Available online at www.sciencedirect.com
SCIENCE DIRECT®
Progress in Neuro-Psychopharmacology & Biological Psychiatry 27 (2003) 411–417
Progress In Neuro-Psychopharmacology & Biological Psychiatry
www.elsevier.com/locate/pnpbp

SNAP-25 reduction in the hippocampus of patients with schizophrenia

Peter M. Thompson[a,*], Shirley Egbufoama[b], Marquis P. Vawter[c]

[a]Mood and Anxiety Disorders Division, Department of Psychiatry, University of Texas Health Science Center at San Antonio (UTHSCSA), 7703 Floyd Curl Drive, Mail Code 7792, San Antonio, TX 78229-3900, USA
[b]Cellular Neurobiology, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Baltimore, MD, USA
[c]Department of Psychiatry, University of California, Irvine, Irvine, CA, USA

Accepted 22 January 2003

Abstract

In this study, the authors sought to replicate the findings of reduced synaptosomal associated protein 25 kDa (SNAP-25) immunoreactivity in the hippocampus of patients with schizophrenia. The authors also measured *N*-methyl-D-aspartate (NMDA) receptor 1 (NR1) receptor subunit to determine if glutamatergic synapses were involved with the loss of SNAP-25. We found 49% less SNAP-25 immunointensity in the schizophrenic group ($n=7$) compared to the control ($n=8$) or bipolar groups ($n=4$) ($P=.004$). There was no change in NMDA NR1 levels in the three groups. The authors confirm the previous report of less SNAP-25 immunoreactivity in the hippocampus using a different cohort of patients with schizophrenia. It also appears that NMDA NR1 was unchanged, indicating that the overall level of NMDA glutamatergic synapses in hippocampus is normal. These data add to evidence suggesting that in schizophrenia the molecular pathology of the hippocampus involves presynaptic components.

Keywords: Bipolar illness; Exocytosis; NMDA; NR1; Schizophrenia; SNAP-25

1. Introduction

The hippocampus is a structure responsible for information processing and memory formation (Zola-Morgan and Squire, 1990). Pathology in the hippocampus is implicated in the altered cognitive functioning seen with schizophrenia (Kuperberg and Heckers, 2000). One of the consistent neuroimaging findings supportive of this hypothesis is decreased hippocampus volume (Copolov et al., 2000). To identify the cellular pathology responsible for the volume loss, studies have measured neuron number and neuropil components such as axon and dendrite densities. The results of these studies are contradictory (Harrison, 1999). Half of the studies find changes in neuronal morphology or loss of neurons and half find no pathology. However, the consensus is that if cell loss is involved the loss is not due to necrosis.

In a molecular approach to identify pathology, investigators measured specific neuronal proteins and mRNA that may be involved with schizophrenia. Using these approaches, both glutamatergic neurotransmission and presynaptic synaptic vesicular docking proteins have been implicated in schizophrenic pathology of the hippocampus. There appears to be diminished amounts of presynaptic components and some types of glutamate receptors subunits (Browning et al., 1993; Eastwood et al., 1995; Eastwood and Harrison, 2000; Vawter et al., 1999; Webster et al., 2001; Meador-Woodruff et al., 2001; Kerwin and Meldrum, 1990; Tsai et al., 1995; Gao et al., 2000).

Glutamatergic neurotransmission involves several types of receptors that may be involved with some aspects of schizophrenia symptomatology (Javitt et al., 1997). One of the receptor subtypes that is specifically implicated is the ionotropic *N*-methyl-D-aspartate (NMDA) receptor. The functional receptor is heteromeric and composed of at least one NMDA receptor 1 (NR1) and one or more of the NR2 subunits (Masu et al., 1993). The NMDA receptor in the hippocampus is found predominately though not exclusively in the postsynaptic area of apical dendrites and soma (Kohama and Urbanski, 1997; Conti et al., 1999; Petralia et al., 1994). When this receptor is activated, it produces

Abbreviations: NMDA, *N*-methyl-D-aspartate; NR1–5, NMDA receptors 1–5; PMI, postmortem interval; SNAP-25, synaptosomal associated protein 25 kDa.

* Corresponding author. Tel.: +1-210-567-6816; fax: +1-210-567-3759.
E-mail address: Thompsonp@uthscsa.edu (P.M. Thompson).

0278-5846/03/$ – see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0278-5846(03)00027-7

symptoms found in schizophrenia (Bunney et al., 1995). In tissue from patients with schizophrenia NMDA receptor binding is intact and the NR1 subunit mRNA in some areas may be reduced (Gao et al., 2000).

Synaptosomal associated protein 25 kDa (SNAP-25) is a protein that is involved with regulated exocytosis (Bark et al., 1995) and is located on the presynaptic nerve terminus (Oyler et al., 1989). In a study of the level of this protein in the hippocampus of patients with schizophrenia, Young et al. (1998) found that SNAP-25 was reduced.

Brain lesion studies offer some reference to understand these findings. In dopaminergic systems when the presynaptic cell is lost, the postsynaptic receptors may either upregulate (Creese et al., 1977; Zhang et al., 2001) or downregulate (Levesque et al., 1995). In the hippocampus, lesions of the perforant pathway lead to loss of the presynaptic afferent components. Morphologically, there is initial loss of axons and dendrites followed by reactive sprouting in an attempt to reconnect (Matthews et al., 1976). These cellular changes are reflected in protein changes. Following the lesion, presynaptic SNAP-25 initially decreases then increases (Geddes et al., 1990). Postsynaptically, the NMDA glutamate receptors are lost (Nicolle et al., 1997). Some time later, with sprouting, the receptors increase (Adams et al., 2001; Gazzaley et al., 1997). However, dendritic sprouting does not reach prelesion levels at 90 days posttrauma (Nieto-Sampedro et al., 1982). This observation suggests that the morphology stabilizes but does not entirely correct the damage. If a cell loss process occurs in schizophrenia, postmortem analysis of the presynaptic and postsynaptic components may show similar results as the lesions studies excluding gliosis.

In this study, we sought to replicate the findings of Young et al. (1998). The authors also hypothesized that if a cell loss process is involved with schizophrenia, both presynaptic and postsynaptic proteins will be reduced compared to normal controls. To test this hypothesis, we measured the level of SNAP-25 and NMDA NR1 glutamatergic receptor subunit in hippocampus of patients who had schizophrenia.

2. Methods

2.1. Postmortem hippocampal tissue

The collection and preparation of the hippocampal tissue has been previously reported (Vawter et al., 1998b). Briefly, the tissue was obtained from the National Institute of Mental Health Neuroscience Center at Saint Elizabeth's Hospital brain collection. A neuropathologist examined the brain tissue to identify gross structural abnormalities. Psychiatric diagnosis was made by retrospective chart review by two psychiatrists using Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R) criteria (Williams et al., 1992). 1.5 cm coronal slabs through the entire cerebrum of each human brain were rapidly dissected from the amygdala through the posterior hippocampus (Kleinman et al., 1995). The hippocampus proper then was dissected out from the adjacent entorhinal cortex or parahippocampal gyrus on a platform cooled with dry ice. The right and left hippocampi from each brain were dissected, and the bilateral hippocampal tissue was combined for each subject. The dissected tissue was frozen on dry ice and stored at -80 °C.

Membrane extracts of human hippocampus was prepared as described (Takamatsu et al., 1994), with slight modifications (Vawter et al., 1998a,b). Frozen pulverized whole hippocampal tissue ~ 0.50 g was suspended in 10 ml of cold 0.05 M Tris-buffered saline (TBS, pH 7.4) with protease inhibitors: antipain (4 μg/ml), pepstatin A (2 μg/ml), aprotinin (2 μg/ml), leupeptin (2 μg/ml) and phenyl methyl sulfonyl fluoride (0.1 μg/ml). The solution was homogenized (Tissumizer; Tekmar, Cincinnati, OH) for 5×10 s pulses in a 4 °C ice bath and 30 s cooling interval between pulses. The homogenate was centrifuged for 30 min at $42{,}000 \times g$ at 4 °C. The clear supernatant with visible lipid removed was the "cytosolic" fraction. The pellet was resuspended and washed with cold TBS-protease inhibitor cocktail and recentrifuged at $42{,}000 \times g$ for 30 min at 4 °C. The supernatant was discarded and the pellet was extracted in cold TBS + protease inhibitor + 1% NP-40 detergent. The solution was stirred at 4 °C for 30 min and then centrifuged at $42{,}000 \times g$ for 30 min. The supernatant was labeled "membrane extract." Protein concentration was measured bicinchoninic acid assay (Pierce).

The brain pH was determined from cerebellar tissue (0.4–0.8 g) that was homogenized in 10 times the tissue volume in ddH_2O (pH 7.0). The homogenate pH was measured with a calibrated pH meter.

2.2. Western blotting

Eighty micrograms of the crude membrane fraction was electrophoresed on a 7.5% SDS-PAGE gel, run for 5.5 h at 40 mA and cooled to 4 °C. Protein from the gels was then electroblotted to Hybond supersupported 0.45 μm nitrocellulose membrane (Amersham) in a semidry transfer apparatus (Bio-Rad). Membranes were cut in half and the upper half containing the NMDA NR1 protein was blocked in phosphate-buffered saline (PBS) and 5% nonfat powered milk for 4 h at 4 °C. The membrane was then washed twice in PBS with 0.1% Tween-20 (PBS-T) for 20 min at room temperature (RT). The NMDA NR1 antibody (clone 54.1; Zymed) reacts specifically with a band at 103 kDa and does not cross-react with NMDAR2, NMDAR3, NMDAR4 and NMDAR5. Anti-NMDA NR1 was diluted 1/500 in PBS-T with 1% bovine serum albumin (BSA) and 2.5% nonfat powered milk and incubated for 16 h at 4 °C with the membrane. The membranes were then washed as before and incubated in a 1/1000 dilution with the secondary goat antimouse antibody conjugated to horseradish peroxidase (HRP) for 40 min at RT with shaking. The membranes were

washed and the antigen antibody complex was visualized with enhanced chemiluminescence (Amersham). The band intensity was measured with NIH Image Software v.1.69 from a scanned image (Alpha Innotech) of the membrane. Relative values of NMDA NR1 were determined by comparing the immunointensity of the unknown sample to that of a standard curve. The standard curve was made by applying 20–80 μg of control crude brain extract to the same gel as the experimental samples. Units of measure are immunointensity per microgram total protein.

The lower half of the membrane containing the SNAP-25 band was blocked in 5% nonfat powered milk in TTBS (TBS with 0.1% Tween) for 1 h at RT with shaking. The membrane was then washed 2 times for 10 min in TTBS at RT with shaking. Following this step, the membrane was incubated in a 1/5000 dilution of anti-SNAP-25 antibody (SMI 81, Sternberger Monoclonals) in TTBS for 1 h at RT. Following two more washes, the membrane was incubated with 1/10,000 goat antimouse HRP conjugated IgG (Zymed) for 1 h and then washed as before. Visualization was similar to NMDA NR1. Relative quantitation was determined by the same procedure as NMDA NR1, but immunodetection was with the SMI-81 antibody (Thompson et al., 1998).

2.3. Statistical analysis

The potential confounding factors of postmortem interval (PMI), brain pH and age at the time of death were compared in separate analysis of variance (ANOVA). Gender differences were compared using the Pearson χ^2 test. Differences of NMDA NR1 and SNAP-25 levels between all groups were determined by ANOVA using PMI and storage time as covariants (ANCOVA).

3. Results

There were no differences between groups for gender ($\chi^2 = 2.37$, $df = 2$, $P = .31$), age [$F(2,15) = 0.111$, $P = .89$] and pH [$F(2,15) = 1.5$, $P = .25$] as shown in Table 1. PMI was significantly shorter in the schizophrenia group [$F(2,15) = 4.0$, $P = .04$]. The tissue storage time was considered as a potential confound since the freezer time was different between the three diagnostic groups [$F(2,16) = 19.41$, $P = .00005$]. However, the controls and schizophrenia groups were not significantly different in storage time ($P = .43$), while the bipolar group was stored longer compared to controls or schizophrenia groups ($P < .0005$ for both comparisons).

In the control group, one patient had a detectable level of lidocaine, one with phensuximide and one with morphine. Two patients with schizophrenia showed positive levels of antipsychotic drugs at the time of death. Additionally, one had doxepin and one had nortriptyline. The bipolar group had one individual with detectable alcohol, serax and nardil. In 3 of the total 19 patients, no toxicological information is available (2 = biolar, 1 = schizophrenia).

Table 1
SNAP-25 and NMDA NR1 Western blot measurements and demographic characteristics

	Group		
	Control ($n = 8$)	Bipolar illness ($n = 4$)	Schizophrenia ($n = 7$)
Brain pH (±S.D.)	6.2 ± 0.35	6.4 ± 0.08	6.57 ± 0.25
Tissue storage time (months ± S.D.)	112.5 ± 15.7	196.3 ± 3.7[a]	127.2 ± 32.3
PMI (hours ± S.D.)	25.4 ± 13.8	29.6 ± 7.4	12.5 ± 8.2[b]
Age (year ± S.D.)	49.1 ± 13.6	48.3 ± 11.8	52.3 ± 19.3
Gender (M = males, F = females)	M = 5, F = 3	M = 1, F = 3	M = 5, F = 2
Ethnicity (Black/Caucasian)	6/2	1/3	2/5
NMDA NR1 (relative immunoreactivity units ± S.D.)	0.85 ± 0.35	0.96 ± 0.12	0.84 ± 0.3
SNAP-25 (relative immunoreactivity units ± S.D.)	1.13 ± 0.4	1.18 ± 0.09	0.58 ± 0.2[c]

[a] Bipolar group is more than the control and schizophrenia groups ($P = .00005$).
[b] Schizophrenia group is less than the control and bipolar groups ($P = .04$).
[c] Schizophrenia group is less than control or bipolar groups ($P < .004$).

For protein loading, we initially determined that the optimal range was in the range of 20–80 μg of total protein in order to quantitatively assess NMDAR1 and SNAP-25. For each immunoblot, a standard preparation of human occipital cortex was loaded on each gel in ascending protein load (10, 20, 30, 40 and 50 μg). We assayed 50 μg of total protein for each unknown sample. The densitometry of these readings for each gel indicated linear results for micrograms of protein loaded within the 10–50 μg range and the ECL reaction ($r > .97$ for NMDAR1 and $r > .9$ for SNAP-25 standard curves).

The authors compared the level of SNAP-25 and NMDA NR1 across diagnostic groups with PMI as the covariant. The level of NMDA NR1 protein was not significantly different between groups [$F(2,15) = 0.14$, $P = .87$, control = 0.85 ± 0.35, bipolar illness = 0.96 ± 0.12 and schizophrenia = 0.84 ± 0.3, relative immunointensity]. In contrast, there was a significant difference in mean level of SNAP-25 immunointensity between groups [$F(2,15) = 8.2$, $P = .004$, control = 1.13 ± 0.4, bipolar illness = 1.18 ± 0.09 and schizophrenia = 0.58 ± 0.2] (Fig. 1). Two individuals in the schizophrenia group were treated with neuroleptics before death. Because of the presence of this mediation, the ANCOVA was rerun without these subjects. The SNAP-25 difference remained significant [$F(2,13) = 7.8$, $P = .009$].

The correlation between SNAP-25 and freezer time was low ($r = -.06$) for all combined groups. For individual groups, the correlations between freezer time and SNAP-25 for bipolar ($r = .27$, $P = .72$), schizophrenia ($r = -.54$, $P = .21$) and controls ($r = .31$, $P = .45$) were all modest but not significant. The correlations for the schizophrenia and con-

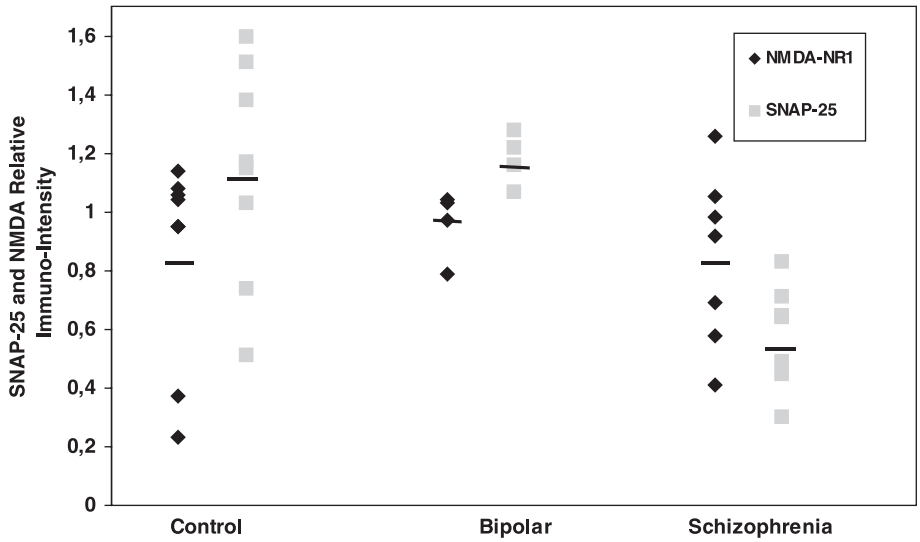

Fig. 1. Relative NMDA NR1 and SNAP-25 immunoreactivity. Horizontal bars represent mean values.

trol groups taken together were $r = -.26$, $P=.35$, indicating that freezer time might influence samples, as greater decreases of SNAP-25 with increasing freezer storage time might be seen in the schizophrenia group. The SNAP-25 analysis of covariance was calculated and the group effect for diagnosis remained significant [$F(1,12) = 10.23$, $P=.007$].

4. Discussion

We demonstrate SNAP-25 immunointensity is 49% less and NMDA NR1 is equivalent in the hippocampus of the schizophrenia group by Western blotting. In the bipolar group, the levels of both SNAP-25 and NMDA NR1 were similar to controls. Several potentially confounding issues affect the interpretation of these data. The first is the small number of subjects in the bipolar group. The small number of subjects in this group may have caused us to underestimate the differences between the groups and commit a type 2 statistical error. Secondly, the PMI was significantly shorter in schizophrenia than the other three groups, and the tissue storage time was longer in the bipolar group. The authors accounted for these differences by using PMI and storage time as covariants in the statistical analysis. If protein degradation were responsible for the results, we would anticipate that the shorter PMI would lead to an increase in SNAP-25 in the schizophrenia. We did not see this. We also found consistent levels of NMDAR1 between groups, suggesting that our protein loading and degradation was not different between groups. Another suggestion that cellular degradation was not a significant factor in our results was the uniform brain pH in all groups. Finally, medication use may alter protein levels. In two members of the schizophrenic group, neuroleptics were used. When these samples were removed from the analysis, the differences remained significant, suggesting that neuroleptics did not affect the SNAP-25 level. Preclinical studies also suggest that neuroleptic exposure does not influence SNAP-25 expression (Nakahara et al., 1998; Eastwood et al., 1997) and may lower NMDA NR1 (Chen et al., 1998).

Young et al. (1998), using an ELISA assay of whole hippocampi, measured SNAP-25 and did not find a statistically significant reduction. In this study, SNAP-25 was identified with SP-12 a polyclonal antibody. The lack of significance in the ELISA assay may be due to the different tissue preparation techniques or binding properties of the SP-12 compared with SMI-81 used in the current report (Honer et al., 1997). This group did find statistically significant reductions in subregions, subiculum, CA1, CA3, CA4 and dentate gyrus using a quantitative immunocytochemical method. The authors speculate that their findings may represent a localized disconnection of the entorhinal cortex innervation of the hippocampus.

Fatemi et al. (2001) looked at hippocampal SNAP-25 in schizophrenia and bipolar illness. They found reduced levels of SNAP-25 in several subregions using immunocytochemistry. The stratum granulosum was significantly reduced 51% (compared to controls, $P=.013$) with nonsignificant reductions in the CA4, stratum moleculare, presubiculum and subiculum in schizophrenia. In bipolar illness, there was a significant 45% reduction in the stratum orins and nonsignificant reductions in several other areas. Our results take a different approach and measure the entire hippocampus rather than individual subregions. With both types of methodology, there appears to be less SNAP-25 immunoreactivity in the hippocampi of individuals with schizophrenia, while in bipolar illness we were not able to confirm their finding. The most likely explanation for not replicating this finding is the small sample size of our bipolar group. In schizophrenia, the reduced SNAP-25 immunoreactivity may represent loss of excitatory axonal connections.

In support of this hypothesis is the finding of reduced NMDA NR1 mRNA in dentate gyrus and a trend for reduction in CA3 region (Gao et al., 2000). In the entorhinal cortex, which supplies the major afferent connections with the hippocampus, NR1 mRNA was also reduced (Hemby et al., 2002). A reduction in mRNA is thought to indicate a corresponding reduction in the level of the protein. However, ligand binding of the NMDA receptors in the total hippocampus is not reduced (Kerwin and Meldrum, 1990). These authors demonstrate that the amount of NMDA receptor binding is preserved. Our current data and the normal glutamate ligand findings suggest that the total numbers of NMDA receptors are unchanged in schizophrenia. However, the current study would have been strengthened by actual measurement of a presynaptic vesicular glutamate transporters such as human brain-specific Na^+-dependent inorganic phosphate transporter or differentiation-associated Na^+/Pi cotransporter (Smith et al., 2001). Thus, the interpretation of our findings could be strengthened by measurement of both preglutamatergic and postglutamatergic markers.

Another explanation of our data and that of reduced volume of hippocampus in schizophrenia is loss of γ-aminobutyric acid (GABA) interneurons. Research in this area indicates increased $GABA_A$ receptors (Benes et al., 1996), normal levels of glutamate decarboxylase (GAD_{65}), possible loss of some nonpyramidal neurons (Benes, 1999) and reduced $GABA_B$ receptors (Mizukami et al., 2000). The Benes group has repeated the GAD 65 and GAD 67 experiments in a large sample. They did not find significant reductions of these GABA synthetic enzymes in schizophrenia but did in bipolar illness. They suggest that loss of interneurons did not account for the pathology found in the hippocampus in schizophrenia (Heckers et al., 2002). This area of research merits further attention; however, extensive loss of GABA interneurons is not apparent.

If there is presynaptic pathology in the neurons innervating the hippocampus, then some of the projections to or from the hippocampus may also be deficient. As a consequence, decreased presynaptic protein and mRNA levels in the temporal and prefrontal cortexes (Mirnics et al., 2000; Eastwood and Harrison, 1995; Sokolov et al., 2000; Glantz and Lewis, 1997; Hemby et al., 2002; Karson et al., 1999), both of which project to and receive efferent projections from the hippocampus (Shepherd, 1998), could be relevant. There is also some indication of abnormal SNARE complex formation in some cases of schizophrenia (Honer et al., 2002). Further, if some pathways are underfunctioning, other pathways may be compensating by excessive neurotransmitter release. There is some support for this hypothesis. Increased presynaptic proteins and mRNA indicating excessive neurotransmitter release has been identified in the prefrontal and cingulate cortex (Thompson et al., 1998; Gabriel et al., 1997; Perrone-Bizzozero et al., 1996).

The mechanism that is responsible for the decreased SNAP-25 is unknown. In an earlier study, we showed that SNAP-25 in the cerebrospinal fluid (CSF) of subjects with schizophrenia was elevated compared to controls (Thompson et al., 1999a). This finding is opposite of what we found in the current postmortem tissue. Given that the intracellular level is reduced and extracellular level is increased may suggest that membrane integrity is involved. Alternatively, less SNAP-25 may cause a dysregulation of the vesicular exocytosis/endocytosis pathway, leading to loss of membrane associated proteins.

5. Conclusion

Two previous reports using quantitative immunocytochemistry reported area-specific hippocampus reductions of SNAP-25. We now add a third report using a separate sample and different methodology. By semiquantitative Western blotting of whole hippocampi, we also show illness-specific reduction of the presynaptic vesicular docking protein SNAP-25. This reduced SNAP-25 does not appear to be due to loss of glutamatergic synapses.

Acknowledgements

We thank William J. Freed, PhD and James Meador-Woodruff, MD, PhD for critical reading of this manuscript. The authors wish to thank Joel E. Kleinman, MD, PhD, Section Chief on Neuropathology, Thomas M. Hyde, MD, PhD and Mary M. Herman, MD and others at the NIMH Clinical Brain Disorders Branch for the generous use of the samples in this study. We also thank John P. Hatch, PhD for statistical analysis help. This work was supported in part by NARSAD Young Investigator Award to PMT and the William Lion Penzner Foundation to MPV.

References

Adams, M.M., Gazzaley, A.H., Morrison, J.H., 2001. Attenuated lesion-induced *N*-methyl-D-aspartate receptor (NMDAR) plasticity in the dentate gyrus of aged rats following perforant path lesions. Exp. Neurol. 172, 244–249.

Bark, I.C., Hahn, K.M., Ryabinin, A.E., Wilson, M.C., 1995. Differential expression of SNAP-25 protein isoforms during divergent vesicle fusion events of neural development. Proc. Natl. Acad. Sci. U. S. A. 92, 1510–1514.

Benes, F.M., 1999. Evidence for altered trisynaptic circuitry in schizophrenic hippocampus. Biol. Psychiatry 46, 589–599.

Benes, F.M., Khan, Y., Vincent, S.L., Wickramasinghe, R., 1996. Differences in the subregional and cellular distribution of GABAA receptor binding in the hippocampal formation of schizophrenic brain. Synapse 22, 338–349.

Browning, M.D., Dudek, E.M., Rapier, J.L., Leonard, S., Freeman, R., 1993. Significant reductions in synapsin but not synaptophysin specific activity in brains of some schizophrenics. Biol. Psychiatry 34, 529–535.

Bunney, B.G., Bunney Jr., W.E., Carlsson, A., 1995. Schizophrenia and glutamate. In: Bloom, F.E., Kuffer, D.J. (Eds.), Psychopharmacology. Raven Press, New York, pp. 1205–1214. Chapter 101.

Chen, A.C., McDonald, B., Moss, S.J., Gurling, H.M., 1998. Gene expression studies of mRNAs encoding the NMDA receptor subunits NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C, and NMDAR2D following long-term treatment with *cis*- and *trans*-flupenthixol as a model for understanding the mode of action of schizophrenia drug treatment. Brain Res. Mol. Brain Res. 54, 92–100.

Conti, F., Barbaresi, P., Melone, M., Ducati, A., 1999. Neuronal and glial localization of NR1 and NR2A/B subunits of the NMDA receptor in the human cerebral cortex. Cereb. Cortex 9, 110–120.

Copolov, D., Velakoulis, D., McGorry, P., Carina, M., Yung, A., Rees, S., Jackson, G., Rehn, A., Brewer, W., Pantelis, C., 2000. Neurobiological findings in early phase schizophrenia. Brain Res., Brain Res. Rev. 31, 157–165.

Creese, I., Burt, D.R., Snyder, S.H., 1977. Dopamine receptor binding enhancement accompanies lesion-induced behavioral supersensitivity. Science 197, 596–598.

Eastwood, S.L., Harrison, P.J., 1995. Decreased synaptophysin in the medial temporal lobe in schizophrenia demonstrated using immunoautoradiography. Neuroscience 69, 339–343.

Eastwood, S.L., Harrison, P.J., 2000. Hippocampal synaptic pathology in schizophrenia, bipolar disorder and major depression: a study of complexin mRNAs. Mol. Psychiatry 5, 425–432.

Eastwood, S.L., Burnet, W.J., Harrison, P.J., 1995. Altered synaptophysin expression as a marker of synaptic pathology in schizophrenia. Neuroscience 66, 309–319.

Eastwood, S.L., Heffernan, J., Harrison, P.J., 1997. Chronic haloperidol treatment differentially affects the expression of synaptic and neuronal plasticity-associated genes. Mol. Psychiatry 2, 322–329.

Fatemi, S.H., Earle, J.A., Stary, J.M.L.S., Sedgewick, J., 2001. Altered levels of the synaptosomal associated protein SNAP-25 in hippocampus of subjects with mood disorders and schizophrenia. NeuroReport 12, 3257–3262.

Gabriel, S.M., Haroutunian, V., Powchik, P., Honer, W.G., Davidson, M., Davies, P., 1997. Increased concentrations of presynaptic proteins in the cingulate cortex of subjects with schizophrenia. Arch. Gen. Psychiatry 54, 559–566.

Gao, X.M., Sakai, K., Roberts, R.C., Conley, R.R., Dean, B., Tamminga, C.A., 2000. Ionotropic glutamate receptors and expression of *N*-methyl-D-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia. Am. J. Psychiatry 157, 1141–1149.

Gazzaley, A.H., Benson, D.L., Huntley, G.W., Morrison, J.H., 1997. Differential subcellular regulation of NMDAR1 protein and mRNA in dendrites of dentate gyrus granule cells after perforant path transection. J. Neurosci. 17, 2006–2017.

Geddes, J.W., Hess, E.J., Hart, R.A., Kesslak, J.P., Cotman, C.W., Wilson, M.C., 1990. Lesions of hippocampal circuitry define synaptosomal-associated protein-25 (SNAP-25) as a novel presynaptic marker. Neuroscience 38, 515–525.

Glantz, L.A., Lewis, D.A., 1997. Reduction of synaptophysin immunoreactivity in the prefrontal cortex of subjects with schizophrenia. Regional and diagnostic specificity (corrected and republished article originally appeared in Arch. Gen. Psychiatry, 1997 July, 54(7), 660–669). Arch. Gen. Psychiatry 54, 943–952.

Harrison, P.J., 1999. The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122, 593–624.

Heckers, S., Stone, D., Walsh, J., Shick, J., Koul, P., Benes, F.M., 2002. Differential hippocampal expression of glutamic acid decarboxylase 65 and 67 messenger RNA in bipolar disorder and schizophrenia. Arch. Gen. Psychiatry 59, 521–529.

Hemby, S.E., Ginsberg, S.D., Brunk, B., Arnold, S.E., Trojanowski, J.Q., Eberwine, J.H., 2002. Gene expression profile for schizophrenia: discrete neuron transcription patterns in the entorhinal cortex. Arch. Gen. Psychiatry 59, 631–640.

Honer, W.G., Falkai, P., Young, C., Wang, T., Xie, J., Bonner, J., Hu, L., Boulianne, G.L., Luo, Z., Trimble, W.S., 1997. Cingulate cortex synaptic terminal proteins and neural cell adhesion molecule in schizophrenia. Neuroscience 78, 99–110.

Honer, W.G., Falkai, P., Bayer, T.A., Xie, J., Hu, L., Li, H., Arango, V., Mann, J.J., Dwork, A.J., Trimble, W.S., 2002. Abnormalities of SNARE mechanism proteins in anterior frontal cortex in severe mental illness. Cereb. Cortex 12, 349–356.

Javitt, D.C., Sershen, H., Hashim, A., Lajtha, A., 1997. Reversal of phencyclidine-induced hyperactivity by glycine and the glycine uptake inhibitor glycyldodecylamide. Neuropsychopharmacology 17, 202–204.

Karson, C.N., Mrak, R.E., Schluterman, K.O., Sturner, W.Q., Sheng, J.G., Griffin, W.S., 1999. Alterations in synaptic proteins and their encoding mRNAs in prefrontal cortex in schizophrenia: a possible neurochemical basis for 'hypofrontality'. Mol. Psychiatry 4, 39–45.

Kerwin, R., Meldrum, B., 1990. Quantitative autoradiographic analysis of glutamate binding sites in the hippocampal formation in normal and schizophrenic brain post mortem. Neuroscience 39, 25–32.

Kleinman, J.E., Hyde, T.M., Herman, M.M., 1995. Methodological issues in the neuropathology of mental illness. In: Bloom, F.E., Kupfer, D.J. (Eds.), Psychopharmacology: the Fourth Generation of Progress. Raven Press, New York, pp. 859–864. Chapter 75.

Kohama, S.G., Urbanski, H.F., 1997. Distribution of glutamate receptor subunits in the primate temporal cortex and hippocampus. Brain Res. 769, 44–56.

Kuperberg, G., Heckers, S., 2000. Schizophrenia and cognitive function. Curr. Opin. Neurobiol. 10, 205–210.

Levesque, D., Martres, M.P., Diaz, J., Griffon, N., Lammers, C.H., Sokoloff, P., Schwartz, J.C., 1995. A paradoxical regulation of the dopamine D3 receptor expression suggests the involvement of an anterograde factor from dopamine neurons. Proc. Natl. Acad. Sci. U. S. A. 92, 1719–1723.

Masu, M., Nakajima, Y., Moriyoshi, K., Ishii, T., Akazawa, C., Nakanashi, S., 1993. Molecular characterization of NMDA and metabotropic glutamate receptors. Ann. N. Y. Acad. Sci. 707, 153–164.

Matthews, D.A., Cotman, C., Lynch, G., 1976. An electron microscopic study of lesion-induced synaptogenesis in the dentate gyrus of the adult rat: II. Reappearance of morphologically normal synaptic contacts. Brain Res. 115, 23–41.

Meador-Woodruff, J.H., Hogg Jr., A.J., Smith, R.E., 2001. Striatal ionotropic glutamate receptor expression in schizophrenia, bipolar disorder, and major depressive disorder. Brain Res. Bull. 55, 631–640.

Mirnics, K., Middleton, F.A., Marquez, A., Lewis, D.A., Levitt, P., 2000. Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. Neuron 28, 53–67.

Mizukami, K., Sasaki, M., Ishikawa, M., Iwakiri, M., Hidaka, S., Shiraishi, H., Iritani, S., 2000. Immunohistochemical localization of gamma-aminobutyric acid(B) receptor in the hippocampus of subjects with schizophrenia. Neurosci. Lett. 283, 101–104.

Nakahara, T., Nakamura, K., Tsutsumi, T., Hashimoto, K., Hondo, H., Hisatomi, S., Motomura, K., Uchimura, H., 1998. Effect of chronic haloperidol treatment on synaptic protein mRNAs in the rat brain. Brain Res. Mol. Brain Res. 61, 238–242.

Nicolle, M.M., Shivers, A., Gill, T.M., Gallagher, M., 1997. Hippocampal *N*-methyl-D-aspartate and kainate binding in response to entorhinal cortex aspiration or 192 IgG-saporin lesions of the basal forebrain. Neuroscience 77, 649–659.

Nieto-Sampedro, M., Bussineau, C.M., Cotman, C.W., 1982. Isolation, morphology, and protein and glycoprotein composition of synaptic junctional fractions from the brain of lower vertebrates: antigen PSD-95 as a junctional marker. J. Neurosci. 2, 722–734.

Oyler, G.A., Higgins, G.A., Hart, R.A., Battenberg, E., Billingsley, M., Bloom, F.E., Wilson, M.C., 1989. The identification of a novel synaptosomal-associated protein, SNAP-25, differentially expressed by neuronal subpopulations. J. Cell Biol. 109, 3039–3052.

Perrone-Bizzozero, N.I., Sower, A.C., Bird, E.D., Benowitz, L.I., Ivins, K.J., Neve, R.L., 1996. Levels of the growth associated protein GAP-43 are selectively increased in association cortices in schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 93, 14182–14187.

Petralia, R.S., Yokotani, N., Wenthold, R.J., 1994. Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the

rat nervous system using a selective anti-peptide antibody. J. Neurosci. 14, 667–696.

Shepherd, G.M., 1998. The Synaptic Organization of the Brain, 4th ed. Oxford University Press, New York.

Smith, R.E., Haroutunian, V., Davis, K.L., Meador-Woodruff, J.H., 2001. Vesicular glutamate transporter transcript expression in the thalamus in schizophrenia. NeuroReport 12, 2885–2887.

Sokolov, B.P., Tcherepanov, A.A., Haroutunian, V., Davis, K.L., 2000. Levels of mRNAs encoding synaptic vesicle and synaptic plasma membrane proteins in the temporal cortex of elderly schizophrenic patients. Biol. Psychiatry 48, 184–196.

Takamatsu, K., Auerbach, B., Gerardy-Schahn, R., Eckhardt, M., Jaques, G., Madry, N., 1994. Characterization of tumor-associated neural cell adhesion molecule in human serum. Cancer Res. 54, 2598–2603.

Thompson, P.M., Sower, A.C., Perrone-Bizzozero, N.I., 1998. Altered levels of the synaptosomal associated protein SNAP-25 in schizophrenia. Biol. Psychiatry 43, 239–243.

Thompson, P.M., Rosenberger, C., Qualls, C., 1999. CSF SNAP-25 in schizophrenia and bipolar illness: a pilot study. Neuropsychopharmacology 21, 717–722.

Tsai, G., Passani, L.A., Slusher, B.S., Carter, R., Baer, L., Kleinman, J.E., Coyle, J.T., 1995. Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. Arch. Gen. Psychiatry 52, 829–836.

Vawter, M.P., Cannon-Spoor, H.E., Hemperly, J.J., Hyde, T.M., VanderPutten, D.M., Kleinman, J.E., Freed, W.J., 1998a. Abnormal expression of cell recognition molecules in schizophrenia. Exp. Neurol. 149, 424–432.

Vawter, M.P., Hemperly, J.J., Hyde, T.M., Bachus, S.E., VanderPutten, D.M., Howard, A.L., Cannon-Spoor, H.E., McCoy, M.T., Webster, M.J., Kleinman, J.E., Freed, W.J., 1998b. VASE-containing N-CAM isoforms are increased in the hippocampus in bipolar disorder but not schizophrenia. Exp. Neurol. 154, 1–11.

Vawter, M.P., Howard, A.L., Hyde, T.M., Kleinman, J.E., Freed, W.J., 1999. Alterations of hippocampal secreted N-CAM in bipolar disorder and synaptophysin in schizophrenia. Mol. Psychiatry 4, 467–475.

Webster, M.J., Shannon, W.C., Herman, M.M., Hyde, T.M., Kleinman, J.E., 2001. Synaptophysin and GAP-43 mRNA levels in the hippocampus of subjects with schizophrenia. Schizophr. Res. 49, 89–98.

Williams, J.B., Gibbon, M., First, M.B., Spitzer, R.L., Davies, M., Borus, J., Howes, M.J., Kane, J., Pope, H.G.J., Rounsaville, B., 1992. The Structured Clinical Interview for DSM-III-R (SCID): II. Multisite test–retest reliability. Arch. Gen. Psychiatry 49, 630–636.

Young, C.E., Arima, K., Xie, J., Hu, L., Beach, T.G., Falkai, P., Honer, W.G., 1998. SNAP-25 deficit and hippocampal connectivity in schizophrenia. Cereb. Cortex 8, 261–268.

Zhang, K., Tarazi, F.I., Baldessarini, R.J., 2001. Nigrostriatal dopaminergic denervation enhances dopamine D(4) receptor binding in rat caudate-putamen. Pharmacol. Biochem. Behav. 69, 111–116.

Zola-Morgan, S.M., Squire, L.R., 1990. The primate hippocampal formation: evidence for a time-limited role in memory storage. Science 250, 288–290.