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The Novel Antipsychotic Drug Lurasidone Enhances NMDA Receptor-Mediated Synaptic Responses

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Running Title: Regulation of NMDA Receptors by Lurasidone

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Abbreviations

NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA receptor; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPAR, AMPA receptor; EPSC, excitatory postsynaptic current; mEPSC, miniature EPSC; PCP, phencyclidine; APD, atypical antipsychotic drug; PFC, prefrontal cortex; NOR, novel object recognition

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Abstract

N-methyl-D-aspartate (NMDA) receptor (NMDAR) hypofunction has been postulated to contribute to the cognitive deficit of schizophrenia. In this study, we examined the effect of lurasidone (trade name Latuda), a newly approved atypical antipsychotic drug (APD), on NMDAR synaptic function in rat frontal cortical pyramidal neurons. *In vivo* administration of lurasidone produced a significant and selective enhancement of NMDAR-mediated synaptic responses and the surface expression of NR2A and NR2B subunits. Lurasidone has high affinity for serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_7 receptors and dopamine D_2 receptors. *In vivo* administration of the 5-HT_7 receptor antagonist SB-269970 mimicked the enhancing effect of lurasidone on NMDAR responses, while the D_2 receptor antagonist haloperidol failed to do so. Previous studies have found that acute administration of lurasidone reverses the cognitive impairment induced by subchronic administration of phencyclidine (PCP), an NMDAR noncompetitive antagonist. In this study, we found that lurasidone, as well as the prototypical atypical APD clozapine, restored NMDAR-mediated synaptic responses to the normal level in the PCP model of schizophrenia. These results suggest that NMDAR is the potential key molecular target of lurasidone, possibility via antagonizing 5-HT_7 receptors, which is consistent with evidence that 5-HT_7 receptor antagonism contributes to cognitive enhancement by atypical APDs in patients with schizophrenia.

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Introduction

Schizophrenia is characterized by positive symptoms (delusions and hallucinations), negative symptoms (e.g. affective flattening, anhedonia, anergia), abnormalities in mood, and deficits in multiple domains of cognition, including working memory, declarative memory, and executive function, often leading to severe functional impairment from the time of diagnosis (Meltzer, 1989; Sawa and Snyder, 2002). Abnormalities in prefrontal cortex (PFC) and temporal cortex are considered to be the most likely basis for the cognitive impairment of schizophrenia (Weinberger et al 1986). Hypofunction of glutamatergic pyramidal neurons in cortex and hypodopaminergic activity are believed to underlie the cognitive deficit of schizophrenia (Tsai and Coyle, 2002; Lewis and Lieberman, 2000). The evidence for the hypoglutamatergic theory includes the ability of noncompetitive NMDA receptor (NMDAR) antagonists, such as phencyclidine (PCP), MK-801 and ketamine, to produce behavioral symptoms and cognitive dysfunction that have some similarity to schizophrenia in normal volunteers and to exacerbate positive and negative symptoms in schizophrenia (Javitt and Zukin, 1991). Acute or subchronic administration of NMDAR antagonists also increases locomotor activity and disrupts prepulse inhibition in rodents, both of which are thought to model schizophrenia symptoms (Jentsch et al., 1997; Jentsch and Roth, 1999). Further, mice with genetic knockdown of the NMDAR subunit NR1, as well as other rodent models in which specific glutamate receptor subtypes are genetically altered, also have phenotypes suggestive of schizophrenia, including increased locomotor activity, stereotypy, and deficits in cognitive and social function (Mohn et al., 1999).

Typical antipsychotic drugs (APDs), e.g. haloperidol and perphenazine, are believed to diminish positive symptoms in patients with schizophrenia through blockade of limbic dopamine D_2 receptors (Creese et al 1976; Sawa & Snyder 2002), but blockade of D_2 receptors in the dorsal striatum produce unwanted extrapyramidal side effects (Meltzer 1992). Clozapine, the prototypical atypical APD, as well as many other atypical APDs, are more potent serotonin 5-HT_{2A} than dopamine D_2 receptor antagonists, which has been suggested to be the basis for some of their advantages over typical APDs, including low EPS (Meltzer et al. 1989; Meltzer and Huang, 2008). Actions at adrenergic and muscarinic receptors may

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also contribute to the efficacy of various atypical APDs (Meltzer et al. 1989). Thus, clozapine and related atypical APDs have been referred to as multireceptor antagonists to reflect the contribution of receptors other than 5-HT_{2A} and D_2 receptors to their efficacy and side effects (Meltzer and Huang, 2008).

Lurasidone is a novel atypical APD recently approved for treatment of schizophrenia by the U.S. Food and Drug Administration. Lurasidone has potent binding affinities for 5-HT_{2A}, 5-HT_7, 5-HT_{1A}, D_2, and noradrenaline α_{2C} receptors (Ishibashi et al 2010). Clinical trials have shown that lurasidone is a safe and effective treatment for schizophrenia patients with minimal extrapyramidal, cardiovascular, and metabolic complications (Citrome 2011; Nakamura et al 2009; Meltzer et al., 2011a). Lurasidone has been reported to improve acute MK-801-induced memory impairment in rats (Enomoto et al 2008; Ishiyama et al 2007), as well as subchronic PCP-induced impairment in novel object recognition (NOR, Meltzer et al. 2011b; Horiguchi et al. 2011). The molecular mechanism for the atypical APDs to improve cognition is not fully known. We sought to test the hypothesis that lurasidone and clozapine may reverse NMDAR hypofunction via their 5-HT_7 receptor antagonism.

Materials and Methods

Animals.

All animal experiments were performed with the approval of the Institutional Animal Care and Use Committee of the State University of New York at Buffalo. Sprague-Dawley (3-4 week-old) rats were used in all experiments. Animals were injected with lurasidone (0.1 mg/kg, s.c.), clozapine (5 mg/kg, s.c.), SB-269970 (1 mg/kg, i.p.) or haloperidol (0.1 mg/kg, i.p.). One hour later, animals were anesthetized by inhaling halothane (Sigma) for ~30 sec and decapitated quickly. In some experiments, PCP (Sigma) was administered (5 mg/kg, i.p.) once daily for 7 days (Wang et al 2006). One day after the last administration of PCP, animals were injected with lurasidone (0.1mg/kg, s.c.) or clozapine (5mg/kg, s.c.), and sacrificed one hour later.

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Slice Preparation.

Brains were removed, iced, blocked, and coronal slices (300 μm) containing prelimbic/ infralimbic regions were obtained with a Vibratome (Leica VP1000S) in the presence of a low Ca^{2+}, HEPES-buffered salt solution (in mM: 140 Na isethionate, 2 KCl, 4 $MgCl_2$, 0.1 $CaCl_2$, 23 glucose, 15 HEPES, pH =7.4, 300-305 mOsm). These slices were then incubated for 1-5 hrs at room temperature (20-22°C) in a $NaHCO_3$-buffered saline bubbled with 95% O_2, 5% CO_2.

Electrophysiological Recordings.

To record evoked synaptic currents in prefrontal cortical slices, the whole-cell voltage clamp technique was used as described previously (Yuen et al., 2005; 2009). The internal solution contained (in mM): 130 Cs-methanesulfonate, 10 CsCl, 4 NaCl, 1 $MgCl_2$, 10 HEPES, 5 EGTA, 2.2 QX-314, 12 phosphocreatine, 5 MgATP, 0.5 Na_2GTP, pH 7.2-7.3, 265-270 mosM. Slices was perfused with ACSF (in mM: 130 NaCl, 26 $NaHCO_3$, 3 KCl, 5 $MgCl_2$, 1.25 NaH_2PO_4, 1 $CaCl_2$, 10 Glucose, pH 7.4, 300 mOsm) bubbled with 95% O_2 and 5% CO_2. CNQX (25 μM) and bicuculline (10 μM) were added when NMDAR-EPSC was recorded, while D-APV (25 μM) and bicuculline (10 μM) were added when AMPAR-EPC was measured. Recordings were conducted at the room temperature. Neurons were visualized with a 40 x water-immersion lens and illuminated with near infrared IR light. All recordings were performed using a Multiclamp 700A amplifier and digitized with Digidata1322A. Tight seals (2-10 GΩ) were generated by applying negative pressure, followed by additional suction to disrupt the membrane and obtain the whole-cell configuration. The access resistance ranged from 8-15 MΩ. Evoked currents were generated with a 50 μs pulse from a stimulation isolation unit controlled by a S48 pulse generator (Astro-Med, Inc., West Warwick, RI). A bipolar stimulating electrode (FHC, Inc., Bowdoinham, ME) was positioned ~100 μm from the neuron under study. The same stimulation intensity was used in individual neurons across groups with various drug treatments, similar to what was described before (Yuen et al., 2009). For NMDAR-EPSC recording, cells (voltage-clamped at -70 mV) were depolarized to +60 mV for 3 s before stimulation to fully relieve the voltage-dependent Mg^{2+} block of NMDAR channels. For AMPAR-EPSC

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recording, cells were constantly held at -70mV. When miniature EPSC (mEPSC) was recorded, TTX (1 μM) was added to ACSF. The capacitance of the recorded neurons ranged from 80-120 pF. Signals were acquired at a bandwidth of 20 KHz (eEPSC) or 2 KHz (mEPSC) and filtered with a 2 KHz low-pass Bessel filter.

To minimize experimental variations between cells, the following criteria were used: (1) layer V medial prefrontal cortex (mPFC) pyramidal neurons with comparable membrane capacitances were selected; (2) the stimulating electrode was positioned at the same location (layer VI, ~100 μm horizontally) from the recording neuron, and the electrode tip was cleaned after every recording to allow precise stimulation capacity; (3) recordings from animals injected with different drugs were interleaved throughout the course of experiments.

Data analyses were performed with Clampfit (Axon instruments), Mini Analysis (Synaptosoft) and Kaleidagraph (Albeck Software). Rise time was measured from 10 to 90% of peak amplitude. No correlation between EPSC amplitude and rise time was found. Because the distribution of EPSC amplitudes in different animals was often found to be non-normal, statistical analysis was performed with Kruskal-Wallis test. For comparisons of data (two groups) with normal distributions, Student *t* test was used.

Biochemical measurement of surface and total proteins.

The surface AMPA and NMDA receptors were detected as previously described (Yuen et al., 2009). In brief, PFC slices were incubated with ACSF containing 1 mg/ml sulfo-*N*-hydroxysuccinimide-LC-Biotin (Pierce Chemical Co., Rockford, IL) for 20 min on ice. The slices were then rinsed three times in Tris-buffered saline to quench the biotin reaction, followed by homogenization in modified radio-immunoprecipitation assay buffer (1% Triton X-100, 0.1% SDS, 0.5% deoxycholic acid, 50 mM $NaPO_4$, 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and 1 mg/ml leupeptin). The homogenates were centrifuged at 14,000 x *g* for 15 min at 4°C, incubated with 50% Neutravidin Agarose (Pierce Chemical Co.) for 2 hr at

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4°C, and bound proteins were resuspended in SDS sample buffer and boiled. Quantitative Western blots were performed on both total and biotinylated (surface) proteins using antibodies against NR2A (1:500, Millipore, 07-632), NR2B (1:500, Millipore, 06-600), NR1 (1:500, Cell Signaling, 5704), GluR1 (1:200, Santa Cruz, sc-13152), GluR2 (1:500, Millipore, MAB397) and actin (1:1000, Santa Cruz, sc-1616).

Results

***In vivo* administration of lurasidone induces a significant enhancement of NMDAR-mediated synaptic responses.**

To examine whether the NMDAR is a target of lurasidone and clozapine, we measured NMDAR-mediated excitatory postsynaptic currents (EPSC) in animals injected with either saline, lurasidone (0.1 mg/kg, sc) or clozapine (5 mg/kg, sc). As shown in **Fig. 1A**, mPFC pyramidal neurons from lurasidone- or clozapine-injected rats showed significantly increased NMDAR-EPSC amplitudes, compared to those from saline-injected rats (saline: 112±5.9pA, n=12; lurasidone: 238±15.1pA, n=13; clozapine: 206±19.8pA, n=9, $p<0.001$, Kruskal-Wallis test). The amplitudes of NMDAR-EPSC induced by a series of stimulus intensities, as shown in the input/output curves of NMDAR-EPSC, also revealed a significant increase in lurasidone-injected rats (**Fig. 1B**, 45-77% increase, lurasidone: n=9; saline: n=6, $p<0.001$, *t* test). Lurasidone administration did not cause significant changes on the NMDAR-EPSC kinetics (decay time constant, saline: 150±10.1ms, n=7; lurasidone: 160±14.9ms, n=7, $p>0.05$, *t* test) or the rise time (saline: 7.83±0.23ms, n=18; lurasidone: 7.79±0.22ms, n=18, $p>0.05$, *t* test). The sensitivity to the NR2B antagonist ifenprodil (5 μM) was also unchanged by lurasidone (**Fig. 1C** and **1D**, saline: 32±2.0% reduction, n=5; lurasidone: 31±2.6% reduction, n=5, $p>0.05$, *t* test).

In contrast to the significance enhancement of NMDAR-EPSC, AMPAR-mediated excitatory postsynaptic currents (AMPAR-EPSC) were not responsive to either lurasidone or clozapine (**Fig. 2A**, saline: 146±9.2pA, n=12; lurasidone: 144±12.0pA, n=10; clozapine: 161±16.7pA, n=11, $p>0.05$, Kruskal-Wallis test). The input/output curves of AMPAR-EPSC amplitude also showed no change in lurasidone- vs. saline-injected rats (**Fig. 2B**, <5% increase, lurasidone: n=9; saline: n=7, $p>0.05$, *t* test). The rise time

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of AMPAR-EPSC was also unchanged by lurasidone administration (saline: 4.59±0.21ms, n=19; lurasidone: 4.52±0.22ms, n=20, p>0.05, *t* test). The slow ePSC rise time was consistent with previous results recorded in CA1 pyramdial neurons (Xia et al., 2005), which may reflect asynchrony of release rather than heavy filtering or compromised degree of voltage control. Furthermore, miniature EPSC (mEPSC), an AMPAR-mediated synaptic response resulting from quantal release of single glutamate vesicles, was unchanged by lurasidone administration (**Fig. 2C** and **2D**, saline: 24±0.8pA, 2.1±0.2Hz, n=7; lurasidone: 25±0.7pA, 2.0±0.2Hz, n=6, p>0.05, *t* test). No change was found on the mEPSC rise time either (saline: 1.52±0.09ms, n=5; lurasidone: 1.44±0.05ms, n=6, p>0.05, *t* test). The lack of changes in evoked or miniature AMPAR-EPSC ruled out the possibility of changes in presynaptic glutamate release by these APDs. The ratio of NMDAR-EPSC to AMPAR-EPSC was significantly higher in individual neurons from lurasidone-injected rats than those from saline-injected rats (**Fig. 2E**, saline: 1.0±0.1, n=15; lurasidone: 1.7±0.1, n=13, p<0.01, *t* test). Taken together, our results suggest that *in vivo* administration of lurasidone or clozapine selectively enhances postsynaptic NMDAR function.

In vivo administration of lurasidone induces a significant enhancement of the surface expression of NMDAR subunits.

Next we examined the potential mechanism underlying the enhancement of NMDAR-EPSC by lurasidone. One possibility is the increased surface delivery of NMDA receptors after administration of this antipsychotic drug. To test it, we performed surface biotinylation and Western blotting experiments to detect the surface and total level of NMDAR and AMPAR subunits. Animals were injected with saline or lurasidone (0.1 mg/kg, s.c.). One hour later, animals were sacrificed and brains were sliced. Following one hour of recovery, cortical slices were harvested for the biochemical assay. As shown in **Fig. 3A-C**, lurasidone-injected rats showed a significant increase in surface NR2A and NR2B subunits of NMDA receptors, compared to saline-injected rats (surface NR2A: 3.3±0.7 fold of control, n=7 pairs; surface NR2B: 3.1±0.8 fold of control, n=5 pairs; p<0.05, *t* test). No significant increase was found in surface NR1 subunits (1.2±0.5 fold of control, n=6 pairs, p>0.05, *t* test). The level of surface GluR1 and GluR2

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subunits of AMPA receptors was also unchanged by lurasidone (surface GluR1: 1.2±0.2 fold of control, n=8 pairs; surface GluR2: 1.1±0.2 fold of control, n=4 pairs, p>0.05, *t* test). The total level of these receptor subunits remained similar in saline- vs. lurasidone-injected animals (total NR2A/2B/1: 0.9±0.2 fold of control, n=7 pairs; total GluR1/2: 1.0±0.1 fold of control, n=6 pairs), which rules out the possibility of new glutamate receptor synthesis. These results suggest that *in vivo* lurasidone administration selectively increases the surface level of NMDAR NR2 subunits, which may account for the potentiation of NMDAR-mediated synaptic responses in frontal cortex.

Lurasidone increases NMDAR function through 5-HT$_7$ receptor antagonism.

We next examined potential receptors underlying the enhancement by lurasidone of NMDAR-EPSC. *In vitro* functional assays demonstrate that lurasidone acts as an antagonist at D_2 and 5-HT$_7$ receptors and as a partial agonist at the 5-HT$_{1A}$ receptor (Ishibashi et al 2010). Our previous studies have found that a 5-HT$_{1A}$ agonist decreases NMDAR-EPSC (Yuen et al., 2005), whereas a 5-HT$_{2A}$ agonist or antagonist alone have no effect on NMDAR-EPSC (Yuen et al 2008). Thus, we focused on the role of 5-HT$_7$ and D_2 receptors in the effect of lurasidone on NMDAR-EPSC. As shown in **Fig. 4**, *in vivo* administration of the selective 5-HT$_7$ antagonist SB-269970 (1 mg/kg, i.p., Gasbarri et al., 2008) produced significantly increased NMDAR-EPSC (saline: 111±6.2pA, n=19; SB: 274±25.5pA, n=20, p<0.001, Kruskal-Wallis test), mimicking the effects of clozapine and lurasidone. Note that a subset of neurons (5 out of 20) had no response to the 5-HT$_7$ antagonist, consistent with the previous report that 5-HT$_7$ is not expressed in every mature PFC pyramidal neurons (Beique et al 2004). Moreover, co-injection of lurasidone (0.1 mg/kg) plus SB-269970 (1 mg/kg) did not produce an additive enhancement of NMDAR-EPSC (231±32.6pA, n=18) at these doses. On the other hand, *in vivo* administration of the selective D_2 antagonist haloperidol (0.1 mg/kg, i.p.) failed to change NMDAR-EPSC (112±7.9pA, n=10). These results suggest that 5-HT$_7$ antagonism may underlie the lurasidone-induced enhancement of NMDAR function.

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Lurasidone rescues the NMDAR hypofunction in a PCP model of schizophrenia.

Since lurasidone reversed the effect of subchronic PCP administration on novel object recognition (Horiguchi et al, 2011) and was shown here to potently increase NMDAR function, we further tested its ability to restore NMDAR hypofunction in PCP-treated rats . Thus, we administered lurasidone following subchronic treatment of PCP (5 mg/kg, i.p., 7 days). One day after PCP withdrawal, animals were given one injection of lurasidone (0.1 mg/kg, s.c.) and tested 1-4 hours later. As shown in **Fig. 5A**, PCP- treated animals showed significantly diminished NMDAR-EPSC (saline: 128±12.8pA, n=12; PCP: 66±6.5pA, n=15, $p<0.001$, Kruskal–Wallis test). Administration of lurasidone increased NMDAR-EPSC in saline-injected animals (lurasidone: 207±13.3pA, n=14), and restored NMDAR-EPSC in the PCP-treated animals (PCP+lurasidone: 168±20.3pA, n=12). A similar rescue was observed with *in vivo* administration of clozapine (5 mg/kg, s.c., saline+clozapine: 244±9.6pA, n=14; PCP+clozapine: 227±18.2pA, n=9, **Fig. 5B**). These results suggest that lurasidone, like clozapine, is capable of reversing the NMDAR hypofunction induced by repeated PCP treatment, which is a widely studied animal model of schizophrenia.

Discussion

Despite the ability of lurasidone and clozapine to improve cognition in animal models of schizophrenia (Enomoto et al 2008; Ishiyama et al 2007; Nakamura et al 2009; Singdha et al. 2010; Horiguchi et al. 2011), little is known about the molecular and cellular mechanism underlying this action. Repeated exposure to lurasidone increases the mRNA and protein levels of BDNF (Fumagalli et al., 2011), an important determinant of synaptic plasticity of glutamatergic synapses, consistent with the idea that antipsychotic treatment may change the expression, trafficking, and interaction of essential components of glutamatergic synapses (Fumagalli et al., 2008; Iasevoli et al., 2010). This study has provided the first electrophysiological evidence showing that *in vivo* administration of lurasidone or clozapine produces a significant enhancement of NMDAR-mediated EPSC in PFC neurons. Moreover,

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administration of a single dose of lurasidone or clozapine restored NMDAR responses in subchronic PCP-treated rats.

Similar to the pharmacological profile of clozapine (Meltzer, 1994), lurasidone has high binding affinity to various monoamine receptors, such as 5-HT_{2A}, 5-HT_7, 5-HT_{1A}, D_2, and α_{2C} receptors (Meyer et al., 2009; Ishibashi et al., 2010). Drugs that affect several 5-HT receptors, e.g. 5-HT_{2A} antagonists and 5-HT_{2C} agonists, are effective to prevent the effects of NMDAR noncompetitive blockers on locomotor activity (Marquis et al. 2007) and to restore NOR in the subchronic PCP model (Meltzer et al., 2011b). Our previous studies have found that selective agonists or antagonists for 5-HT_{1A} or 5-HT_{2A} receptors either reduce NMDAR-EPSC or have no effect (Yuen et al., 2005; 2008). In this study, we show that the selective 5-HT_7 antagonist SB-269970 mimics the enhancing effect of lurasidone on NMDAR-EPSC, while the D_2 antagonist haloperidol (a typical APD) is ineffective. These results suggest that antagonism of 5-HT_7 receptors may contribute to the ability of some atypical APDs to potentiate NMDAR function. The electrophysiological results reported here are consistent with the behavioral effects of these compounds in schizophrenia models. Horiguchi et al. (2011) have found that lurasidone, clozapine and SB-269970, but not haloperidol, improve the impairment in NOR induced by subchronic PCP treatment. Moreover, the ability of lurasidone to reverse the PCP-induced NOR deficit is blocked by the 5-HT_7 agonist AS19 (Horiguchi et al. 2011).

Emerging evidence suggests that NMDAR trafficking, which is regulated by interactions with PDZ proteins and tyrosine phosphorylation, plays a key role in controlling NMDAR function at synapses (Wenthold et al., 2003). Our biochemical evidence indicates that the surface levels of NR2A and NR2B subunits of NMDA receptors are selectively and significantly elevated after lurasidone administration. Since the availability of NR2 subunits determines the number of functional NMDARs at synapses, our results suggest that the potential molecular mechanism underlying the enhancing effect of *in vivo* administration of lurasidone on NMDAR synaptic responses is the increased delivery or decreased internalization of synaptic NMDA receptors.

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Pharmacological data suggest that the effect of lurasidone on NMDARs is likely to be through a mechanism involving 5-HT_7 receptor antagonism. 5-HT_7 is a G_s-coupled GPCR that stimulates type 1 and type 8 Ca^{2+}/calmodulin-sensitive adenylyl cyclases (Baker et al., 1998). The 5-HT_7 receptor is enriched in brain regions mediating complex cognitive processes, such as the limbic system, hippocampus, amygdala and PFC (Beique et al 2004; Ruat et al 1993). 5-HT_7 expression and function also correlate with neuronal depolarization in the developing rat PFC (Beique et al 2004). 5-HT_7 receptor knockout or blockade of the 5-HT_7 receptor enhances learning and memory (Gasbarri et al 2008). A growing body of evidence supports the concept for targeting 5-HT_7 antagonism as a possible mechanism for the treatment of cognitive deficits and a potential target for novel anxiolytic and antidepressant drugs (Mnie-Filali et al., 2009; Hedlund et al., 2005; Abbas et al. 2010; Horiguchi et al. 2011). In conclusion, the results reported here suggest that the pro-cognitive effect of 5-HT_7 receptor antagonism (Gasbarri et al 2008; Horiguchi et al. 2011) may result from enhancement of NMDAR function.

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Authorship Contribution

Participated in research design: Yan, Yuen, Meltzer

Conducted experiments: Yuen, Li, Wei

Performed data analysis: Yuen, Li, Wei

Wrote or contributed to the writing of the manuscript: Yan, Meltzer, Horiguchi

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Footnotes

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Figure Legends

Figure 1. *In vivo* administration of lurasidone induces a significant enhancement of NMDAR-EPSC. **A,** Dot plots showing the amplitude of NMDAR-EPSC in PFC pyramidal neurons from rats injected with saline, lurasidone (0.1 mg/kg, sc), or clozapine (5 mg/kg, sc). Inset: Representative NMDAR-EPSC. Scale bar: 50pA, 100ms. **B,** Summarized input-output curves of NMDAR-EPSC in response to a series of stimulation intensity in saline- vs. lurasidone-injected rats. *: $p<0.001$, Kruskal-Wallis test. **C,** Plot of normalized NMDAR-EPSC showing the effect of ifenprodil (an NR2B antagonist, 5 μM) in PFC neurons from saline- vs. lurasidone-injected rats. **D,** Bar graphs summarizing the percentage reduction of NMDAR-EPSC amplitude by ifenprodil in saline- vs. lurasidone-injected rats.

Figure 2. *In vivo* administration of lurasidone does not alter AMPAR-EPSC. A, Dot plots showing the amplitude of AMPAR-EPSC in PFC pyramidal neurons from rats injected with saline, lurasidone (0.1 mg/kg, sc), or clozapine (5 mg/kg, sc). Inset: Representative AMPAR-EPSC traces. Scale bar: 50pA, 20ms. **B,** Summarized input-output curves of AMPAR-EPSC in response to a series of stimulation intensity in saline- vs. lurasidone-injected rats. **C,** Cumulative plot of mEPSC amplitudes in PFC neurons from saline- vs. lurasidone-injected rats. Inset: Representative mEPSC traces. Scale bars: 10pA, 5s. **D,** Bar graphs showing the mEPSC amplitude and frequency in PFC neurons from saline- vs. lurasidone-injected rats. **E,** Bar graphs showing the NMDAR-EPSC/AMPAR-EPSC ratio in PFC neurons from saline- vs. lurasidone-injected rats. *: $p<0.01$, *t* test. Inset: Representative NMDAR-EPSC and AMPAR-EPSC traces recorded in the same neurons. Scale bars: 50pA, 100ms (NMDA), 20ms (AMPA).

Figure 3. *In vivo* administration of lurasidone significantly increases the surface expression of NMDA receptor NR2 subunits. (A-C) Immunoblots and quantification analysis of the surface and total AMPAR and NMDAR subunits in cortical slices from saline vs. lurasidone-injected rats. *: $p<0.05$ *t* test.

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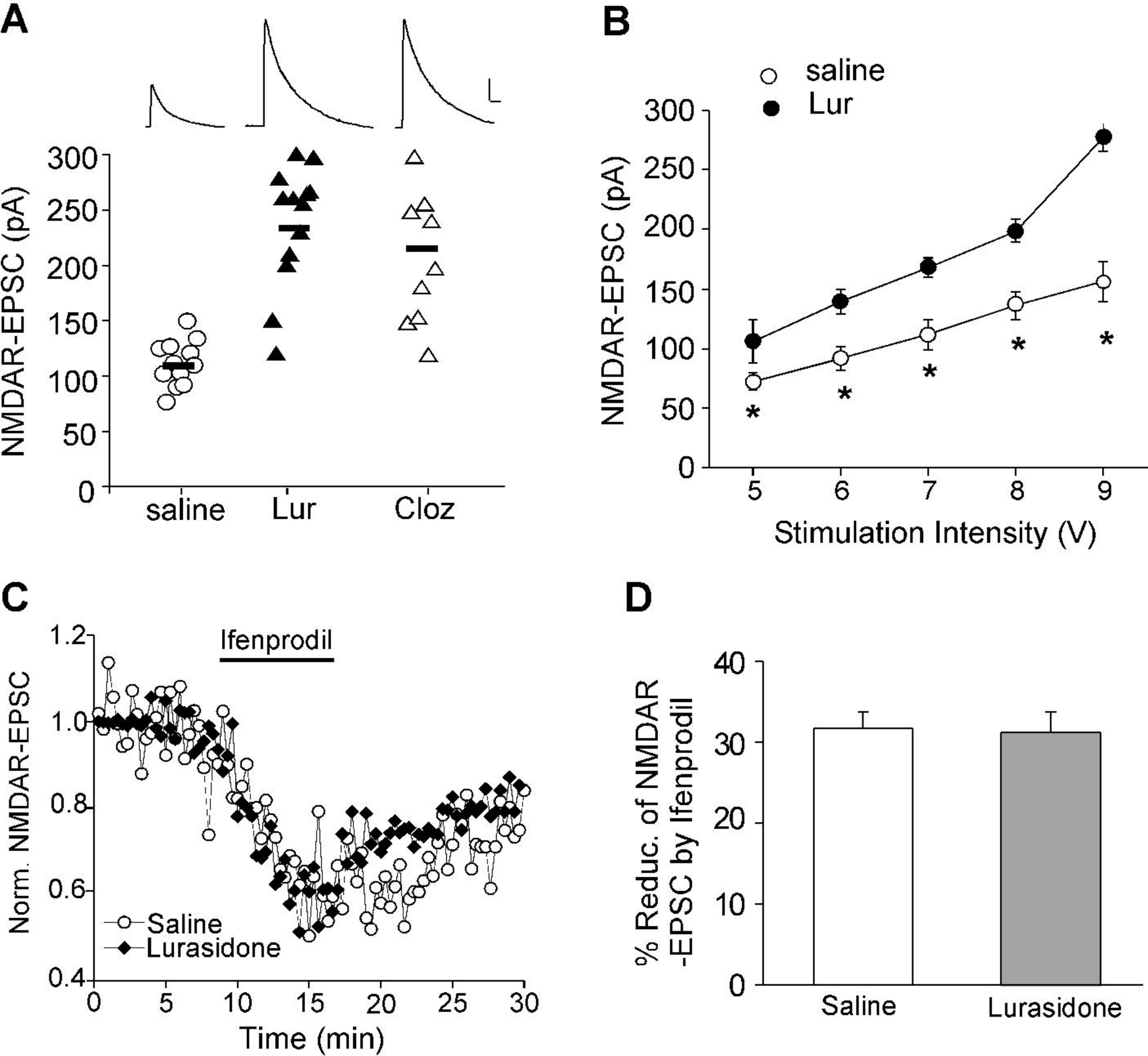
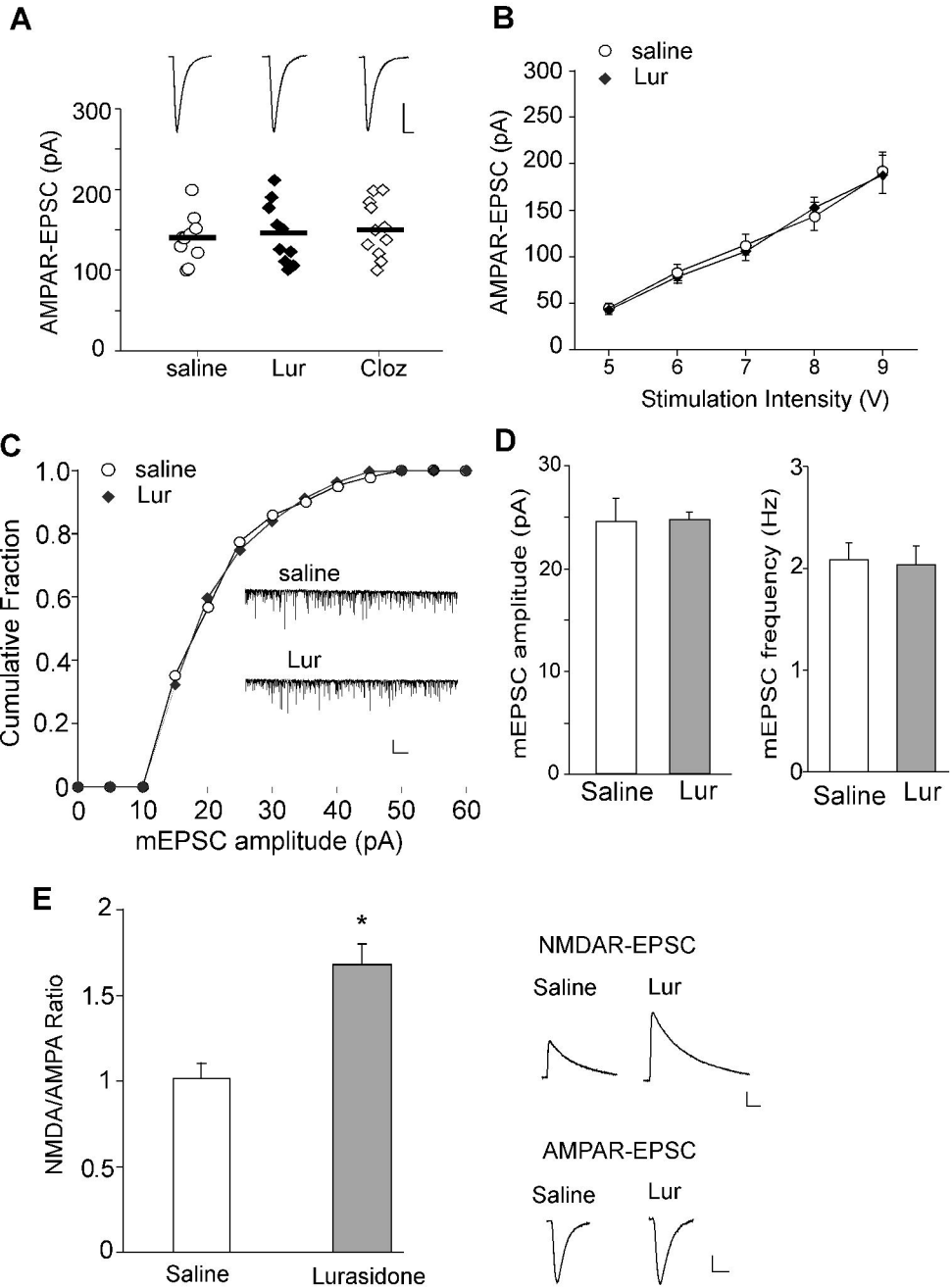
Figure 4. The enhancing effect of lurasidone on NMDAR-EPSC is mimicked by antagonizing 5-HT$_7$ receptors. A, Dot plot showing the amplitude of NMDAR-EPSC in PFC pyramidal neurons from animals injected with saline, the 5-HT$_7$ antagonist SB-269970 (1 mg/kg, ip), lurasidone (0.1 mg/kg, sc) plus SB-269970, or the D_2 antagonist haloperidol (0.1 mg/kg, ip). **B,** Representative NMDAR-EPSC traces in rats injected with different agents. Scale bar: 50pA, 100ms.

Figure 5. Lurasidone reverses NMDAR hypofunction in the PCP model of schizophrenia. A, B, Dot plots showing the amplitude of NMDAR-EPSC in PFC pyramidal neurons from PCP-treated animals injected with lurasidone (0.1 mg/kg, sc, A) or clozapine (5mg/kg, sc, B). Inset: Representative NMDAR-EPSC traces. Scale bar: 50pA, 100ms.

Figure 1


A
NMDAR-EPSC (pA)
300
250
200
150
100
50
0
saline
Lur
Cloz
B
saline
Lur
NMDAR-EPSC (pA)
300
250
200
150
100
50
0
5
6
7
8
9
Stimulation Intensity (V)
C
Ifenprodil
Norm. NMDAR-EPSC
1.2
1.0
0.8
0.6
0.4
Saline
Lurasidone
0
5
10
15
20
25
30
Time (min)
D
% Reduc. of NMDAR -EPSC by Ifenprodil
40
30
20
10
0
Saline
Lurasidone


A
AMPAR-EPSC (pA)
300
200
100
0
saline
Lur
Cloz
B
saline
Lur
AMPAR-EPSC (pA)
300
250
200
150
100
50
0
5
6
7
8
9
Stimulation Intensity (V)
C
saline
Lur
Cumulative Fraction
1.0
0.8
0.6
0.4
0.2
0
saline
Lur
0
10
20
30
40
50
60
mEPSC amplitude (pA)
D
mEPSC amplitude (pA)
30
20
10
0
Saline
Lur
mEPSC frequency (Hz)
3
2
1
0
Saline
Lur
E
NMDA/AMPA Ratio
2
1.5
1
0.5
0
*
Saline
Lurasidone
NMDAR-EPSC
Saline
Lur
AMPAR-EPSC
Saline
Lur

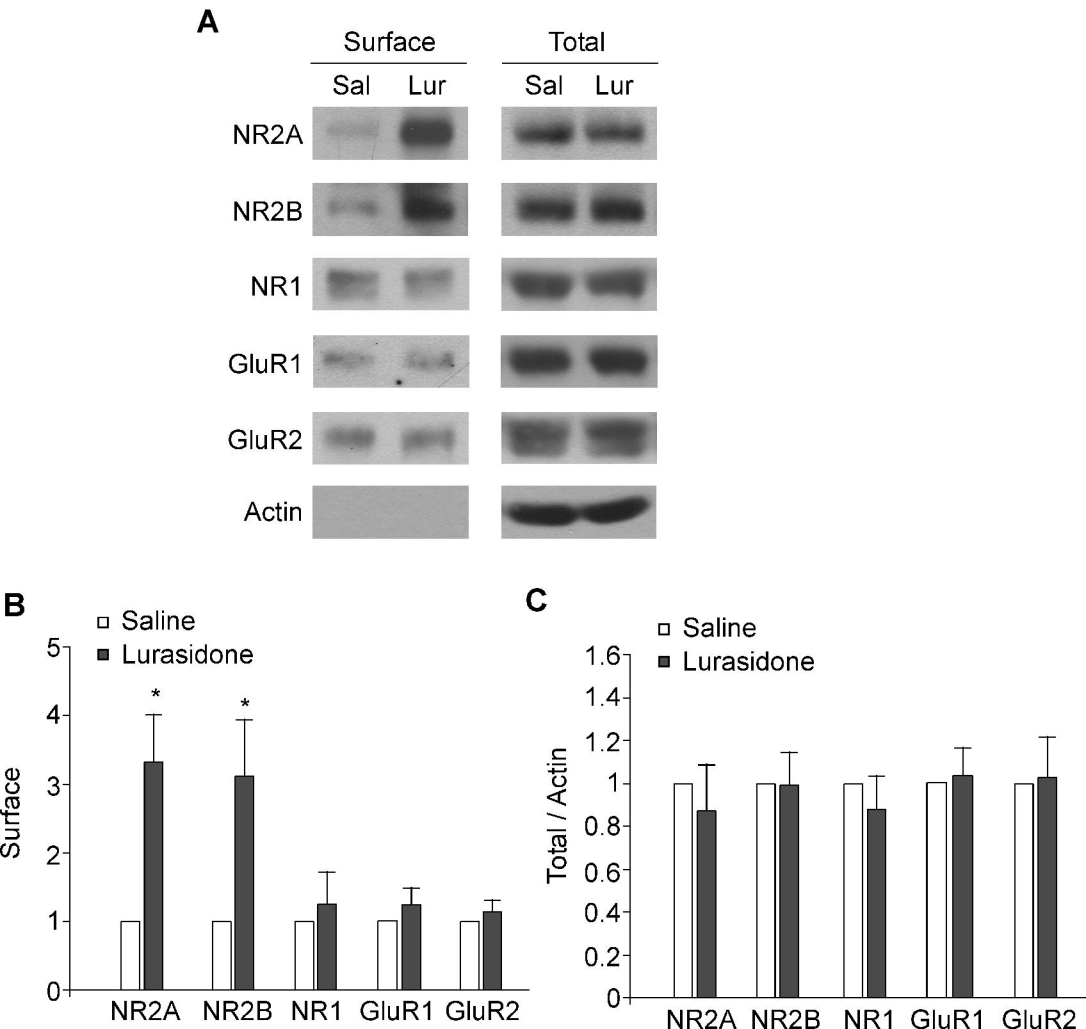
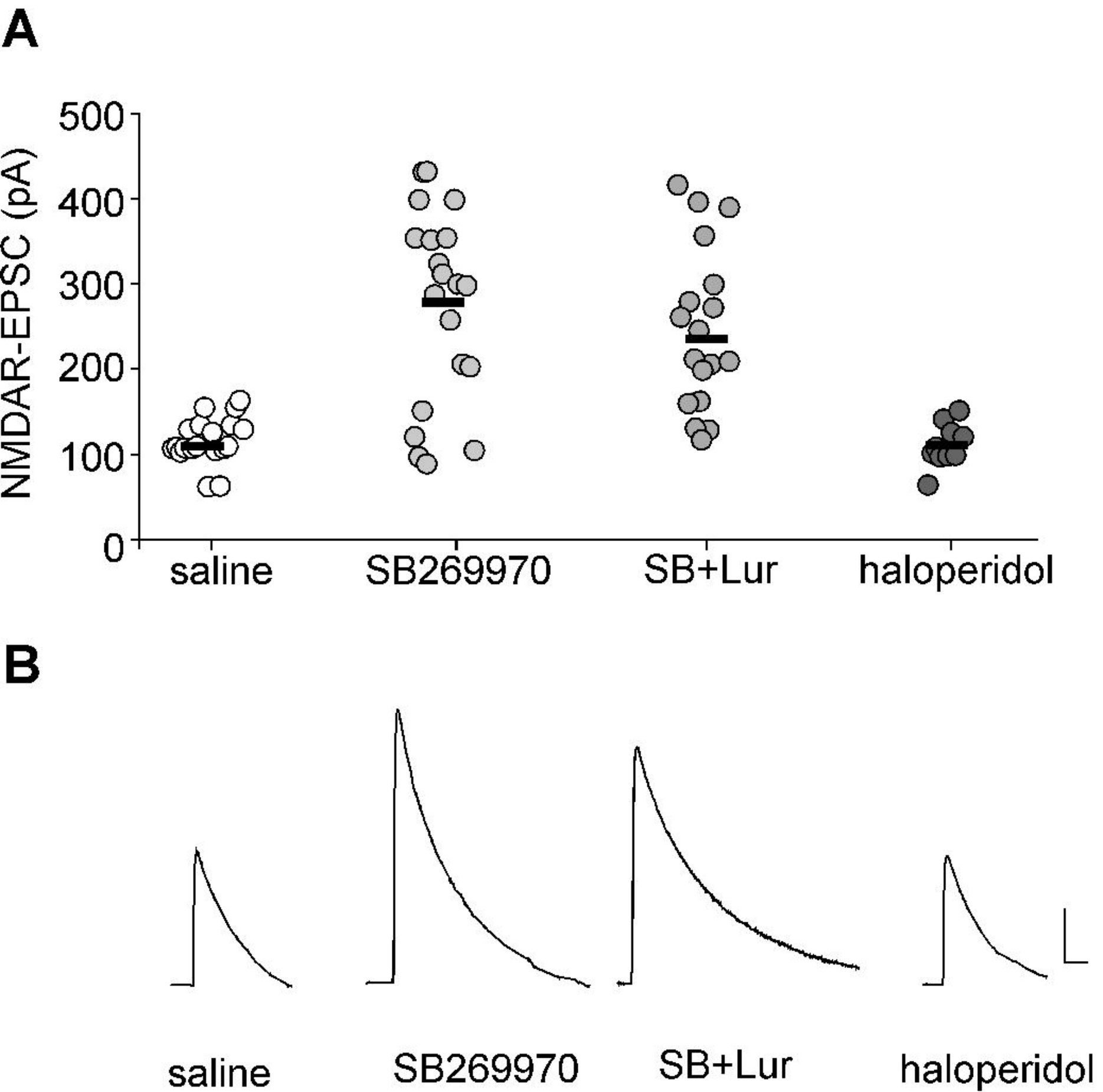
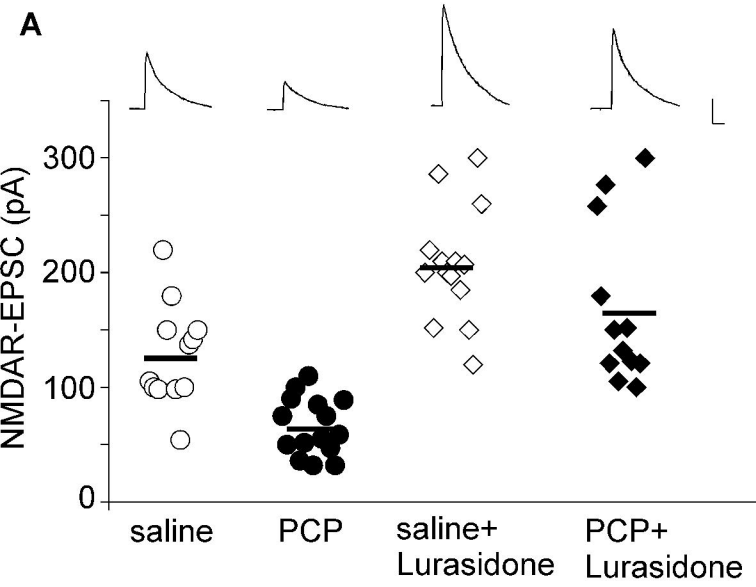
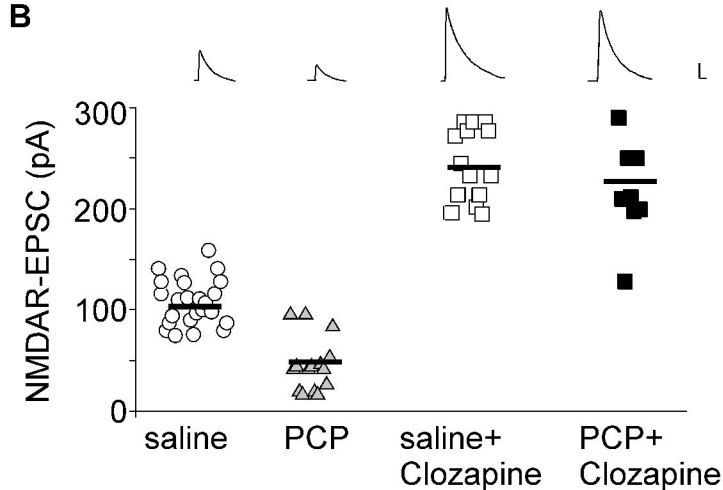

A
Surface
Total
Sal
Lur
Sal
Lur
NR2A
NR2B
NR1
GluR1
GluR2
Actin
B
Saline
Lurasidone
Surface
5
4
3
2
1
0
*
*
NR2A
NR2B
NR1
GluR1
GluR2
C
Saline
Lurasidone
Total / Actin
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
NR2A
NR2B
NR1
GluR1
GluR2

Figure 4


A
NMDAR-EPSC (pA)
500
400
300
200
100
0
saline
SB269970
SB+Lur
haloperidol
B
saline
SB269970
SB+Lur
haloperidol


A
NMDAR-EPSC (pA)
300
200
100
0
saline
PCP
saline+
Lurasidone
PCP+
Lurasidone


B
NMDAR-EPSC (pA)
300
200
100
0
saline
PCP
saline+
Clozapine
PCP+
Clozapine