Published in final edited form as:

Neurosci Lett. 2011 October 10; 503(3): 215–219. doi:10.1016/j.neulet.2011.08.039.

Developmental localization of NMDA receptors, Src and MAP kinases in mouse brain

Xiangning Jiang¹, Renatta Knox^{1,2}, Praneeti Pathipati¹, and Donna Ferriero^{1,3}

¹Department of Neurology; University of California, San Francisco, CA, 94143

²Biomedical Science Graduate Program; University of California, San Francisco, CA, 94143

³Pediatrics; University of California, San Francisco, CA, 94143

Abstract

Activation of NMDA receptors (NMDAR) is associated with divergent downstream signaling leading to neuronal survival or death that may be regulated in part by whether the receptor is located synaptically or extrasynaptically. Distinct activation of the MAP kinases ERK and p38 by synaptic and extrasynaptic NMDAR is one of the mechanisms underlying these differences. We have recently shown that the Src family kinases (SFKs) play an important role in neonatal hypoxic-ischemic brain injury by regulating NMDAR phosphorylation. In this study, we characterized the distribution of NMDAR, SFKs and MAP kinases in synaptic and extrasynaptic membrane locations in the postnatal day 7 and adult mouse cortex. We found that the NMDAR, SFKs and phospho-NR2B were predominantly at synapses, whereas striatal-enriched protein tyrosine phosphatase (STEP) and its substrates ERK and p38 were much more concentrated extrasynaptically. NR1/NR2B was the main subunit at extrasynaptic membrane with concomitant NR2B phosphorylation at tyrosine (Y) 1336 in the immature brain. STEP expression increased, while p38 decreased with development in the extrasynaptic membrane. These results suggest that SFKs and STEP are poised to differentially regulate NMDAR-mediated signaling pathways due to their distinct subcellular localization, and thus may contribute to the age-specific differences seen in vulnerability, pathology and consequences of hypoxic–ischemic brain injury.

Keywords

neonatal; p38; ERK; STEP; NR2B; phosphorylation

Introduction

There are striking differences between neonatal and adult brain in response to hypoxic-ischemic (HI) brain injury. Due to overexpression of glutamate receptors to promote activity-dependent neuronal plasticity, the neonatal brain is more excitable and prone to oxidative stress than the adult brain [9, 11]. Recent studies show that the *N*-methyl-D-aspartate receptors (NMDAR), which have long been considered as a critical mediator for

© 2011 Elsevier Ireland Ltd. All rights reserved.

Corresponding author: Xiangning Jiang, MD. Ph.D. Department of Neurology, Box 0663 University of California, San Francisco 521 Parnassus Ave, Room C215, San Francisco, CA 94143-0663 Tel: 415-502-5822 Fax: 415-502-5821 xiangning.jiang@ucsf.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

excitotoxic cell death, are also able to initiate neuronal survival depending on whether they are synaptically or extrasynaptically located [4, 6, 19]. Synaptic NMDAR stimulation boosts intrinsic antioxidant defenses [16], activates the Ras-extracellular signal regulated kinase (ERK)-cAMP response element binding protein (CREB) pathway and translation of prosurvival proteins [23], whereas stimulation of extrasynaptic NMDAR induces pro-apoptotic proteins through an ERK-CREB shut-off pathway [7, 8] and activation of p38 [22]. Interestingly, coupling of NMDAR to intracellular signaling pathways is developmentally regulated as well [5, 18, 24]. This raises the question whether the NMDAR and its associated proteins are localized differentially in synaptic membrane components in neonatal and adult brain, which allows for specificity of the signaling cascades.

NMDAR are heteromeric complexes of the NR1, NR2 (2A-2D) and NR3 subunits. The NR1 subunit is essential for functional NMDAR channels, whereas the four NR2 subunits modulate channel activity and functional properties of the receptors. We have previously shown that, following neonatal HI, the Src family kinases (SFKs) are activated in the postsynaptic densities (PSDs) and interact with the NR2A and NR2B subunits [10]. Protection from specific SFKs inhibition implicates SFKs in the injury seen in neonatal HI. SFKs, especially Fyn, mediate tyrosine phosphorylation of NR2B at three major sites: tyrosine (Y) 1472, Y1252 and Y1336 [14]. By contrast, the striatal-enriched tyrosine phosphatase (STEP) dephosphorylates NR2B at Y1472 and reduces activity of its substrates ERK and p38 [1, 13, 15]. A recent study demonstrates in mature hippocampal slices that phosphorylation of Y1472 and Y1336 is associated with synaptic and extrasynaptic enrichment of NR2B, respectively [2]. This points to the possibility that SFKs and STEP may regulate NMDAR trafficking on the cell surface by phosphorylation or dephosphorylation of different residues. These data can not be applied to the immature brain, so it is important to determine whether SFKs modulation of NMDAR and downstream MAP kinases are uniquely affected by their subcellular localization in the developing brain.

In the present study, we characterized the distribution of NMDAR, Src and MAP kinases in synaptic and extrasynaptic membranes of neonatal and adult mouse brain to begin to investigate the mechanisms underlying differences between synaptic versus extrasynaptic NMDAR signaling.

Material and Methods

All animal experiments were approved by the institutional animal care and use committee at the University of California San Francisco and every effort was made to minimize animal suffering and reduce the number of animals used. Cortical tissue was dissected from the brains of postnatal day 7 (P7) and adult (around P48) C57BL/6 mice. Purification of synaptic and extrasynaptic membrane proteins was performed according to Goebel-Goody and colleagues' procedure [2] using a subcellular fractionation approach followed by extraction with Triton X-100. In brief, cortical tissue was homogenized in ice-cold sucrose buffer containing 0.32M sucrose, 10mM Tris-HCl (pH 7.4), 1mM EDTA, 1mM EGTA and protease and phosphatase inhibitors (Complete mini and Phospho-Stop cocktail tablets, Roche, Indianapolis, IN). A low-speed (1,000×g) centrifugation was performed to remove the nuclear fraction and tissue debris. The resulting supernatant (S1) was spun at 10,000×g for 15 minutes to yield a crude membrane fraction (P2). The supernatant (S2) was then centrifuged at 100,000×g for 60 min to separate cytoplasmic protein (S3) and intracellular light membrane fraction (P3). The P2 was subsequently resuspended in 120 µl sucrose buffer, and mixed with 8 volumes of 0.5% Triton X-100 buffer containing 10mM Tris-HCl (pH 7.4), 1mM EDTA, 1mM EGTA and protease and phosphatase inhibitors. The mixture was homogenized again with 30 pulses of a glass pestle and rotated at 4°C for 30 min

followed by centrifugation at 32,000×g for 30 min in a TL-100 tabletop ultracentrifuge (Beckman). The resultant pellet (TxP) containing Triton X-insoluble PSD proteins was considered as the synaptic membrane compartment. The supernatant (TxS) containing proteins soluble in Triton X-100 and not tightly bound to the PSD was defined as the extrasynaptic membrane compartment. The S3 and TxS fractions were further concentrated by adding 8 volumes of 100% acetone and incubated at −20 °C overnight. The precipitated protein was spun at 3000×g at 4°C for 15 min and dried at room temperature for 15 min. All the pellets were dissolved in TE buffer (100 mM Tris-HCl, 10 mM EDTA) with 1% SDS. The samples were sonicated, boiled for 5 min and stored at −80 °C until use. Protein concentration was determined by the bicinchoninic acid method (Pierce).

For Western blot analysis, an equal amount of cytoplasmic (S3), extrasynaptic (TxS) and synaptic (TxP) protein (7 µg) from P7 and adult mice was applied to 4–12% Bis-Tris SDS polyacrylamide gel electrophoresis (Invitrogen, Carlsbad, CA) and transferred to polyvinylidene difluoride membrane as described elsewhere [10]. The blots were probed with the following primary antibodies overnight at 4°C: NR1 (1:1,000; BD Pharmingen, San Diego, CA), NR2A (1:500; Upstate Cell Signaling Solutions, Lake Placid, NY), NR2B (1:2,000; BD), Fyn (1:800; Santa Cruz Biotechnology, Santa Cruz, CA), Src (1:500; Upstate), the phospho-site specific antibodies against NR2B Tyr1252, Tyr1336, and Tyr1472 (1:800; PhosphoSolutions, Inc. Aurora, CO), ERK (1:2,000; Cell signaling Technology, Danvers, MA), p38 (1:200; Cell signaling), and STEP (1:500; Upstate). The following antibodies were used to verify synaptic and extrasynaptic membrane purity: PSD-95 (1:2,000; Upstate), P97 ATPase (1:1,000; Fitzgerald Industries International, Concord, MA), EEA1 (1:200; Cell signaling) and Rab11 (1:500; Cell signaling). Appropriate secondary horseradish peroxidase-conjugated antibodies (1:2,000, Santa Cruz) were used, and signal was visualized with enhanced chemiluminescence (Amersham). Image J software was used to measure the mean optical densities (OD) and areas of protein signal on radiographic film after scanning.

To quantify the protein expression from Western blot analysis, the OD values from each blot were normalized to P7 synaptic values. For STEP and p38, the blots were normalized to P7 extrasynaptic values. Two-tailed Student's t-tests were used to compare protein expression between P7 and adult animals in cytoplasmic, synaptic and extrasynaptic membrane fractions. Statistical significance was determined as $p < 0.05$. Data are presented as mean $\pm$ SD from three independent experiments.

Results

Purity of the synaptic and extrasynaptic membranes

The purity of the subcellular compartments was assessed by Western blotting (Fig. 1). Synaptic markers used were proteins representative of PSD (PSD-95, NR1 and NR2A). For identification of extrasynaptic membrane proteins, we used antibodies against EEA1 and Rab11, which are involved in early endosomal transport and receptor endocytic recycling that take place at extrasynaptic sites [12, 17]. The p97 ATPase (also called valosin-containing protein) is bound to Golgi and endoplasmic reticulum membrane and was used as a marker for intracellular light membrane (P3). Consistent with previous studies with the same approach [2], PSD95 was exclusively in synaptic membrane (TxP); NR1 and NR2A were most enriched in synaptic membrane. EEA1 and Rab11 were not expressed in TxP, but most concentrated in extrasynaptic fractions (TxS). p97 ATPase was predominantly present in the intracellular light membrane fraction (P3). These results confirmed that synaptic and extrasynaptic membranes were enriched without contamination with other subcellular components.

Membrane Localization of NMDARs in P7 and adult mouse cortex

NR1, NR2A and NR2B were all concentrated in synaptic membranes in both P7 and adult brains (Fig. 2a). In the synaptic fraction, the expression of NR2B decreased ($p=0.0155$, P7 vs. adult), while NR2A increased ($p=0.0487$, P7 vs. adult), with development (Fig. 2a-c). NR1 remained constant at both ages. Extrasynaptically, there was significantly higher expression of NR1 ($p=0.0348$) and NR2B ($p=0.0276$) at P7 than that in adult brain, suggesting that NR1/NR2B is the main NMDAR subtype at extrasynaptic sites at P7. NR2B is also the main subunit to be tyrosine phosphorylated by Fyn in the PSDs, so we chose to examine the localization and expression of NR2B that is phosphorylated at three major tyrosine residues by Fyn. pY1472NR2B and pY1252NR2B were located predominantly in synapses at both ages and increased significantly with development (Fig. 2a, 2d, $p=0.0432$ for NR2BY1472, $p=0.0188$ for NR2BY1252, P7 vs. adult). In regard to pY1336NR2B, although was more enriched synaptically (Fig. 2a, 2c), it was the major phosphorylated NR2B form located extrasynaptically at P7 (12.56% of total pY1336NR2B) with no extrasynaptic expression in the adult.

Membrane localization of SFKs and STEP in P7 and adult mouse cortex

We further examined the subcellular localization of Src, Fyn and STEP, which are the best-characterized tyrosine kinases and phosphatase involved in phospho-regulation of NMDAR and both are changed following neonatal HI. Fyn was more concentrated at synapses and decreased with age in both synaptic and extrasynaptic membranes (Fig. 3a-b, $p=0.0006$ for synaptic Fyn; $p=0.0048$ for extrasynaptic Fyn, P7 vs. adult). Src was equally distributed between synaptic and extrasynaptic membranes with lower levels in the adult brain than at P7 (Fig. 3a-b, $p=0.035$ for synaptic Src; $p=0.0346$ for extrasynaptic Src, P7 vs. adult). We used a STEP antibody that detects the three major alternatively spliced variants (61, 46 and 38kD). Membrane-associated STEP61 was located extrasynaptically and was 1.7-fold higher in adult animals compared to P7 mice (Fig. 3a, 3d, $p=0.0233$). Other STEP isoforms with lower molecular weights were detected in the cytoplasmic fractions and expressed at higher levels in adult animals, too.

Membrane localization of ERK and p38 in P7 and adult mouse cortex

The concentration of ERK and p38 was highest in the cytoplasmic fraction compared with extrasynaptic and synaptic fractions (Fig. 3a). There was no change with age in cytoplasmic ERK or p38. In membranes, ERK was more enriched extrasynaptically with a small fraction in the synaptic membranes. ERK1, but not ERK2, decreased with development at synaptic membranes ($p=0.0017$, P7 vs. adult). p38 was enriched extrasynaptically and not detectable in the synaptic fraction (Fig. 3a, 3d). Expression of extrasynaptical p38 was higher at P7 than that in adult ($p=0.0013$).

Discussion

This is the first study to examine the distribution of the NMDAR, Src and MAP kinases in synaptic and extrasynaptic membranes in the developing brain and to investigate their age-related expression on the cell surface. Our major findings are: 1). At all ages, membrane-associated NMDAR and Src kinases are predominantly at synapses, whereas STEP and its substrates ERK and p38 are much more concentrated extrasynaptically. 2). There is a developmental switch from NR2B to NR2A expression in synaptic membranes with more NR1/NR2B expression in extrasynaptic membranes in the developing brain. 3). While Fyn and Src protein levels decrease with age, NR2BY1472 and NR2BY1252 mediated by these kinases are significantly higher in the adult animals. At P7, phosphorylation of NR2B at Y1336 is associated with extrasynaptic NMDAR. 4). The developmental increase in STEP is accompanied by the decrease in p38 extrasynaptically. The results imply developmental

changes in expression at different locations in the coupling of NMDAR to downstream signaling mechanisms.

NR2B and Fyn are expressed at much higher levels at P7 in both synaptic and extrasynaptic membranes, suggesting the importance of Fyn in regulating NR2B in the developing brain. Fyn modulates NMDAR internalization and their lateral movement on the surfaces by phosphorylation at specific tyrosine residues. Most studies focus on NR2B Y1472, as it is the major site and the main form involved in synaptic plasticity. Little is known about the physiological function of NR2B Y1252 and NR2BY1336. From our study, although NR2B Y1472, Y1252 and Y1336 are all enriched in the synapses, NR1/NR2B is the main subunit occupying extrasynaptic sites with concomitant phosphorylation at Y1336 in the immature brain. This is in agreement with a recent study in adult hippocampal slices showing phosphorylation of Y1336 is associated with extrasynaptic enrichment of NR2B [2]. Other studies suggested that Y1336 phosphorylation enhances calpain-mediated extrasynaptic NR2B cleavage at C terminus, which may affect the ability of NR2B binding to associated proteins and thus change downstream signaling complexes [21]. This site also mediates activation of phosphatidylinositol 3-kinase (PI3K) and p38 dephosphorylation in mature hippocampal cultures following extrasynaptic NR2B stimulation, suggesting a possible protective role against NMDA toxicity [20]. This phenomenon was not observed in immature cultures since NR2B Y1336 was not increased under the same condition [20]. We found elevated NR2B Y1472, Y1252 and Y1336 expression early after neonatal HI at P7 (unpublished data), but whether and how these modifications link to their surface locations after HI and the subsequent downstream NMDAR signaling still remain unknown.

STEP61, the membrane-associated isoform, was found primarily at extrasynaptic sites in both P7 and adult brain. Correspondingly, STEP substrates ERK and p38 are mostly associated with extrasynaptic membranes. The extrasynaptic localization of STEP and p38 is consistent with a recent study from adult mouse cortical tissue [22], it also supports the preference of p38 activation and STEP cleavage following extrasynaptic NMDAR stimulation or in vitro ischemia [22]. STEP61 cleavage was also found in a neonatal P7 rat HI model [3]. Compared to the adult brain, P7 animals have lower STEP and higher p38 available at extrasynaptic sites; this may be related to the greater susceptibility of neonates to HI or other brain injury involving excitotoxicity.

ERK, another STEP substrate, while more concentrated extrasynaptically, has been reported to be activated by synaptic NMDAR stimulation and shut-off by extrasynaptic NMDAR. Complex mechanisms are involved in ERK regulation, so ERK activity is determined by whether activation or inhibition dominates. The functional significance of extrasynaptic ERK and p38 is not clear. It is possible that cytosol ERK and p38 are translocated to different cell compartments including membranes to interact with specific signal proteins in response to different stimuli.

In conclusion, our study demonstrates a developmental regulation in localization and expression of NMDAR, Src and MAP kinases in synaptic and extrasynaptic membranes in mouse cortical tissue. Protein localization could contribute to, but is unlikely to fully account for the differences between synaptic versus extrasynaptic NMDAR signaling. Determining whether pro-death or pro-survival signaling following NMDAR activation predominates will allow for identification of more specific therapeutic targets for neonatal HI.

Acknowledgments

This work was supported by: NINDS R21 NS059613 to Dr. Xiangning Jiang, NINDS F31 NS073145 to Renatta Knox, and NINDS RO1 NS33997 to Dr. Donna Ferriero.

References

1. Braithwaite SP, Adkisson M, Leung J, Nava A, Masterson B, Urfer R, Oksenberg D, Nikolic K. Regulation of NMDA receptor trafficking and function by striatal-enriched tyrosine phosphatase (STEP). *Eur J Neurosci.* 2006; 23:2847–56. [PubMed: 16819973]
2. Goebel-Goody SM, Davies KD, Linger R.M. Alvestad, Freund RK, Browning MD. Phosphoregulation of synaptic and extrasynaptic N-methyl-D-aspartate receptors in adult hippocampal slices. *Neuroscience.* 2009; 158:1446–59. [PubMed: 19041929]
3. Gurd JW, Bissoon N, Nguyen TH, Lombroso PJ, Rider CC, Beesley PW, Vannucci SJ. Hypoxia-ischemia in perinatal rat brain induces the formation of a low molecular weight isoform of striatal enriched tyrosine phosphatase (STEP). *J Neurochem.* 1999; 73:1990–4. [PubMed: 10537057]
4. Hardingham GE. Coupling of the NMDA receptor to neuroprotective and neurodestructive events. *Biochem Soc Trans.* 2009; 37:1147–60. [PubMed: 19909238]
5. Hardingham GE, Bading H. Coupling of extrasynaptic NMDA receptors to a CREB shut-off pathway is developmentally regulated. *Biochim Biophys Acta.* 2002; 1600:148–53. [PubMed: 12445470]
6. Hardingham GE, Bading H. Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat Rev Neurosci.* 2010; 11:682–96. [PubMed: 20842175]
7. Hardingham GE, Fukunaga Y, Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat Neurosci.* 2002; 5:405–14. [PubMed: 11953750]
8. Ivanov A, Pellegrino C, Rama S, Dumalska I, Salyha Y, Ben-Ari Y, Medina I. Opposing role of synaptic and extrasynaptic NMDA receptors in regulation of the extracellular signal-regulated kinases (ERK) activity in cultured rat hippocampal neurons. *J Physiol.* 2006; 572:789–98. [PubMed: 16513670]
9. Jensen FE. The role of glutamate receptor maturation in perinatal seizures and brain injury. *Int J Dev Neurosci.* 2002; 20:339–47. [PubMed: 12175872]
10. Jiang X, Mu D, Biran V, Faustino J, Chang S, Rincon CM, Sheldon RA, Ferriero DM. Activated Src kinases interact with the N-methyl-D-aspartate receptor after neonatal brain ischemia. *Ann Neurol.* 2008; 63:632–41. [PubMed: 18384166]
11. Johnston MV. Excitotoxicity in perinatal brain injury. *Brain Pathol.* 2005; 15:234–40. [PubMed: 16196390]
12. Mu FT, Callaghan JM, Steele-Mortimer O, Stenmark H, Parton RG, Campbell PL, McCluskey J, Yeo JP, Tock EP, Toh BH. EEA1, an early endosome-associated protein. EEA1 is a conserved alpha-helical peripheral membrane protein flanked by cysteine “fingers” and contains a calmodulin-binding IQ motif. *J Biol Chem.* 1995; 270:13503–11. [PubMed: 7768953]
13. Munoz JJ, Tarrega C, Blanco-Aparicio C, Pulido R. Differential interaction of the tyrosine phosphatases PTP-SL, STEP and HePTP with the mitogen-activated protein kinases ERK1/2 and p38alpha is determined by a kinase specificity sequence and influenced by reducing agents. *Biochem J.* 2003; 372:193–201. [PubMed: 12583813]
14. Nakazawa T, Komai S, Tezuka T, Hisatsune C, Umemori H, Semba K, Mishina M, Manabe T, Yamamoto T. Characterization of Fyn-mediated tyrosine phosphorylation sites on GluR epsilon 2 (NR2B) subunit of the N-methyl-D-aspartate receptor. *J Biol Chem.* 2001; 276:693–9. [PubMed: 11024032]
15. Nguyen TH, Liu J, Lombroso PJ. Striatal enriched phosphatase 61 dephosphorylates Fyn at phosphotyrosine 420. *J Biol Chem.* 2002; 277:24274–9. [PubMed: 11983687]
16. Papadia S, Soriano FX, Leveille F, Martel MA, Dakin KA, Hansen HH, Kaindl A, Siffringer M, Fowler J, Stefovskaya V, McKenzie G, Craigmiles M, Corriveau R, Ghazal P, Horsburgh K, Yankner BA, Wyllie DJ, Ikonomidou C, Hardingham GE. Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. *Nat Neurosci.* 2008; 11:476–87. [PubMed: 18344994]
17. Park M, Penick EC, Edwards JG, Kauer JA, Ehlers MD. Recycling endosomes supply AMPA receptors for LTP. *Science.* 2004; 305:1972–5. [PubMed: 15448273]

18. Sala C, Rudolph-Correia S, Sheng M. Developmentally regulated NMDA receptor-dependent dephosphorylation of cAMP response element-binding protein (CREB) in hippocampal neurons. *J Neurosci.* 2000; 20:3529–36. [PubMed: 10804193]
19. Soriano FX, Hardingham GE. Compartmentalized NMDA receptor signalling to survival and death. *J Physiol.* 2007; 584:381–7. [PubMed: 17690142]
20. Waxman EA, Lynch DR. N-methyl-D-aspartate receptor subtype mediated bidirectional control of p38 mitogen-activated protein kinase. *J Biol Chem.* 2005; 280:29322–33. [PubMed: 15967799]
21. Wu HY, Hsu FC, Gleichman AJ, Baconguis I, Coulter DA, Lynch DR. Fyn-mediated phosphorylation of NR2B Tyr-1336 controls calpain-mediated NR2B cleavage in neurons and heterologous systems. *J Biol Chem.* 2007; 282:20075–87. [PubMed: 17526495]
22. Xu J, Kurup P, Zhang Y, Goebel-Goody SM, Wu PH, Hawasli AH, Baum ML, Bibb JA, Lombroso PJ. Extrasynaptic NMDA receptors couple preferentially to excitotoxicity via calpain-mediated cleavage of STEP. *J Neurosci.* 2009; 29:9330–43. [PubMed: 19625523]
23. Zhang SJ, Steijaert MN, Lau D, Schutz G, Delucinge-Vivier C, Descombes P, Bading H. Decoding NMDA receptor signaling: identification of genomic programs specifying neuronal survival and death. *Neuron.* 2007; 53:549–62. [PubMed: 17296556]
24. Zhou X, Moon C, Zheng F, Luo Y, Soellner D, Nunez JL, Wang H. N-methyl-D-aspartate-stimulated ERK1/2 signaling and the transcriptional up-regulation of plasticity-related genes are developmentally regulated following in vitro neuronal maturation. *J Neurosci Res.* 2009; 87:2632–44. [PubMed: 19396876]

Highlights

- There is a developmental switch from NR2B to NR2A expression in synaptic membranes with more NR1/NR2B expression in extrasynaptic membranes in the developing brain.
- While Fyn and Src protein levels decrease with age, NR2BY1472 and NR2BY1252 mediated by these kinases are significantly higher in the adult animals.
- At P7, phosphorylation of NR2B at Y1336 is associated with extrasynaptic NMDA receptors.
- The developmental increase in STEP is accompanied by the decrease in p38 extrasynaptically.

Fig.1. Characterization of synaptic and extrasynaptic membranes. Representative Western blots with each subcellular fractions (S3: cytoplasmic fraction; P1: nuclear fraction; P3: intracellular membrane fraction; TxS: extrasynaptic membrane fraction; TxP: synaptic membrane fraction) probed with the PSD markers PSD95, NR1 and NR2A; the early endosomal markers EEA1 and Rab11 and the ER marker p97ATPase.

Fig. 2.

Localization of the NMDAR and phospho-NR2B at different subcellular fractions in P7 and adult mouse cortex. Equal amount of protein from cytoplasmic (cyto-), extrasynaptic (extra-) and synaptic (synap-) fractions was used for Western blotting. Primary antibodies are indicated on the left of the blots (2a). For each protein, the OD values were normalized to P7 synaptic values. Data are presented on the right (2b-2d) as mean $\pm$ SD from 3 independent experiments. *: $p < 0.05$ versus adult values from the same fraction.

Fig. 3.

Localization of the Src and MAP kinases, STEP at different subcellular fractions in P7 and adult mouse cortex. Equal amount of protein from cytoplasmic (cyto-), extrasynaptic (extra-) and synaptic (synap-) fractions was used for Western blotting. Primary antibodies are indicated on the left of the blots (3a). For Fyn, Src and ERK (3b-3c), the OD values were normalized to P7 synaptic values. For STEP and p38 (3d), the OD values were normalized to P7 extrasynaptic values. Data are presented on the right (3b-3d) as mean $\pm$ SD from 3 independent experiments. *: $p < 0.05$ versus adult values from the same fraction.