT), as well as SR^{-/-} and GlyT1^{+/-} offspring. The animals were housed in groups of four in polycarbonate cages and maintained on a 12:12 h light/dark cycle in a temperature (22°C) and humidity controlled colony. The animals were given access to food and water *ad libitum*. All animal procedures were approved by the McLean Hospital Institutional Animal Care and Use Committee.

5.2. Cellular fractionation protocol

Adult (10–14 weeks old) and juvenile (3–4 weeks olds) male mice were sacrificed and their brains were quickly removed. A dissection block (Braintree Scientific) was used to take a ~1.5mm PFC section approximately 2.9mm–1.5mm anterior to bregma (Paxinos & Franklin, 2001) that was then cut at the rhinal fissure (ventral portion of the section was discarded). Both lobes of the hippocampus were also collected. The tissue was weighed to obtain wet weight values, flash frozen in isopentane, and stored at –80°C until analysis.

The fractionation procedure used here was modified from a previously published protocol (Fumagalli et al., 2008). Tissue was homogenized in a glass-glass potter in 0.4 ml of ice-cold 0.32M sucrose containing 1mM HEPES, 1 mM magnesium chloride, 1 mM EDTA, and 1 mM sodium bicarbonate (pH = 7.4). A 50 µl aliquot was reserved, sonicated, and stored at –80°C. This aliquot served as the whole cell lysate (WCL) fraction. The resultant (350 µl) of homogenized tissue was centrifuged at 1000g for 10 minutes at 4°C to separate a pellet enriched in nuclear components from the supernatant (S1). S1 was then centrifuged at 13,000g for 15 minutes at 4°C to obtain a clarified cytosolic fraction (S2). The pellet (P2), which represented a crude membrane fraction, was resuspended in 1 mM HEPES and centrifuged at 100,000g for 1 hour at 4°C. The pellet (P3) was resuspended in buffer containing 75mM potassium chloride and 1% Triton X-100 using a glass-glass potter and centrifuged at 100,000g for 1 hour at 4°C. The pellet (P4) is referred to as the Triton-insoluble fraction (TIF) that is enriched in PSD proteins. The resultant TIF was first resuspended in 60 µl of a 20mM HEPES buffer containing 0.5% digitonin, 0.2% sodium cholate, and 0.5% NP-40, then sonicated on ice for 30 seconds three times (samples were placed on ice between sonications). The protein was stored at –80°C until analysis.

5.3. Western blot analysis

Western blot analyses were performed on WCL (n = 5–12 per genotype) and PSD (n = 5–12 per genotype) protein fractions. Prior to gel loading, WCL and PSD samples were heated to 95°C for 5 and 10 minutes, respectively. Equal volumes of WCL and PSD protein samples (each sample was run in duplicate) were electrophoretically separated on an SDS-10% polyacrylamide gel. Nitrocellulose membranes (Bio-Rad, Hercules, CA) were blocked with 5% nonfat dry milk (Shaw's; Boise, ID) in 0.05% Tween-20/Tris buffered saline and then incubated with primary antibody overnight at 4°C. The primary antibodies (rabbit polyclonal; Abcam, Cambridge, MA) were used at the following dilutions: NR2A (1:2000), NR2B (1:2000), PSD95 (1:8000), GluR1 (1:1000), GluR2 (1:1000), and β-actin (1:8000). NR1 was detected using a mouse monoclonal primary antibody (1:2000; Millipore, Temecula, CA). After incubation with goat anti-rabbit (1:5000; Abcam, Cambridge, MA) or rabbit anti-mouse (1:3000; Abcam, Cambridge, MA) horseradish peroxidase-conjugated secondary antibodies, immunocomplexes were visualized by chemiluminescence using

Western Lightning-ECL (Perkin Elmer; Waltham, MA). Semi-quantitative assessment of protein bands on developed autoradiography film (Midsco, St Louis, MO) was executed by computerized densitometry on a MacIntosh G4 computer (Apple, Cupertino, CA) using Quantity One Quantitation Software (Bio-Rad, Hercules, CA).

5.4. Statistical analysis

Each protein sample was run in duplicate with the average optical density (OD) of the two bands used as the value for that sample, which was then normalized to the wet weight of the tissue (PSD) to provide a value that was representative of tissue volume. WCL OD values were divided by their corresponding β -actin OD values. The mutant values were normalized to their corresponding WT values (% control) within the same gel. The normalized values were then averaged and statistical differences between genotypes were determined using unpaired Student's t-test. $p < 0.05$ was considered statistically significant.

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Fig. 1.

Altered hippocampal NMDAR expression selectively in the PSD of SR^{-/-} mice. (A,B) Postsynaptic density enriched (PSD) and (C,D) whole cell lysate (WCL) hippocampal fractions were collected from adult wild type (WT; black bars; n = 6–12) and mutant (SR^{-/-}; white bars; n = 6–12) mice. The amount of NMDAR subunits (NR1, NR2A, NR2B), postsynaptic density protein of 95kDa (PSD95), and α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor (AMPA) subunits (GluR1, GluR2) were quantified by Western blot analysis. Values are expressed as the optical density (OD) normalized to WT values (% control). Bars represent mean values $\pm$ s.e.m. Asterisk (*) indicates that SR^{-/-} differed significantly from WT ($p < 0.05$).

Fig. 2.

Altered hippocampal NMDAR and AMPAR expression in GlyT1 +/- mice. (A,B) Postsynaptic density enriched (PSD) and (C,D) whole cell lysate (WCL) hippocampal fractions were collected from adult wild type (WT; black bars; n = 6–12) and mutant (GlyT1 +/-; hatched bars; n = 6–12) mice. The amount of NMDAR subunits (NR1, NR2A, NR2B, postsynaptic density protein of 95kDa (PSD95), and α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor (AMPA) subunits (GluR1, GluR2) were quantified by Western blot analysis. Values are expressed as the optical density (OD) normalized to WT values (% control). Bars represent mean values $\pm$ s.e.m. Asterisk (*) indicates that GlyT1 +/- differed significantly from WT ($p < 0.05$).

Table 1

Glutamatergic receptors are altered in the hippocampus of juvenile SR^{-/-} mice.

Hippocampus		NR1		NR2A		NR2B		PSD95	
PSD Fraction		% control	P	% control	P	% control	P	% control	P
WT (n = 7-10)		100 ± 9	0.89	100 ± 9	0.41	100 ± 10	0.52	100 ± 10	0.24
SR ^{-/-} (n = 7-10)		102 ± 10		114 ± 15		111 ± 14		119 ± 12	
WCL Fraction		% control	P	% control	P	% control	P	% control	P
WT (n = 7-10)		100 ± 5	0.45	100 ± 4	0.13	100 ± 4	0.009	100 ± 6	0.64
SR ^{-/-} (n = 7-10)		105 ± 4		110 ± 5		119 ± 5*		105 ± 8	

Postsynaptic density: PSD; whole cell lysate: WCL; wild type: WT; serine racemase knockout: SR^{-/-}; postsynaptic density protein of 95kDa: PSD95

Table 2

Glutamatergic receptors are unaltered in the PFC of SR^{-/-} mice.

Prefrontal Cortex	NR1		NR2A		NR2B		PSD95		GluR1		GluR2	
	% control	p	% control	p	% control	p	% control	p	% control	p	% control	p
PSD Fraction												
WT (n = 6-12)	100 ± 10	0.97	100 ± 9		100 ± 10	0.84	100 ± 12	0.38	100 ± 4	0.43	100 ± 9	0.79
SRKO (n = 6-12)	99 ± 13		103 ± 15		97 ± 13		116 ± 13		116 ± 18		95 ± 15	
WCL Fraction												
WT (n = 5-6)	100 ± 10	0.93	100 ± 6	0.85	100 ± 14	0.74	100 ± 7	0.53	100 ± 6	0.84	100 ± 4	0.55
SRKO (n = 6)	99 ± 12		98 ± 7		106 ± 7		94 ± 6		97 ± 12		108 ± 11	

Postsynaptic density: PSD; whole cell lysate: WCL; wild type: WT; serine racemase knockout: SR^{-/-}; postsynaptic density protein of 95kDa: PSD95

Table 3

Glutamatergic receptors are unaltered in the PFC of GlyT1 $+/-$ mice.

Prefrontal Cortex	NR1		NR2A		NR2B		PSDP5		GluR1		GluR2	
	% control	p	% control	p	% control	p	% control	p	% control	p	% control	p
PSD Fraction												
WT (n = 5–11)	100 ± 8	0.35	100 ± 6	0.3	100 ± 8	0.52	100 ± 4	0.98	100 ± 14	0.88	100 ± 9	0.83
GlyT1 $+/-$ (n = 5–11)	113 ± 11		112 ± 10		106 ± 6		99 ± 18		104 ± 18		103 ± 12	
WCL Fraction												
WT (n = 6–11)	100 ± 9	0.73	100 ± 7	0.83	100 ± 6	0.79	100 ± 5	0.36	100 ± 4	0.84	100 ± 5	0.65
GlyT1 $+/-$ (n = 6–11)	96 ± 7		98 ± 7		98 ± 5		93 ± 5		98 ± 5		104 ± 7	

Postsynaptic density: PSD; whole cell lysate: WCL; wild type: WT; glycine transporter heterozygote: GlyT1 $+/-$; postsynaptic density protein of 95kDa: PSD95