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Dynamic regulation of glutamatergic post-synaptic activity in rat prefrontal cortex by repeated administration of antipsychotic drugs.

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List of non-standard abbreviations:

aCaMKII= Calcium Calmoduline kinase type II

NMDA receptor= N-Methyl-D-Aspartate receptor

AMPA= α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor

ANOVA= analysis of variance

MAGUK= membrane-associated guanylate kinases

FGA = first generation antipsychotic

SGA = second generation antipsychotic

TIF= Triton Insoluble Fraction

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Abstract

Antipsychotics are the mainstay for the treatment of schizophrenia. Although these drugs act at several neurotransmitter receptors, they are expected to elicit different neuroadaptive changes at structures relevant for schizophrenia. Since glutamatergic dysfunction plays a role in the pathophysiology of schizophrenia, we focused our analysis on glutamatergic neurotransmission following repeated treatment with antipsychotic drugs. Rats were exposed to a 2 week pharmacological treatment with the first generation antipsychotic haloperidol and the second generation antipsychotic olanzapine. By using western blot and immunoprecipitation techniques, we investigated the expression, trafficking and interaction of essential components of glutamatergic synapse in rat prefrontal cortex. Prolonged treatment with haloperidol, but not olanzapine, dynamically affects glutamatergic synapse by selectively reducing the synaptic level of the obligatory NMDA subunit NR1, the regulatory NMDA subunit NR2A and its scaffolding protein PSD95 as well as the trafficking of GluR1 to the membrane. Additionally, haloperidol alters total as well as phosphorylated levels of CaMKII at synaptic sites and its interaction with regulatory NMDA subunit NR2B. Our data suggest that the glutamatergic synapse is a vulnerable target for prolonged haloperidol treatment. The global attenuation of glutamatergic function in prefrontal cortex might explain, at least in part, the cognitive deterioration observed in haloperidol-treated patients.

Introduction

Antipsychotics are the cornerstone for the treatment of schizophrenia. There is a general agreement indicating that first generation antipsychotics (FGAs) are efficacious against positive symptoms, whereas second generation antipsychotics (SGAs) may also ameliorate negative symptoms and cognitive deterioration (Keefe et al., 2006), although recent evidence has challenged this notion (Lieberman, 2007). Nevertheless, full therapeutic activity with FGAs and SGAs can only be achieved after long-term administration, implying that adaptive changes may be required in specific systems or circuitries involved in disease symptomatology (Meltzer, 1996).

Although the prevailing hypothesis for the pathophysiology of schizophrenia tends to correlate the disorder with a dysfunction of dopaminergic neurotransmission (Duncan et al., 1999), a glutamatergic hypothesis of schizophrenia has been put forward, suggesting that a deficit of glutamate neurotransmission might underlie specific aspects of this mental disorder (Jentsch and Roth, 1999; Pilowsky et al., 2006). This hypothesis stems from the evidence that pharmacological (phencyclidine) or genetic (NR1 knockdown mice) reduction of NMDA receptor function yields a remarkable similarity to psychotic states and cognitive deficits observed in schizophrenic patients (Kristiansen et al., 2007; Lindsley et al., 2006; Mohn et al., 1999; Olney et al., 1999).

The above evidence has fueled the investigation of the changes brought about by repeated antipsychotic drug treatment on the glutamatergic system. Evidence exists that available pharmacotherapy shows a differential ability in modulating glutamatergic neurotransmission, primarily via the modulation of ionotropic receptor expression (Fitzgerald et al., 1995; Healy and Meador-Woodruff, 1997; O'Connor et al., 2006; Riva et al., 1997; Schmitt et al., 2003; Tarazi et al., 2003; Tascedda et al., 1999). The different impact of SGAs vs. FGAs is also in agreement with the observation that only the former agents are able to revert PCP-induced disruption of ‘prepulse inhibition’ (Bakshi and Geyer, 1995; Keith et al.,

1991), a valuable tool to measure deficits in gating of cognitive and sensory information which is reduced in schizophrenic patients (for a review, see Braff et al., 2001).

However, it is known that glutamate function is finely tuned at synaptic level through the clustering of ionotropic receptor subunits (AMPA and NMDA) and scaffolding proteins in the post-synaptic densities, a key mechanism to activate selected intracellular signalling pathways and regulate the function at excitatory synapses. On this basis, we incorporated long-term treatments with two representative members of antipsychotic drugs, i.e. the FGA haloperidol and the SGA olanzapine, in order to evaluate the plasticity of the glutamatergic synapse in prefrontal cortex, a region that contributes most to the cognitive impairments observed in schizophrenic patients (Weinberger et al., 2001). Toward this goal, we focused our analysis on investigating the expression and interaction of proteins forming NMDA glutamate receptor complexes at post-synaptic density as well as AMPA subunit expression and trafficking in rat prefrontal cortex.

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Materials and Methods

Materials

General reagents were purchased from Sigma-Aldrich (Milano, Italy). Molecular biology reagents were obtained from Celbio (Pero, Milan, Italy), and Sigma (Milan, Italy). Olanzapine was a generous gift from Eli Lilly (Sesto Fiorentino, Italy) whereas haloperidol was purchased from Sigma-Aldrich (Milano, Italy).

Animal treatments

Male Sprague-Dawley rats (Charles River, Calco, Italy) weighing 225-250 g were used throughout the experiments. Animals were allowed to adapt to laboratory conditions for two weeks before any treatment and handled 5 minutes a day during this period; in addition, they were maintained under a 12 hours light/12 hours dark cycle with food and water available ad libitum. Animals received daily injections of either vehicle (saline), the FGA haloperidol (1 mg/kg) or the SGA olanzapine (2 mg/kg, twice daily) for 14 days and were sacrificed 24 hrs after the last drug injection. Vehicle, haloperidol or olanzapine were administered subcutaneously. The length of the treatment and the time of sacrifice were consistent with our previous experiments showing adaptive changes with psychotropic drugs (Fumagalli et al., 2006). The doses of haloperidol and olanzapine were chosen in accordance with their receptor occupancy (Bymaster et al., 1996; Richelson, 1996; Schotte et al., 1996) and in order to achieve plasma levels within a therapeutic range for the treatment of schizophrenia (Andersson et al., 2002). All animal handling and experimental procedures were performed in accordance with the EC guidelines (EEC Council Directive 86/609 1987) and with the Italian legislation on animal experimentation (Decreto Legislativo 116/92).

Preparation of Protein Extracts

Brain regions were immediately dissected, frozen on dry ice and stored at -80°C. The prefrontal cortex (defined as Cg1, PL, and IL subregions corresponding to the plates 6–10) was dissected from 2-mm thick slices, according to the atlas of Paxinos and Watson (Watson,

1996) whereas hippocampus (including both ventral and dorsal parts) was dissected from the whole brain. Different subcellular fractions were prepared as previously described (Gardoni et al., 2003). Tissues were homogenized in a teflon-glass potter in ice-cold 0.32 M sucrose containing 1 mM Hepes, 1 mM MgCl2, 1 mM EDTA, 1 mM NaHCO3 and 0.1 mM phenylmethylsulfonyl fluoride (PMSF), at pH 7.4, in presence of a complete set of protease and phosphatase inhibitors. The homogenized tissue was centrifuged at 1,000 x g for 10 min, in order to separate a pellet (P1), enriched in nuclear components from the supernatant (S1). The resulting supernatant (S1) was centrifuged at 13,000 x g for 15 min to obtain a clarified fraction of cytosolic proteins (S2). The pellet (P2), corresponding to a crude membrane fraction, was resuspended in 1 mM Hepes plus protease and phosphatase inhibitors and centrifugated at 100,000 x g for 1h. The pellet (P3) was resuspended in buffer containing 75 mM KCl and 1% Triton X-100 in a glass-glass potter and centrifuged at 100,000 x g for 1 h. The resulting supernatant (S4), referred as Triton X-100 soluble fraction (TSF), was stored at -20°C; the pellet (P4), referred as Triton X-100 insoluble fraction (TIF), was homogenized in a glass–glass potter in 20 mM Hepes, protease and phosphatase inhibitors and stored at -20°C in presence of glycerol 30 %.

Total protein content was measured in the subcellular fractions by the Bio-Rad Protein Assay (Bio-Rad, Milano, Italy).

Western Blot Analysis

Western blot analyses were performed in homogenate, TIF and S2 fraction. Equal amount of proteins (10 μg for S2 and homogenate; 5 μg for TIF) were electrophoretically run on a sodium dodecyl sulfate (SDS)-8% polyacrilamide gel under reducing conditions. Nitrocellulose membranes (Bio-Rad) were blocked with 10% nonfat dry milk in TBS/0,1 % Tween-20 buffer and then incubated with primary antibody. The conditions of the primary antibodies are the following: phospho-NR2B (Ser1303) (Upstate, 1:1000 in 5 % albumin), NR2B (Zymed, 1:1000 in 3% nonfat dry milk), NR2A (Zymed, 1:1000 in 3% nonfat dry milk), NR1 (Zymed, 1:1000 in 3% nonfat dry milk), PSD95 (Affinity Bioreagents, 1:4000 in

3% nonfat dry milk), SAP102 (Affinity Bioreagents, 1:1000 in 5% nonfat dry milk), p-αCaMKII(Thr286) (Affinity Bioreagents, 1:2500 in 3% nonfat dry milk), αCaMKII (Chemicon, 1:10000 in 3% nonfat dry milk), p-GluR1(Ser831) (Chemicon, 1:1000 in 5% albumin), GluR1 (Chemicon, 1:2000 in 5% albumin), GluR2 (Chemicon, 1:2000 in 5% albumin), β-actin (Sigma-Aldrich, 1:10000 in 3% non fat dry milk). After 3 washes of 10 minutes in TBS/Tween-20, the blots were incubated 1 hour at room temperature with horseradish peroxidase-conjugated secondary antibody (anti-rabbit or anti-mouse IgG, by Sigma) and immunocomplexes were visualized by chemiluminescence utilizing the ECL Western Blotting kit (Amersham Life Science, Milano, Italy) according to the manufacturer's instructions.

The blots were first probed with antibodies against the phosphorylated forms of the protein and then stripped with SDS 2%, 100mM β-mercaptoethanol and 62.5 mM Tris-HCl pH 6.7 at 50°C for 30 minutes and then re-probed with antibodies against total proteins of same type. Results were standardized to a β-actin control protein, which was detected by evaluating the band density at 43 kDa.

Immunoprecipitation experiments

Aliquots of 100 μg of crude membrane fraction (P2) were incubated with antibodies against NR2B (Zymed, dilution 1:25) overnight at 4°C, in buffer RIA 1X (NaCl 200 mM, EDTA 10 mM, Na2HPO4 10 mM, Nonidet P-40 0,5 % at pH 7,4, in presence of a complete set of protease inhibitors) and SDS 1 %. Protein A beads (Sigma) were added to each samples and incubated at 4°C for 3 hours. Samples were centrifugated and protein A beads were collected and washed 3 times in RIPA 1X (RIA 1X plus SDS 0,1 %). Sample buffer 3X (50 μl for each sample) was added and the final mixture was boiled for 5 minutes. Protein A beads were pelleted by centrifugation at 14,000 x g and supernatants were loaded on a sodium dodecyl sulfate (SDS)-7% polyacrilamide gel under reducing conditions. Nitrocellulose membranes (Bio-Rad) were blocked with 10% nonfat dry milk in TBS/0,1 % Tween-20

buffer and then incubated with primary antibody for p-αCaMKII(Thr286) (Promega, dilution 1:500 in 3 % nonfat dry milk) and αCaMKII (Santa Cruz, dilution 1:1000 in 3 % nonfat dry milk). After rinsing the membranes in TBS/Tween-20, anti-rabbit horseradish peroxidase-conjugated secondary antibody was added at 1:500 dilution and immunocomplexes were visualized by chemiluminescence utilizing the ECL Western Blotting kit (Amersham Life Science, Milano, Italy) according to the manufacturer's instructions. Data were normalized for NR2B (Zymed, dilution 1:250 in 3 % nonfat dry milk).

Statistical Analysis

Expression and phosphorylation state of the proteins of interest were measured using the Quantity One software from Biorad. The mean value of the control group within a single experiment was set at 100 and the data of animals injected with olanzapine or haloperidol were expressed as 'percentages' of saline-treated animals. Statistical evaluation of the changes in the phosphorylation state or expression of proteins produced by antipsychotics in prefrontal cortex was performed using a one-way analysis of variance (ANOVA) followed by Fisher PLSD. Significance for all tests was assumed at $p<0.05$.

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Results

We performed an analysis of key components of glutamatergic post-synaptic transmission following prolonged antipsychotic treatment by investigating the expression of proteins and their phosphorylation state in a subcellular fraction enriched in post-synaptic densities (TIF, Triton Insoluble Fraction). The effectiveness of the preparation was confirmed by the use of protein markers for specific subcellular compartments (Fig. 1). As reported by Gardoni and colleagues (2003), the scaffolding protein PSD95 and NMDA receptor subunits NR2A and NR2B were found enriched in crude synaptosomal fraction (P2) and TIF, with a lower abundance in total homogenate (H), while they were not detectable in the cytosolic compartment (S2) or in the TSF (Triton Soluble Fraction). As expected, the expression of synaptophysin, a synaptic vesicle membrane protein expressed in the presynaptic compartments, was concentrated in P2 and TSF, whereas weakly detected in S2 fraction and TIF. Finally, αCaMKII was distributed throughout the fractions although strongly enriched in TIF as previously reported (Gardoni et al., 2003).

We first examined if antipsychotic drug treatment might affect the levels of synaptic NMDA receptors in rat prefrontal cortex. We found that repeated administration of haloperidol significantly reduced NR1 (-19%) and NR2A (-25%) expression in the TIF, without altering the levels of NR2B (Fig. 2A). Under the same experimental conditions, olanzapine did not produce any change in the synaptic levels of NMDA receptor subunits (Fig. 2A). In order to evaluate the regional specificity of the effects on synaptic NMDA receptors, we analyzed the expression of NMDA receptor subunits in the hippocampus. Figure 2B shows that both haloperidol and olanzapine did not alter NMDA receptor subunit levels in the TIF of this brain region. Since haloperidol shows a regional specific effect on cortical NMDA receptors, we focused our attention on molecular mechanisms underlying NMDA activation in prefrontal cortex.

Because synaptic expression of NMDA subunits is strictly dependent upon binding with scaffolding proteins (Scott et al., 2001; Steigerwald et al., 2000), we next analyzed two membrane-associated guanylate kinases (MAGUKs), synapse associated protein 102

(SAP102) and postsynaptic density 95 (PSD95). Western blot analysis revealed that haloperidol, but not olanzapine, caused a significant decrease of PSD95 (-22%) with no effect on SAP102 (Fig. 3).

Changes in synaptic NMDA composition following haloperidol treatment might alter receptor kinetics and calcium permeability (Monyer et al., 1994). Since the major target of calcium influx through synaptic NMDA receptors is represented by αCaMKII, we reasoned that the changes produced by haloperidol on NMDA receptors could influence the expression and activation state of this kinase. As shown in Figure 4A, prolonged administration of haloperidol significantly reduced total (-25%) as well as p-αCaMKII(Thr286) (-21%) levels in the TIF. This effect was confined to the synaptic compartment, since no significant changes were observed in the crude homogenate (Fig. 4B). Conversely, olanzapine did not modify αCaMKII expression and phosphorylation in any cellular compartment (Fig. 4).

It is known that, following autophosphorylation, αCaMKII interacts with NR2B subunit, thus ensuring a Ca^{++}/calmodulin (CaM) independent kinase activity (Bayer et al., 2001). In order to establish possible changes in αCaMKII-NR2B interaction, we immunoprecipitated NR2B (whose expression is not altered by the pharmacological treatment, see Figure 2), and measured both p-αCaMKII(Thr286) as well as αCaMKII levels. Our results indicate that haloperidol, but not olanzapine, reduced such interactions (-33% for p-αCaMKII(Thr286) and -42% for αCaMKII) (Fig. 5). p-αCaMKII(Thr286), after interacting with NR2B, phosphorylates this NMDA subunit in Ser1303 (Omkumar et al., 1996); however, western blot experiments revealed that such phosphorylation was not modified by either olanzapine or haloperidol (Fig. 6).

Several lines of evidence have demonstrated that, within the post-synaptic density (PSD), αCaMKII is also involved in the modulation of AMPA receptors. In particular, αCaMKII governs GluR1 trafficking to synaptic sites (Hayashi et al., 2000). Hence, in order to investigate the possible consequences of reduced synaptic αCaMKII levels on AMPA receptor trafficking, we examined two AMPA receptor subunits, namely GluR1 and GluR2,

in different cellular fractions from the prefrontal cortex of rats chronically treated with haloperidol or olanzapine. We found that synaptic levels (TIF) of GluR1 expression were significantly reduced by repeated haloperidol treatment (-20%), but not olanzapine (Fig. 7A). In order to establish if this effect was due to a deficit of protein synthesis rather than trafficking mechanisms, we analyzed GluR1 levels in the homogenate and cytosolic fraction of prefrontal cortex. No changes were observed in crude homogenate after treatment with both antipsychotics, whereas haloperidol significantly increased the levels of GluR1 in the cytosolic compartment (+ 74%) (Fig. 7A) but not of GluR2 in all cellular fractions investigated (Fig. 7B). Besides altering protein trafficking, chronic haloperidol treatment reduced GluR1 phosphorylation on the Ser 831 residue by αCaMKII (-26%) (Fig. 8).

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Discussion

We here report notable disparities between haloperidol and olanzapine in the modulation of ionotropic glutamate receptors in the postsynaptic compartment of rat prefrontal cortex. While the SGA olanzapine preserves glutamatergic function at PSD, we found that repeated administration of haloperidol markedly affects post-synaptic density organization and function.

While previous studies on the topic have looked at the effects of antipsychotic treatment on mRNA levels or total protein expression (Fitzgerald et al., 1995; Healy and Meador-Woodruff, 1997; O'Connor et al., 2006; Riva et al., 1997; Schmitt et al., 2003; Tarazi et al., 2003; Tascedda et al., 1999), our study examines how antipsychotics may regulate these 'glutamatergic' proteins at synaptic level, how they are dynamically trafficked in the cell and how they differentially interact with each other to regulate phosphorylation states and, ultimately, the functional activity of glutamatergic receptors. We here report for the first time that antipsychotics can modulate the glutamatergic system, namely the expression of receptor subunits, scaffolding and signalling proteins, in an enriched post-synaptic fraction (TIF) of prefrontal cortex, which has direct implications for glutamatergic function and responsiveness. We found that haloperidol reduced the synaptic levels of the NMDA obligatory subunit NR1 and the regulatory subunit NR2A, but not NR2B, which might lead to altered kinetic properties of the ionotropic receptor (Monyer et al., 1994; Nabekura et al., 2002). The decrease of synaptic NMDA receptor expression after haloperidol treatment is supported by the evidence that the expression of the scaffolding protein PSD95 is also reduced. To this end, a lower expression of NMDA receptor subunits and scaffolding proteins is also observed in prefrontal cortex of schizophrenics (Kristiansen et al., 2006; Ohnuma et al., 2000; Toyooka et al., 2002). Interestingly, the diminished availability of active NMDA receptor is not observed in hippocampus, thus highlighting the regional selectivity of the changes brought about by haloperidol. The evidence that the expression of NR1, NR2A and PSD95 is not altered in the crude homogenates (data not shown) allows us to suggest that

haloperidol affects the synaptic expression of these proteins, likely through an alteration of their trafficking to the synaptic compartment.

NMDA receptor activation drives calcium entry into the cell that, on its turn, activates different intracellular pathways. Hence, it is feasible to hypothesize that haloperidol might attenuate post-synaptic responses associated with calcium influx. A primary target of post-synaptic calcium elevation through NMDA receptor is αCaMKII, which immediately undergoes autophoshorylation in Thr286 and interacts with the regulatory subunit NR2B locking itself in an activated state, thus prolonging its synaptic activity (Bayer et al., 2001). We found that haloperidol reduced total as well as p-αCaMKII(Thr286) levels in the synaptic compartment, but not in the crude homogenate, of prefrontal cortex, suggesting that kinase recruitment at post-synaptic sites is compromised, presumably due to an altered trafficking rather than to a reduction of αCaMKII synthesis. Accordingly, the reduction of αCaMKII, in its total and phosphorylated form, after chronic haloperidol leads to a significant decrease of its interaction with NR2B, as indicated by immunoprecipitation experiments, which might impair NMDA function at synaptic level. The observation that NR2B phosphorylation at Ser 1303 by αCaMKII (Omkumar et al., 1996) was not altered after haloperidol administration, suggests that treatment with the FGA alters the trafficking of the kinase to the post-synaptic compartments rather than its activity on the NMDA complex.

Conversely, reduced synaptic levels of αCaMKII in haloperidol-treated rats were paralleled by a significant decrease of GluR1 levels in the same fraction and a concomitant increase in the cytosol. Since GluR1 trafficking is dependent on synaptic activity and αCaMKII (Gao et al., 2006; Hayashi et al., 2000), reduced levels of this kinase at excitatory synapse might impair the trafficking of this AMPA receptor subunit from the cytosol to the synaptic compartment. To this end, the specificity of this effect is suggested by the observation that GluR2 incorporation into synapse is not altered by the treatment, possibly because this receptor subunit may be constitutively delivered to the membrane, independently from neuronal activity (Passafaro et al., 2001).

Taken as a whole, it is reasonable to assume that the changes produced by repeated treatment with haloperidol converge to determine a global dysfunction of glutamatergic synapse in rat prefrontal cortex. The decreased availability of NR1, NR2A, PSD95 and αCaMKII at post-synaptic density as well as reduced delivery of GluR1 into the synapse, might represent a deleterious mechanism altering synaptic plasticity within prefrontal cortex (Haucke, 2000; Migaud et al., 1998). However, the observation that dysregulation of PSD95 may lead to altered plasticity following chronic exposure to cocaine (Yao et al., 2004) points to this scaffolding protein as a common mediator of addictive and psychiatric disorders and provides additional mechanistic evidence for monoaminergic modulation of glutamatergic neurotransmission that could play a role in the herein reported effects of haloperidol.

The temporal sequence of these events remains elusive, however we favour the possibility that, as a first step, prolonged treatment with haloperidol reduces synaptic NMDA subunit expression and alters its composition, thus limiting NMDA–mediated transmission. Such effect might compromise calcium influx that, in turn, leads to a diminished recruitment of αCaMKII to synaptic sites. Reduced availability of synaptic αCaMKII might be responsible for alterations in GluR1 trafficking to post-synaptic density, leading to an impaired function of synaptic AMPA receptors (Fig. 9).

Although this study has been conducted in 'normal animals', our data suggest the possibility that haloperidol might 'worsen' the function of the glutamatergic system, which can already be defective in schizophrenia (Jentsch et al., 1999).

To sum up, our data provide evidence that haloperidol induces an orchestrated deficiency of glutamatergic post-synaptic functions. Although we are aware that prefrontal cortex is only one part of a much larger and complicated circuit that governs cognitive functions, given the prominent role of glutamate in cognition it is conceivable to hypothesize that such changes may contribute to learning and memory deterioration observed in humans after exposure to the drug (Castner and Williams, 2007; Mouri et al., 2007; Zirnheld et al., 2004).

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Footnotes

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Disclosure/Conflict of Interest

None of the authors has any potential conflict of interests nor financial interests to disclose.

Figure Legends

Figure 1: Characterization of the biochemical fractionation method used in the present study. The procedure for the subcellular extraction is described in Materials and Methods. The isolated biochemical fractions from prefrontal cortex extracts were separated by SDS-PAGE and the blots were probed with antibodies against NR2A/2B, PSD95, αCaMKII and synaptophysin. H= homogenate; S2= cytosolic fraction; P2= crude synaptosomal fraction; P4= Triton Insoluble Fraction (TIF); S4= Triton Soluble Fraction.

Figure 2: Modulation of synaptic levels of NMDA receptor subunits in prefrontal cortex and hippocampus after prolonged antipsychotic drug treatment.

The upper panels show representative immunoblots of NMDA receptor subunits as well as β-actin in the TIF of rat prefrontal cortex (A) and hippocampus (B) after repeated treatment with haloperidol (H) and olanzapine (O), as compared to vehicle-injected animals (S). Rats (n=6 for each group) were killed 24 hours after the last injection. The lower panels show the quantitative data, (mean ± S.E.M), expressed as % of control rats for both prefrontal cortex and hippocampus. *$p<0.05$ vs. controls (one-way ANOVA followed by Fisher PLSD).

Figure 3: Modulation of synaptic levels of NMDA receptor scaffolding proteins in prefrontal cortex after prolonged antipsychotic drug treatment.

The upper panel shows a representative immunoblot of PSD95 and SAP102 in the TIF of rat prefrontal cortex after repeated treatment with haloperidol (H) and olanzapine (O), as compared to vehicle-injected animals (S). Rats (n=6 for each group) were killed 24 hours after the last drug injection. The lower panel shows the quantitative data, (mean ± S.E.M), expressed as % of control rats. *$p<0.05$ vs. controls (one-way ANOVA followed by Fisher PLSD).

Figure 4: Modulation of p-αCaMKII(Thr286) and total αCaMKII levels in rat prefrontal cortex after prolonged antipsychotic drug treatment.

Representative immunoblots of p-αCaMKII(Thr286) and αCaMKII in the TIF (A) and homogenate (B) are shown in the upper panels after repeated treatment with haloperidol (H) and olanzapine (O), as compared to vehicle-injected animals (S). Lower panels show the quantitative data, (mean ± S.E.M), expressed as % of control rats, in TIF and homogenate (n=6 for each group). *p<0.05 and **p<0.01 vs. controls (one-way ANOVA followed by Fisher PLSD).

Figure 5: Modulation of p-αCaMKII(Thr286)-NR2B interaction in rat prefrontal cortex after prolonged antipsychotic drug treatment.

Upper panel shows a representative immunoblot of p-αCaMKII(Thr286) immunoprecipitated with NR2B in the three experimental groups. Lower panel shows the quantitative analysis of p-αCaMKII(Thr286) associated with the regulatory subunit NR2B (n=6 for each group). **p<0.01 vs. controls (one-way ANOVA followed by Fisher PLSD).

Figure 6: Modulation of synaptic p-NR2B(Ser1303) and total NR2B levels in rat prefrontal cortex after prolonged antipsychotic drug treatment.

The upper panel shows a representative immunoblot of p-NR2B(Ser1303) and total NR2B levels as well as □-actin in the TIF of rat prefrontal cortex after repeated treatment with haloperidol (H) and olanzapine (O), as compared to vehicle-injected animals (S). The lower panel shows the quantitative data, expressed as % of control rats, which represent the mean ± S.E.M. of 6 animals for each group. *p<0.05 vs. controls (one-way ANOVA followed by Fischer PLSD).

Figure 7: Modulation of GluR1 and GluR2 AMPA subunits in different cellular fractions of rat prefrontal cortex after prolonged antipsychotic drug treatment.

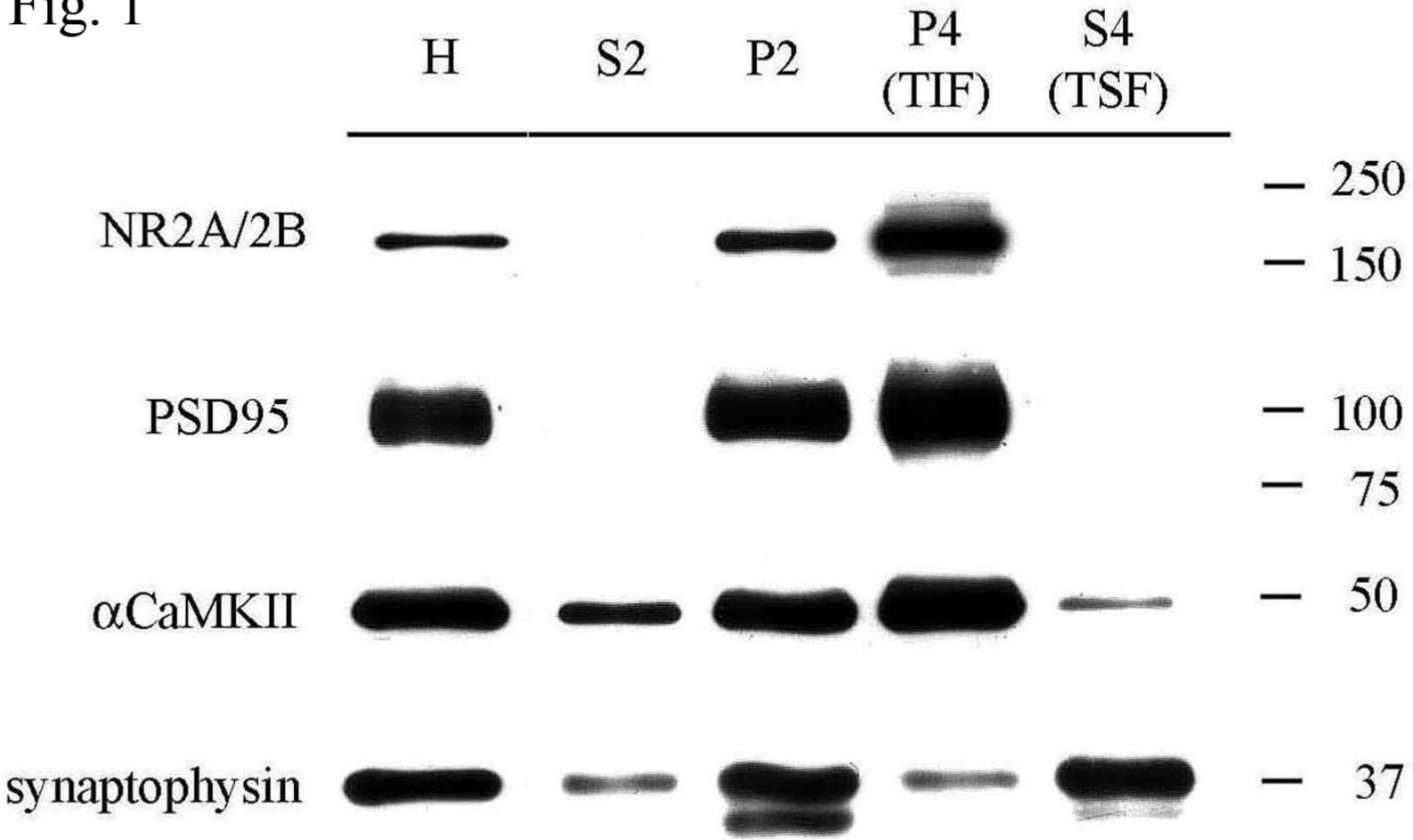
Top panels show representative immunoblots of GluR1 (A) and GluR2 (B) subunit in homogenate, TIF and cytosolic extracts (S2) after repeated treatment with haloperidol (H) and olanzapine (O), as compared to vehicle-injected animals (S). The quantitative data, expressed as % of control rats, represent the mean ± S.E.M. of 6 animals for each group and are shown in the lower panel. *$p<0.05$, **$p<0.01$ vs. controls (one-way ANOVA followed by Fischer PLSD).

Figure 8: Modulation of synaptic p-GluR1(Ser831) levels in rat prefrontal cortex after prolonged antipsychotic drug treatment.

The upper panel shows a representative immunoblot of p-GluR1(Ser831) and β-actin levels in the TIF of rat prefrontal cortex after repeated treatment with haloperidol (H) and olanzapine (O), as compared to vehicle-injected animals (S). The lower panel shows the quantitative data, expressed as % of control rats, which represent the mean ± S.E.M. of 6 animals for each group. *$p<0.05$ vs. controls (one-way ANOVA followed by Fischer PLSD).

Figure 9. Schematic representation of the effects produced by prolonged administration of haloperidol at excitatory synapses.

Fig. 1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P2
P4
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S4
(TSF)
NR2A/2B
PSD95
αCaMKII
synaptophysin
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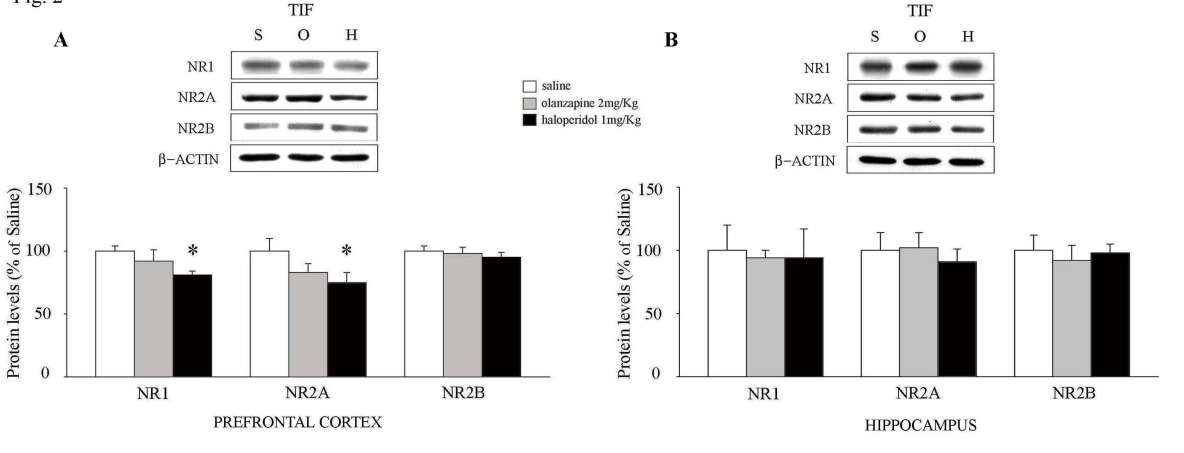
150
100
75
50
37

Fig. 2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O
H
NR1
NR2A
NR2B
β−ACTIN
saline
olanzapine 2mg/Kg
haloperidol 1mg/Kg
Protein levels (% of Saline)
150
100
50
0
*
*
NR1
NR2A
NR2B
PREFRONTAL CORTEX
B
TIF
S
O
H
NR1
NR2A
NR2B
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50
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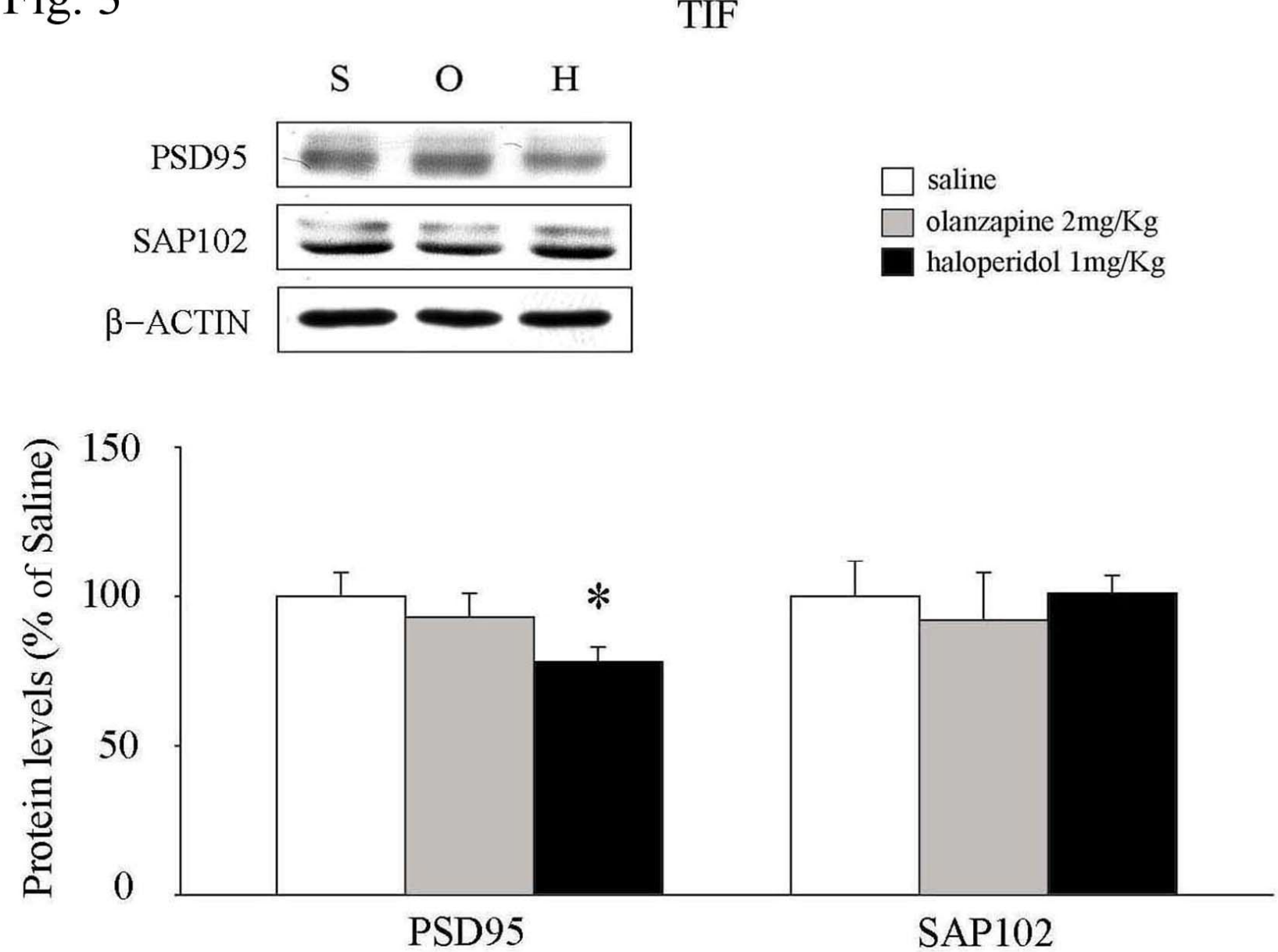
NR1
NR2A
NR2B
HIPPOCAMPUS

Fig. 3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S
O
H
PSD95
SAP102
β−ACTIN
saline
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Protein levels (% of Saline)
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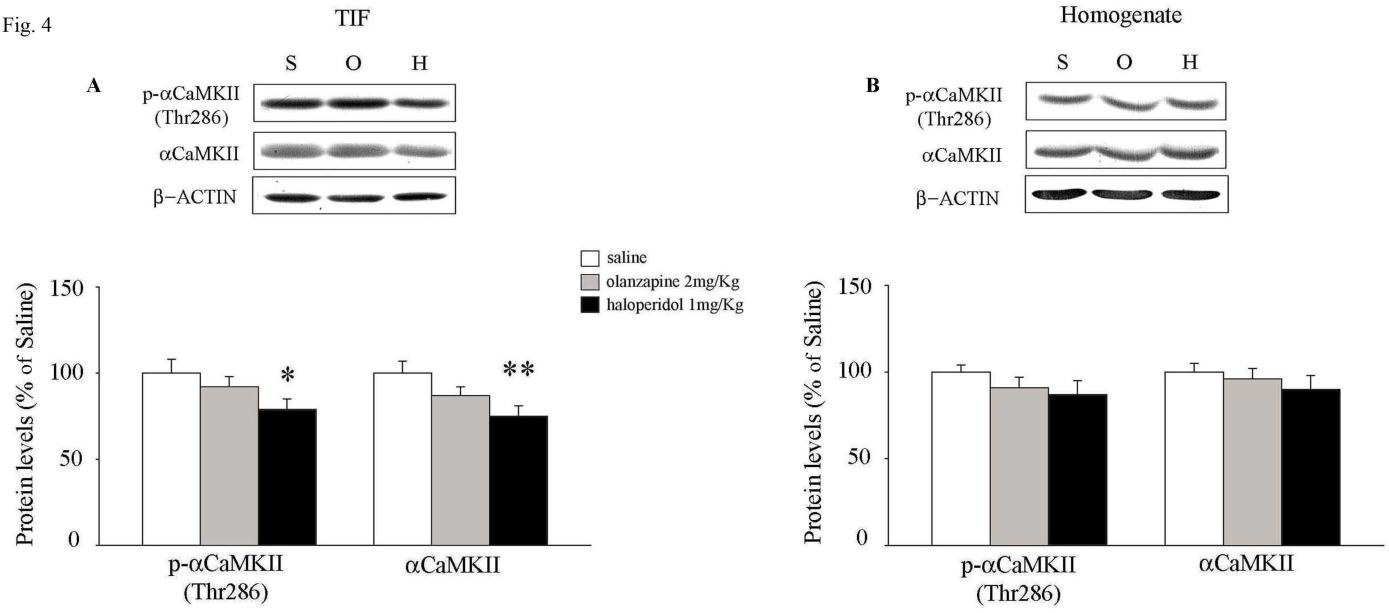
100
50
0
*
PSD95
SAP102

Fig. 4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O
H
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(Thr286)
αCaMKII
β−ACTIN
saline
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haloperidol 1mg/Kg
Protein levels (% of Saline)
150
100
50
0
*
**
p-αCaMKII
(Thr286)
αCaMKII
Homogenate
B
S
O
H
p-αCaMKII
(Thr286)
αCaMKII
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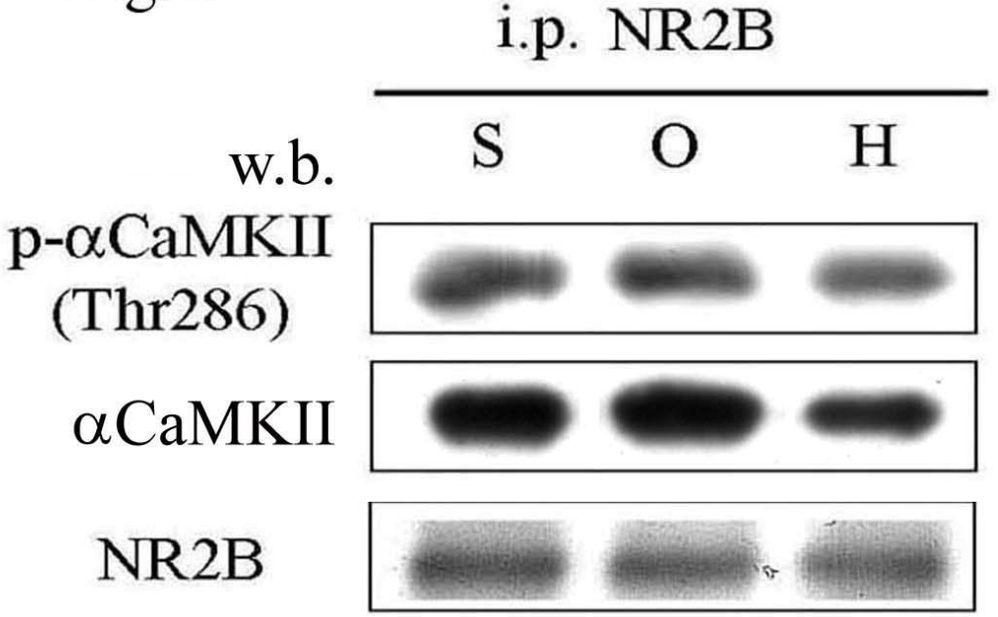
100
50
0
p-αCaMKII
(Thr286)
αCaMKII

Fig. 5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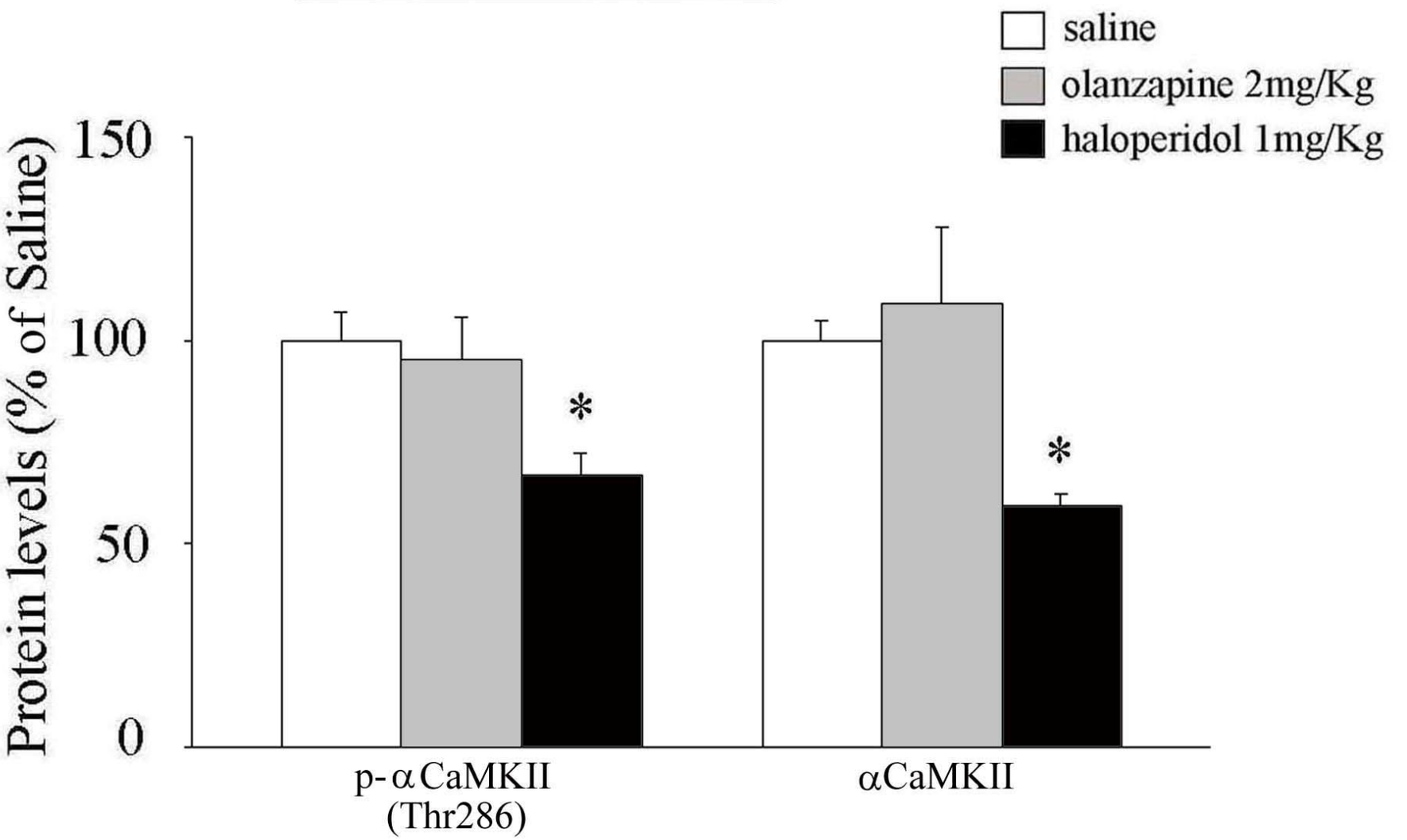
O
H
p-αCaMKII
(Thr286)
αCaMKII
NR2B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haloperidol 1mg/Kg
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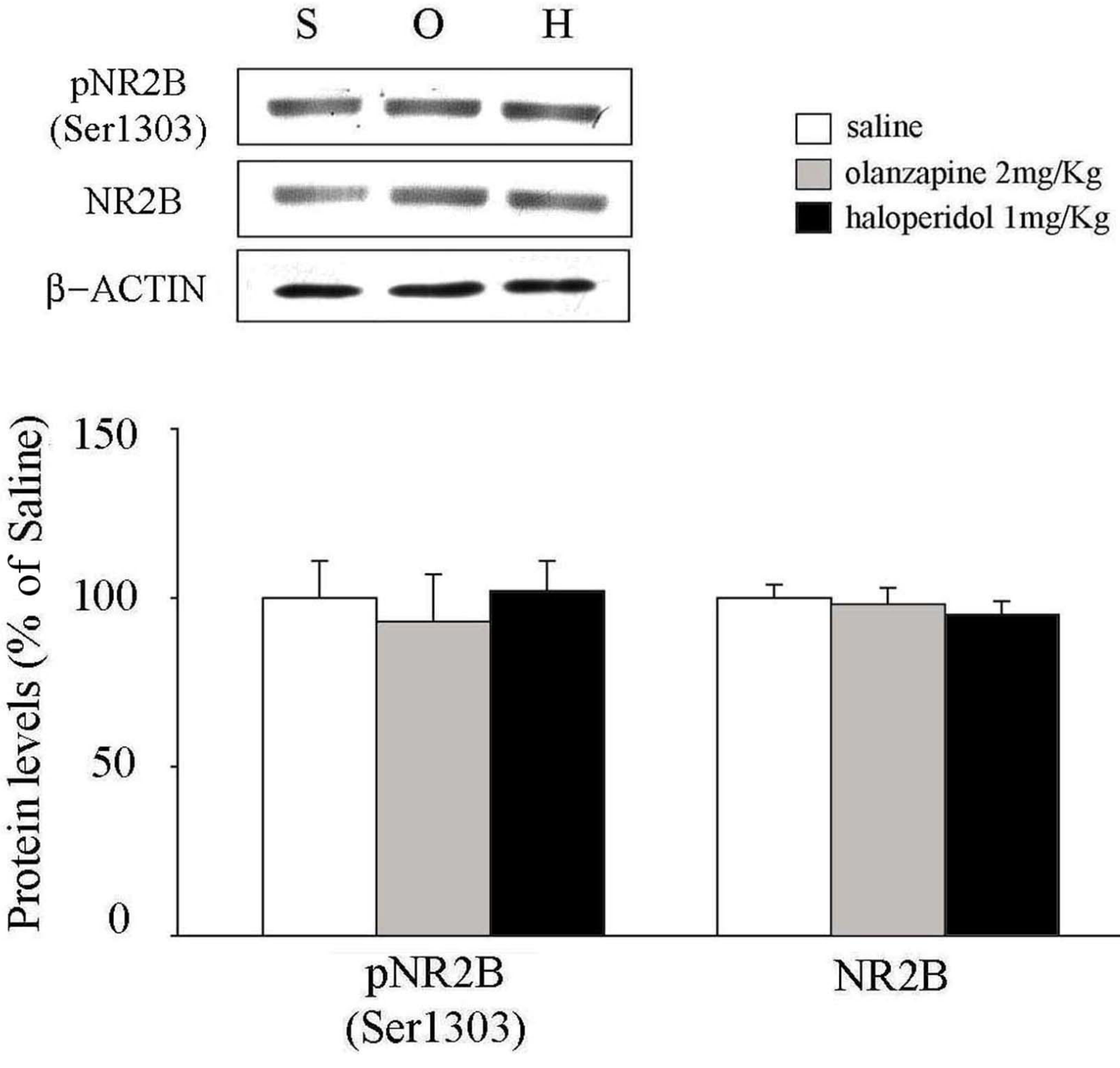
100
50
0
*
*
p-αCaMKII
(Thr286)
αCaMKII

Fig. 6

TIF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NR2B
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olanzapine 2mg/Kg
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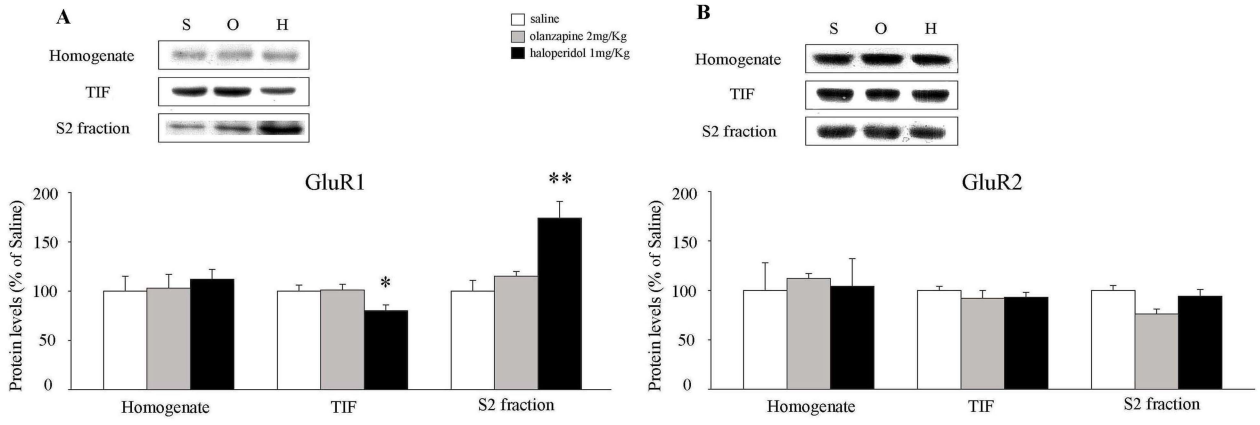
Protein levels (% of Saline)
150
100
50
0
pNR2B
(Ser1303)
NR2B

Fig. 7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S
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H
Homogenate
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S2 fraction
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B
S
O
H
Homogenate
TIF
S2 fraction
GluR1
Protein levels (% of Saline)
200
150
100
50
0
*
**
Homogenate
TIF
S2 fraction
GluR2
Protein levels (% of Saline)
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150
100
50
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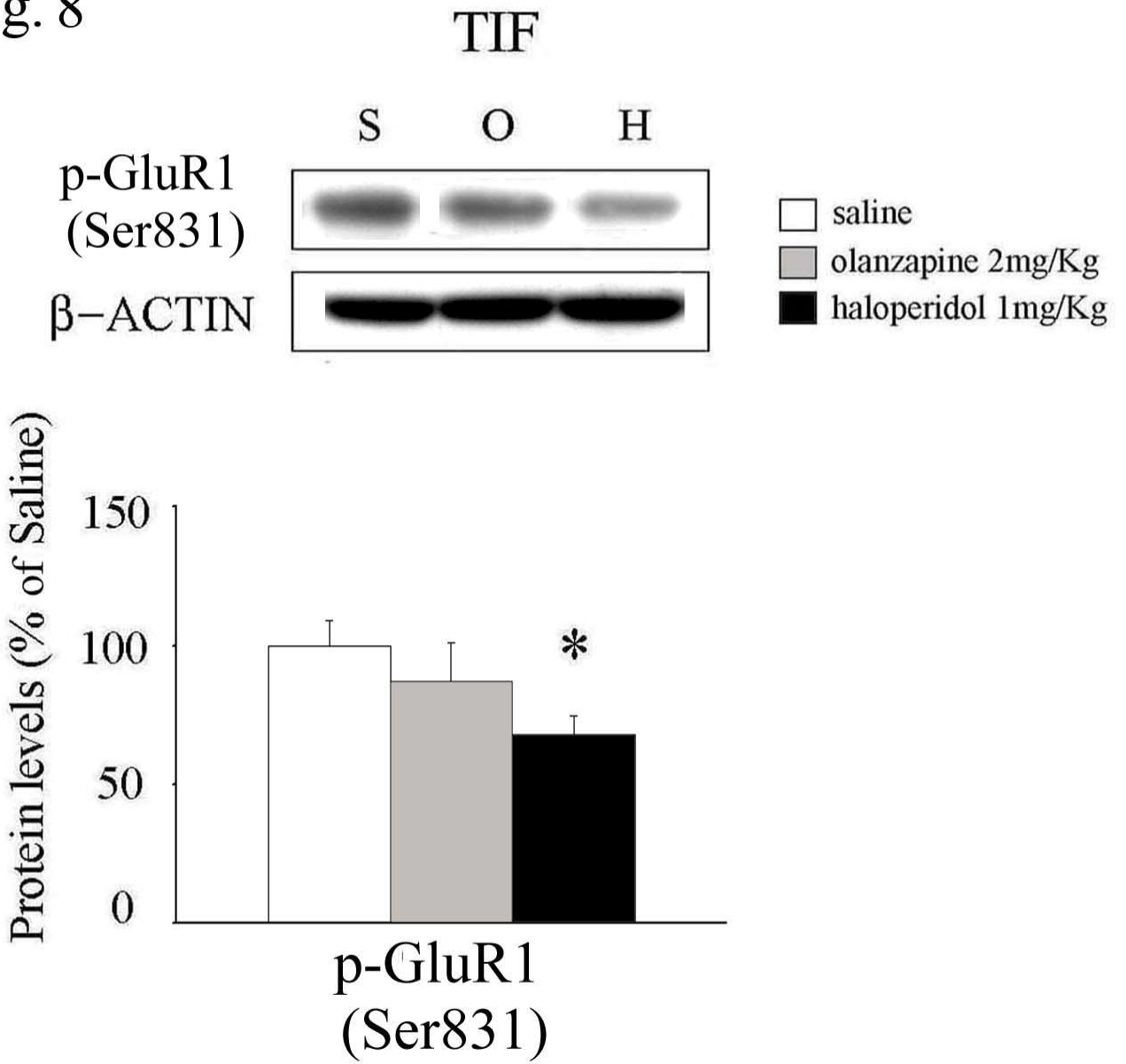
Homogenate
TIF
S2 fraction

Fig. 8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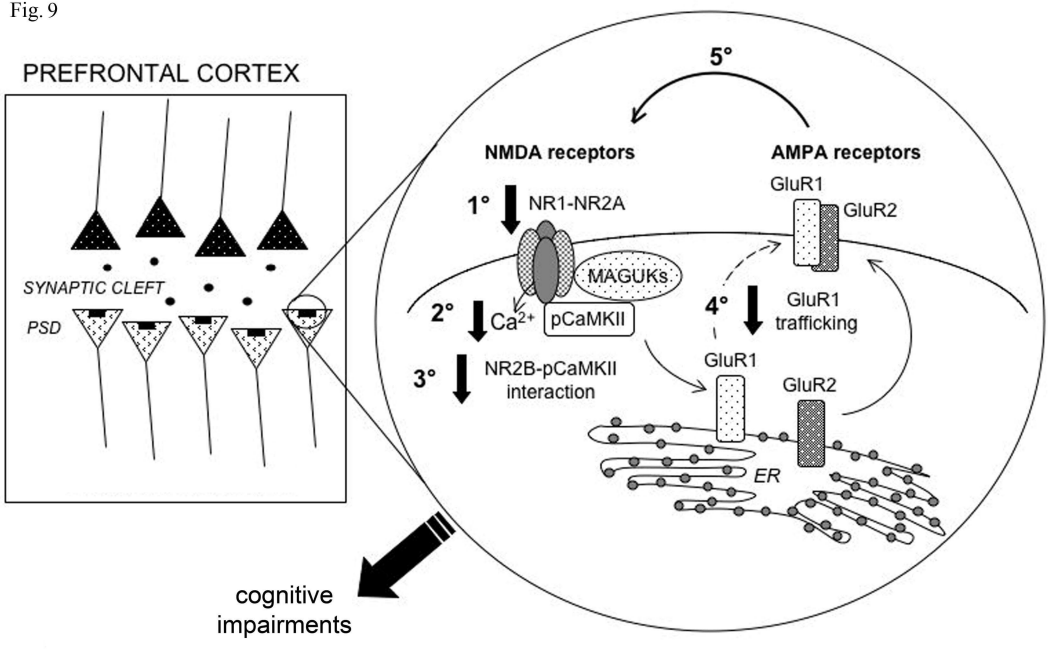
S
O
H
p-GluR1
(Ser831)
β−ACTIN
saline
olanzapine 2mg/Kg
haloperidol 1mg/Kg
Protein levels (% of Saline)
150
100
50
0
*
p-GluR1
(Ser831)

Fig. 9


PREFRONTAL CORTEX
SYNAPTIC CLEFT
PSD
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NMDA receptors
AMPA receptors
1°
NR1-NR2A
GluR1
GluR2
MAGUKs
2°
Ca2+
pCaMKII
4°
GluR1
trafficking
3°
NR2B-pCaMKII
interaction
GluR1
GluR2
ER
cognitive
impairments