Schizophrenia Research 136 (2012) 96–103

Contents lists available at SciVerse ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Inhibition of glycogen synthase kinase-3 attenuates psychotomimetic effects of ketamine

Ming-Huan Chan [a,1], Pao-Hsiang Chiu [a,b,1], Chia-Yu Lin [a], Hwei-Hsien Chen [a,*]

[a] Department of Pharmacology, Tzu Chi University, 701, Section 3, Chung-Yang Road, Hualien, 970, Taiwan
[b] Taipei Veterans General Hospital, No.201, Sec. 2, Shipai Rd., Beitou District, Taipei City, 11217, Taiwan

ARTICLE INFO

Article history:
Received 30 June 2011
Received in revised form 6 January 2012
Accepted 18 January 2012
Available online 9 February 2012

Keywords:
GSK-3
NMDA antagonist
Locomotor
Cognition
Prepulse inhibition
Schizophrenia

ABSTRACT

N-methyl-D-aspartate (NMDA) glutamate receptors mediate fast neurotransmission and regulate synaptic plasticity in the brain. Disruption of NMDA receptor-mediated signaling by noncompetitive antagonists, such as PCP or ketamine, evokes psychotomimetic behaviors, although the cellular mechanisms by which hypofunctional NMDA receptor signaling drives behavioral pathology are still unclear. Activation of glycogen synthase kinase-3 (GSK-3) has been implicated in the cellular neurotoxicity of NMDA receptor antagonists. Accordingly, in the present study we examined the ability of GSK-3 inhibitors, SB216763 and 1-azakenpaullone, to reverse the behavioral aberrations induced by ketamine. Male NMRI mice received intracerebroventricular (i.c.v.) injection of the GSK-3 inhibitors, SB216763 and 1-azakenpaullone, 5 min prior to ketamine administration. Locomotor activity, rotarod performance, prepulse inhibition, novel object recognition, and duration of loss of righting reflex were monitored. GSK-3 inhibitors attenuated ketamine-induced locomotor hyperactivity, motor incoordination, sensorimotor impairment, and cognitive deficits, but did not affect ketamine anesthesia. These data support an important role of GSK-3 in the expression of behavioral aberrations associated with NMDA receptor hypofunction, and suggest that GSK-3 inhibitors may ameliorate certain behavioral and cognitive dysfunctions in patients with schizophrenia.

1. Introduction

Hypofunctional N-methyl-D-aspartate (NMDA) receptor signaling, particularly in the prefrontal cortex, has been implicated in the cognitive and behavioral disturbances seen in schizophrenia (Javitt, 2007). Exposure to non-competitive NMDA receptor antagonists, such as PCP and ketamine, induces a broad range of antipsychotic-sensitive and schizophrenia-like behavioral abnormalities in healthy individuals (Krystal et al., 1994; Adler et al., 1998; Newcomer et al., 1999) and exacerbates the same symptoms in schizophrenic patients (Breier et al., 1997; Lahti et al., 2001). These schizophrenia-like behaviors have been attributed to the blockade of synaptic NMDA receptors by these drugs. However, the molecular and cellular events precipitated by blockade of synaptic NMDA receptors that drive these behavioral disturbances are still unclear.

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase with diverse physiological functions in mediating intracellular signaling and regulating neuronal plasticity, gene expression, and cell survival (Grimes and Jope, 2001). GSK-3 encompasses two isoforms, GSK-3α and GSK-3β. Recent studies have implicated GSK-3β signaling in schizophrenia (Kozlovsky et al., 2000; Emamian et al., 2004; Lovestone et al., 2007; Freyberg et al., 2010). Moreover, GSK-3β is a target for several antipsychotics that decrease the activity of GSK-3β by causing the phosphorylation of GSK-3β at Ser9 (Kozlovsky et al., 2006). In fact, the NMDA receptor antagonists PCP and MK-801 have been found to increase GSK-3β activity both in vitro (Elyaman et al., 2002) and in vivo (Lei et al., 2008). Reduction of GSK-3β activity by selective inhibitors or depletion of GSK-3β by siRNA attenuates the neurotoxicity produced by ketamine or PCP (Takadera et al., 2006; Shang et al., 2007; Lei et al., 2008). However, there is no evidence that the behavioral aberrations associated with NMDA receptor hypofunction are caused by GSK-3β activation. In the present study, we examined whether two relatively potent and selective GSK-3 inhibitors, SB 216763 and 1-azakenpaullone, which inhibit GSK3 β with IC_{50} values of 34 nM and 18 nM, respectively (Coghlan et al., 2000; Kunick et al., 2004), can reverse ketamine-induced behavioral aberrations in mice. These experiments support the notion that GSK-3β activation contributes to the psychotomimetic effects of NMDA antagonists, and that inhibition of GSK-3β is a logical therapeutic goal in the management of schizophrenia.

2. Materials and methods

2.1. Animals and drugs

Male NMRI and ICR mice (8–9 weeks, 33–40 g) were supplied by the Laboratory Animal Center of Tzu Chi University (Hualien, Taiwan)

* Corresponding author at: Institute of Pharmacology and Toxicology, Tzu Chi University, 701, Section 3, Chung-Yang Road, Hualien, 970, Taiwan.
[1] The first two authors contributed equally to this study.

0920-9964/$ – see front matter
doi:10.1016/j.schres.2012.01.024

and BioLASCO Taiwan Co. (Taiwan), respectively, and housed 4 to 5 per cage in a 12-hour light/dark cycle (7:00 AM, lights on; 7:00 PM, lights off) with ad libitum access to water and food. The experimental protocol was approved by Tzu Chi University Review Committee for the Use of Animals. ICR mice were used in prepulse inhibition test since basal levels of prepulse inhibition in NMRI mice are very low. NMRI mice were used in all other behavioral tests. Each animal was used in one experiment.

Ketamine (Sigma, USA) was dissolved in saline. The doses of ketamine used for behavioral tests and anesthesia were based on our previous studies (Sou et al., 2006; Chan et al., 2008) and preliminary observations. SB 216763 and 1-azakenpaullone (Tocris, UK) were dissolved in dimethyl sulfoxide (DMSO) and diluted in artificial cerebrospinal fluid (ACSF) containing (in mM) NaCl(120), KCl(5), $CaCl_2$(1.5), $MgCl_2$(0.8), Na_2HPO_4 (1.4), and NaH_2PO_4 (0.25) at pH 7.4. Control mice received 5 μl 1% DMSO in ACSF. SB 216763 (10–500 pmol/5 μl) and 1-azakenpaullone (10–500 pmol/5 μl) were administered by intracerebroventricular (i.c.v.) injections 5 min prior to ketamine or saline administration (i.p.).

Intracerebroventricular injections were performed following a procedure established previously (Sou et al., 2006). Briefly, each conscious mouse was grasped firmly by the loose skin behind the head, and its snout was gently pushed into the mouth of a barrel of 5 ml syringe that had been horizontally fixed on the edge of a table. The animal was injected at the bregma with a 10-μl Hamilton syringe fitted with a 26-gage needle that was inserted to a depth of 2.4 mm. Bregma was located about 1–3 mm rostral to a line drawn between the anterior base of the ears by feeling the suture line by lightly rubbing with the point of needle. The volume of i.c.v. injections was 5 μl. Insertion of the needle and injection of vehicle had minimal effects on the mice. Immediately after removal of the needle, the animals remained quiet for approximately 30 s before resuming normal activity. All experiments were carried out during the light phase (0900–1600 h). At the end of the experiment, mice were sacrificed by chloralhydrate overdose (500 mg/kg, i.p.), and the precise location of injection was ascertained by injection of 1% methylene blue solution followed by visual inspection of the lateral brain ventricle.

2.2. Locomotor activity

The animals were moved from their home cages, weighed, and placed into an activity cage (Columbus Auto-Track System, Version 3.0 A, Columbus Institute, OH) in a dark environment for a 2-hour habituation. The observation period started immediately after ketamine (20 mg/kg) or saline injection. The distance (cm) traveled was recorded for 30 min. A 75% alcohol solution was used to clean the inner surface of the apparatus between trials to remove potentially interfering odors left by the previous mouse.

2.3. Prepulse inhibition test

SR-LAB (San Diego Instruments, San Diego, CA) acoustic startle chambers were used. SR-LAB software controlled the delivery of all stimuli to the animals and recorded the response. The animals were randomly assigned to groups and initially moved from their home cage, weighed, and placed into the startle chamber restrainers for a 30-min habituation period. Following administration of ketamine (30 mg/kg), the experiment started with a 5-min adaptation period during which the animals were exposed to 67 dB background noise. This background noise was continued throughout the session. The startle session began with five initial startle stimuli (120 dB bursts of white noise, 40 ms duration). After the initial stimuli, mice received five different trial types: pulse alone trials (120 dB bursts of white noise, 40 ms duration); three prepulse and pulse trials in which 76, 81 or 86 dB white noise bursts (9, 14, and 19 dB above background, respectively) of 20 ms duration, preceded 120 dB pulse by 100 ms prepulse onset to pulse onset; and no-stimuli trials during which only background noise was applied. Each trial type was presented 5 times in randomized order. The intertrial interval was 7–23 s, and the entire test lasted 15 min. Prepulse inhibition was calculated as the percent inhibition of the startle amplitude evoked by the pulse alone: % PPI = ((magnitude on pulse alone trial − magnitude on prepulse and pulse trial) / magnitude on pulse alone trial) × 100.

2.4. Rotarod motor coordination test

Motor coordination was assessed using an automated rotarod apparatus (TSE systems, Bad Homburg, Germany). A computer recorded the latency to fall in seconds. The mice were trained on the rotarod at a constant speed of 20 rpm until all the mice were able to stay on the rod at least for 3 min. Then, the mice were tested 10, 15, 20, and 25 min after administration of ketamine (20 mg/kg) or saline.

2.5. Novel object recognition test

The novel object recognition test was carried out as described previously (Beaulieu et al., 2009). The experimental apparatus consisted of a Plexiglas open-field box (50 × 50 × 25 cm high) located in a sound-attenuated room and illuminated with a 20-W bulb.

The novel object recognition test procedure consisted of three sessions: habituation, training, and retention. The animals were videotaped in both training and retention sessions. Each mouse was individually habituated to the box with 10 min of exploration in the absence of objects for three consecutive days (habituation session, days 1–3). During the training session (day 4), each animal was placed in the test box for a 5-min habituation period before two objects were introduced into two corners. Each animal was allowed to explore in the box for 5 min. An animal was considered to be exploring the object when its head was facing the object at a distance of 1 cm or less or was touching or sniffing the object. The time spent exploring each object was recorded by an experimenter blinded to the identity of the treatments. During the retention sessions (day 5), the animals were placed into the same box 24 h after the training session, but one of the familiar objects introduced during the training session was replaced with a novel object. The animals were allowed to explore freely for 5 min, and the time spent exploring each object was recorded as described above. A preference index in the retention session, i.e., the ratio of the amount of time spent exploring the novel object to the total time spent exploring both objects, was used as a measure of cognitive function. In the training session, the preference index was calculated as a ratio of the time spent exploring the object that was replaced by the novel object in the retention session to the total exploring time. Ketamine (30 mg/kg) or saline was administered 20 min prior to initiating the training session.

2.6. Loss of righting reflex test (LORR)

Loss of righting reflex was used as an indication of anesthesia. After an intraperitoneal injection of ketamine (120 mg/kg), mice were placed in a clean cage until the righting reflex was lost. They were then placed in the supine position until recovery. Recovery of the righting reflex was defined as the ability to perform three successive rightings. The duration of the LORR was recorded.

2.7. Statistical analyses

Results are presented as mean ± SEM. The data of righting reflex were analyzed by one-way ANOVA. Two-way ANOVA was used to analyze data from locomotor activity, startle amplitude, and novel object recognition tests. The data from rotarod test and PPI were analyzed by mixed-design ANOVA with testing time or prepulse

intensity as the within-subjects factor. Multiple comparisons were performed using the Student–Newman–Keuls test. $P<0.05$ was considered statistically significant.

3. Results

3.1. Locomotor activity

Pretreatment with SB216763 and 1-azakenpaullone attenuated ketamine-induced locomotor hyperactivity (Fig. 1). In the experiment for testing the effect of SB216763 on ketamine-induced locomotor hyperactivity, ANOVA revealed a main effect of ketamine ($F_{1,42}=8.792$, $P<0.01$) and SB216763 ($F_{2,42}=5.503$, $P<0.01$). In addition, there was a significant interaction between ketamine and SB216763 ($F_{2,42}=7.273$, $P<0.01$).

In the experimental set of 1-azakenpaullone, analysis of the results revealed a main effect of ketamine ($F_{1,42}=19.589$, $P<0.001$) and 1-azakenpaullone ($F_{2,42}=12.713$, $P<0.001$), and a significant interaction between the two ($F_{2,42}=15.127$, $P<0.001$).

3.2. PPI test

Pretreatment with SB216763 and 1-azakenpaullone attenuated ketamine-induced disruption of PPI (Fig. 2). Two-way ANOVA revealed that ketamine, SB216763, and 1-azakenpaullone did not affect startle amplitude to pulse alone (Fig. 2B, D). As for PPI, ANOVA with prepulse intensity as repeated measures revealed a significant between-subject effect of ketamine ($F_{1,42}=5.627$, $P<0.05$), a significant within-subject effect of prepulse intensity ($F_{2,84}=89.84$, $P<0.001$), and a significant SB216763 × ketamine interaction ($F_{2,42}=5.557$, $P<0.01$). Post hoc tests revealed that pretreatment of SB216763 significantly attenuated the ketamine-induced disruption of PPI (Fig. 2A). In the experimental set of 1-azakenpaullone, the ANOVA revealed a significant between-subject effect of ketamine ($F_{1,42}=14.82$, $P<0.05$), a significant within-subject effect of prepulse intensity ($F_{2,84}=54.572$, $P<0.001$), and a significant 1-azakenpaullone × ketamine interaction ($F_{2,42}=4.429$, $P<0.05$). Multiple comparisons revealed that pretreatment of 1-azakenpaullone significantly attenuated the ketamine-induced disruption of PPI (Fig. 2C).

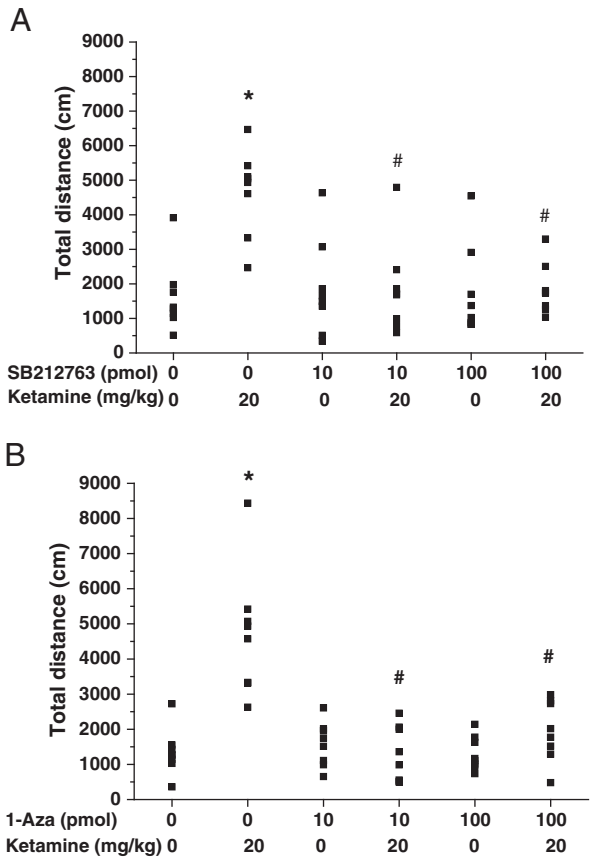

Fig. 1. Effects of SB216763 and 1-azakenpaullone on ketamine-induced locomotor hyperactivity. Mice were pretreated with vehicle, SB216763 (10 or 100 pmol, i.c.v.) (A) or 1-azakenpaullone (10 or 100 pmol, i.c.v.) (B), and the 30-min distance traveled was recorded after administration of ketamine (20 mg/kg, i.p.). All values are expressed as the mean ± SEM ($n=8$). Data were analyzed by two-way ANOVA. *$P<0.05$, compared with the vehicle/saline, #$P<0.05$ vs. vehicle/ketamine (post hoc Student–Newman–Keuls test).

3.3. Rotarod test

Pretreatment with SB216763 and 1-azakenpaullone improved ketamine-induced motor incoordination in rotarod test (Fig. 3). In the experiment for assessing the effect of SB216763 and ketamine on rotarod performance, the ANOVA revealed significant between-subject effects of ketamine ($F_{1,42}=132.07$, $P<0.001$) and SB216763 ($F_{2,42}=6.11$, $P<0.01$), within-subject effect of testing time ($F_{3,126}=120.15$, $P<0.001$) and significant interaction effects of ketamine × SB216763 ($F_{2,42}=6.11$, $P<0.01$), ketamine × time ($F_{6,126}=120.15$, $P<0.001$), SB216763 × time ($F_{6,126}=5.47$, $P<0.01$), and time × SB216763 × ketamine ($F_{6,126}=5.47$, $P<0.01$). Post hoc tests indicated that SB216763 (100 pmol) at high dose significantly reduced the ketamine-induced motor incoordination (Fig. 3A). As for 1-azakenpaullone experimental set, the ANOVA revealed significant between-subject effects of ketamine ($F_{1,42}=184.03$, $P<0.001$), 1-azakenpaullone ($F_{2,42}=8.104$, $P<0.001$), within-subject effect of testing time ($F_{3,126}=129.48$, $P<0.001$), and significant interaction effects of ketamine × 1-azakenpaullone ($F_{2,42}=8.104$, $P<0.001$), ketamine × time ($F_{6,126}=120.15$, $P<0.001$), 1-azakenpaullone × time ($F_{6,126}=7.29$, $P<0.001$), and time × 1-azakenpaullone × ketamine ($F_{6,126}=7.29$, $P<0.001$). Post hoc tests indicated that 1-azakenpaullone (100 pmol) significantly attenuated the ketamine-induced motor incoordination (Fig. 3B).

3.4. Novel object recognition test

Preference for novelty was defined as spending greater than 50% of the time with the novel object during the novel object recognition test. There was no significant difference in the exploratory preferences for the treatment groups in the training session (Fig. 4A, C). In the retention session, ANOVA showed that ketamine and SB216763 had significant effects and interaction on exploratory preferences (ketamine: $F_{1,36}=28.82$, $P<0.001$; SB216763 $F_{2,36}=18.201$, $P<0.001$; interaction: $F_{2,36}=31.268$, $P<0.001$). Post hoc tests indicated that SB216763 (10 and 100 pmol) improved the ketamine-induced cognitive deficits (Fig. 4A). In the experimental set of 1-azakenpaullone, ketamine and 1-azakenpaullone had significant effects and interaction on exploratory preferences (ketamine: $F_{1,36}=9.194$, $P<0.01$; 1-azakenpaullone $F_{2,36}=6.024$, $P<0.01$; interaction: $F_{2,36}=12.858$, $P<0.001$). Post hoc tests indicated that pretreatment of 1-azakenpaullone (10 and 100 pmol) significantly attenuated the ketamine-induced cognitive deficits (Fig. 4C). There was no significant difference for total exploration between groups in these two set of experiments (Fig. 4B and D).

3.5. Loss of righting reflex

Ketamine (120 mg/kg, i.p.) caused a loss of righting reflex for 20–25 min. As shown in Fig. 5, pretreatment with SB216763 (100 and 500 pmol) or 1-azakenpaullone (100 and 500 pmol) did not affect the duration of ketamine-induced anesthesia.

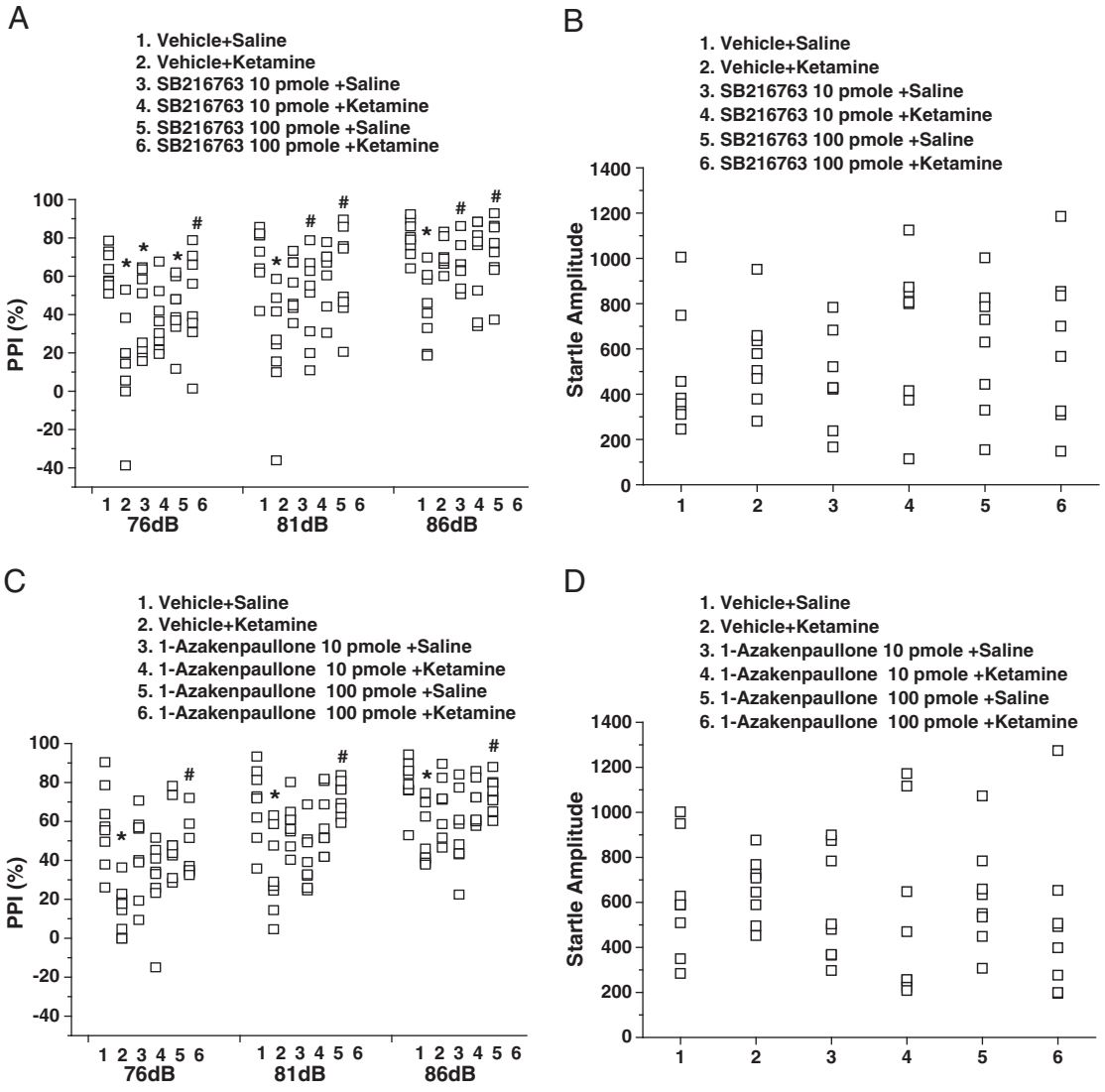

Fig. 2. Effects of SB216763 and 1-azakenpaullone on ketamine-induced PPI deficits. Mice were pretreated with vehicle, SB216763 (10 or 100 pmol, i.c.v.) (A, B) or 1-azakenpaullone (10 or 100 pmol, i.c.v.) (C, D). Startle amplitude and PPI were measured 15 min after ketamine (30 mg/kg, i.p.) administration. All values are expressed as the mean ± SEM (n = 8). Data were analyzed by two-way ANOVA (startle amplitude) and mixed design ANOVA (PPI). *P<0.05, compared with the vehicle/saline, #P<0.05 vs. vehicle/ketamine (post hoc Student–Newman–Keuls test).

4. Discussion

The present study demonstrates that two selective GSK-3 inhibitors, SB 216763 and 1-azakenpaullone, attenuate the psychotomimetic behavioral responses induced by subanesthetic doses of ketamine. However, ketamine anesthesia is not affected by GSK-3 inhibition. The primary mechanism of ketamine action is blockade of the NMDA receptor channel, although it also stimulates high affinity state of dopamine D2 receptors (Seeman and Kapur, 2003; Seeman et al., 2005). Moreover, ketamine anesthesia is thought to be mediated by inhibition of glutamate and nicotinic receptors and activation of GABAA receptors and potassium 'leak' channels, such as TREK-1 (Irifune et al., 2000; Solt and Forman, 2007). It has been reported that subanesthetic doses of ketamine increase glutamate and dopamine outflow in the prefrontal cortex (Liu and Moghaddam, 1995; Moghaddam et al., 1997; Smith et al., 1998), whereas anesthetic doses of ketamine decrease glutamate levels in the prefrontal cortex (Moghaddam et al., 1997). Consistent with these distinct neurochemical responses to subanesthetic and anesthetic doses of ketamine, our data show that the GSK-3 inhibitors suppress the subanesthetic ketamine-induced psychotomimetic behaviors, but not ketamine-induced anesthesia. A recent study reported that inhibition of GSK-3 is necessary for the rapid antidepressant effect of low level of ketamine in the mouse model of learned helplessness (Beurel et al., 2011). Thus, GSK-3 appears to play distinct modulatory roles in the signaling pathways underlying the antidepressant and psychotomimetic effects elicited by different doses of ketamine.

Subanesthetic doses of ketamine induce schizophrenia-like symptoms in healthy humans (Krystal et al., 1994; Adler et al., 1998). The cellular mechanisms of NMDA receptor hypofunction and subsequent neurochemical changes that trigger the psychotomimetic behaviors are unclear. It has been shown that injection of NMDA receptor antagonists such as MK-801 and PCP transiently increases GSK-3β activity and increases the active (phospho-tyrosine-216) form of GSK-3β and decreases the inactive (phospho-serine-9) forms of GSK-3β in the rat forebrain (Elyaman et al., 2002; Lei et al., 2008). However, it has also been reported that ketamine and PCP, at low doses, increase phospho-Ser9-GSK-3ß in the frontal cortex, striatum, and hippocampus (Svenningsson et al., 2003; Beurel et al., 2011). Therefore, it is important to clarify the dose-dependent effects of ketamine on GSK-3 activity.

GSK-3 inhibitors significantly attenuate ketamine-induced psychotomimetic behaviors, suggesting inhibition of GSK-3 as a strategy

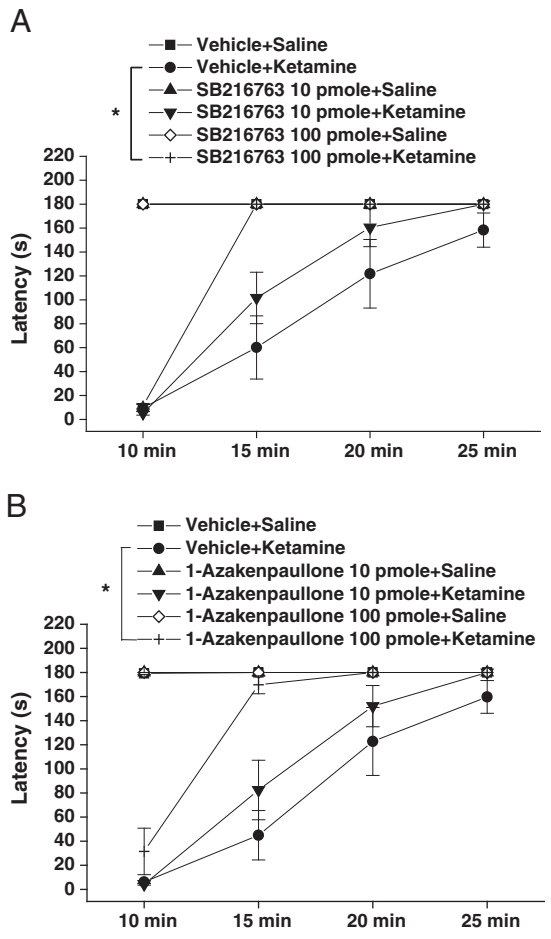

Fig. 3. Effects of SB216763 and 1-azakenpaullone on ketamine-induced motor incoordination in the rotarod test. Mice were pretreated with vehicle, SB216763 (10 or 100 pmol, i.c.v.) (A) or 1-azakenpaullone (10 or 100 pmol, i.c.v.) (B) and the latency to fall was recorded 10, 15, 20, and 25 min after administration of ketamine (20 mg/kg, i.p.). All values are expressed as the mean ± SEM (n = 8). Data were analyzed by mixed design ANOVA. *P<0.01, compared with the vehicle/saline, #P<0.01 vs. vehicle/ketamine (post hoc Student–Newman–Keuls test).

to treat psychosis associated with NMDA receptor hypofunction. NMDA receptor signaling is a site of action for GSK-3β with respect to reducing ketamine-induced psychotomimetic behaviors. Inhibition of GSK-3β causes a long-lasting reduction of NMDA receptor-mediated synaptic currents in cortical pyramidal neurons by increasing Rab5-mediated and PSD-95-regulated NMDA receptor internalization via a clathrin/dynamin-dependent process (Chen et al., 2007). In contrast, several other studies failed to demonstrate that GSK-3 inhibitors produce clear effects on the basal NMDA receptor-mediated transmissions (Peineau et al., 2007; Zhu et al., 2007; Li et al., 2009). Alternatively, modulation of ketamine-induced psychotomimetic behaviors by GSK-3 inhibitors might be exerted at the neural circuit level. It has been speculated that the psychotomimetic features associated with NMDA receptor hypofunction are related to the disinhibitory effects caused by reduced activity of GABAergic interneurons followed by an increase in extracellular glutamate and serotonin in the prefrontal cortex (Bubenikova-Valesova et al., 2008). Increased extracellular glutamate or serotonin in the prefrontal cortex could contribute to the psychotropic effects of ketamine and PCP, since AMPA receptor antagonists, mGluR2/3 agonists, or $5HT_{2A}$ antagonists abolish various behavioral effects of NMDA antagonists. GSK-3 inhibitors cause a significant reduction of the amplitude of miniature excitatory postsynaptic current (mEPSC), a measure of the unitary strength of synaptic AMPARs that is associated with decreased surface and synaptic GluR1 clusters on dendrites and increased internalization of GluR1 (Wei et al., 2010). Treatment with the mGluR2/3 agonist LY379268 at a dose that attenuates PCP-induced locomotor hyperactivity increases phosphorylated GSK-3, thereby reducing GSK-3 activity in the prefrontal cortex (Sutton and Rushlow, 2011). Similarly, blockade of 5-HT_{2A} receptors reduces the GSK-3 activity (Li et al., 2004).

Atypical antipsychotics, including clozapine, olanzapine or ziprasidone, reduce locomotor hyperactivity (Gleason and Shannon, 1997), cognitive deficits (Abdul-Monim et al., 2003; Idris et al., 2005; Dunn and Killcross, 2007) and disruption of PPI (Brody et al., 2004) induced by NMDA antagonists. The metabotropic glutamate receptor 5 agonist (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) and positive allosteric modulator 3,3′-difluorobenzaldazine (DFB) significantly attenuate ketamine-induced behavioral aberrations (Beaulieu et al., 2009). In fact, antipsychotics (Roh et al., 2007) and activation of the metabotropic glutamate receptor 5 (Liu et al., 2005) have been reported to increase the inactive form of phospho-Ser9-GSK3β. Taken together, AMPA receptor antagonists, mGluR2/3 agonists, 5-HT_{2A} antagonists, atypical antipsychotics, and mGluR5 positive modulating agents that ameliorate the behavioral abnormalities induced by NMDA receptor antagonists all reduce GSK-3 activity. Moreover, GSK-3 inhibitors produce attenuation effects similar to these agents. Accordingly, inhibition of GSK-3 might be a common pathway for these agents in inhibiting psychotomimetic behaviors associated with NMDA hypofunction.

Lithium and valproate, two drugs widely used for treatment of mood disorders, have been shown to directly and/or indirectly reduce GSK-3 activity (Stambolic et al., 1996; Chen et al., 1999). The Ki for lithium inhibition of GSK-3β activity is 2 mM (Klein and Melton, 1996). On the other hand, valproate does not directly inhibit GSK-3β in biochemical assay (Jonathan Ryves et al., 2005). However, neither of these two compounds prevents the disruption of PPI induced by ketamine (Ong et al., 2005) or MK-801 (Umeda et al., 2006), and lithium even exacerbates the MK-801-induced disruption of PPI (Umeda et al., 2006). In fact, lithium inhibits NMDA receptor-mediated signaling by decreasing NR2A and NR2B tyrosine phosphorylation (Hashimoto et al., 2003; Ma and Zhang, 2003), which is consistent with the findings that NMDA receptor antagonists augment the antidepressant (Ghasemi et al., 2010a) and anticonvulsant (Ghasemi et al., 2010b) effects of lithium. It is possible that NMDA receptors are more sensitive to the inhibitory effects of lithium than GSK-3. This might explain why lithium exacerbates rather than attenuates disruption of PPI induced by NMDA receptor antagonists.

Our data show that ketamine-induced locomotor hyperactivity, motor incoordination, sensorimotor gating deficit, and cognitive impairment are alleviated by pretreatment with the specific GSK-3 inhibitors, SB 216763 and 1-azakenpaullone. The present study suggests that inhibition of GSK-3β may be a molecular mechanism by which antipsychotic drugs reverse the deficits associated with dysfunctional glutamatergic transmission. This is consistent with findings with atypical antipsychotic drugs that have GSK-3β inhibitory properties. Administration of subanesthetic ketamine shows phenomenological validity for modeling psychotic behaviors. Accordingly, our findings suggest that GSK-3 inhibitors may be of potential value in treating behavioral disorganization and cognitive impairment in patients with schizophrenia.

SB 216763 and 1-azakenpaullone alone did not interfere with basal levels of locomotor activity, motor coordination, and recognition memory. However, PPI was significantly impaired by SB 216763 and slightly by 1-azakenpaullone. This is consistent with previous report that indicate that SB216763 and another inhibitor, linopirdine, reduce PPI when directly infused into the medial prefrontal cortex (mPFC) (Kapfhamer et al., 2010). Even though GSK-3 inhibitors reduce ketamine-induced impairment in PPI, their negative impact on PPI would be a concern in clinical application. In addition, SB216763 and 1-azakenpaullone simultaneously inhibit GSK-3 α

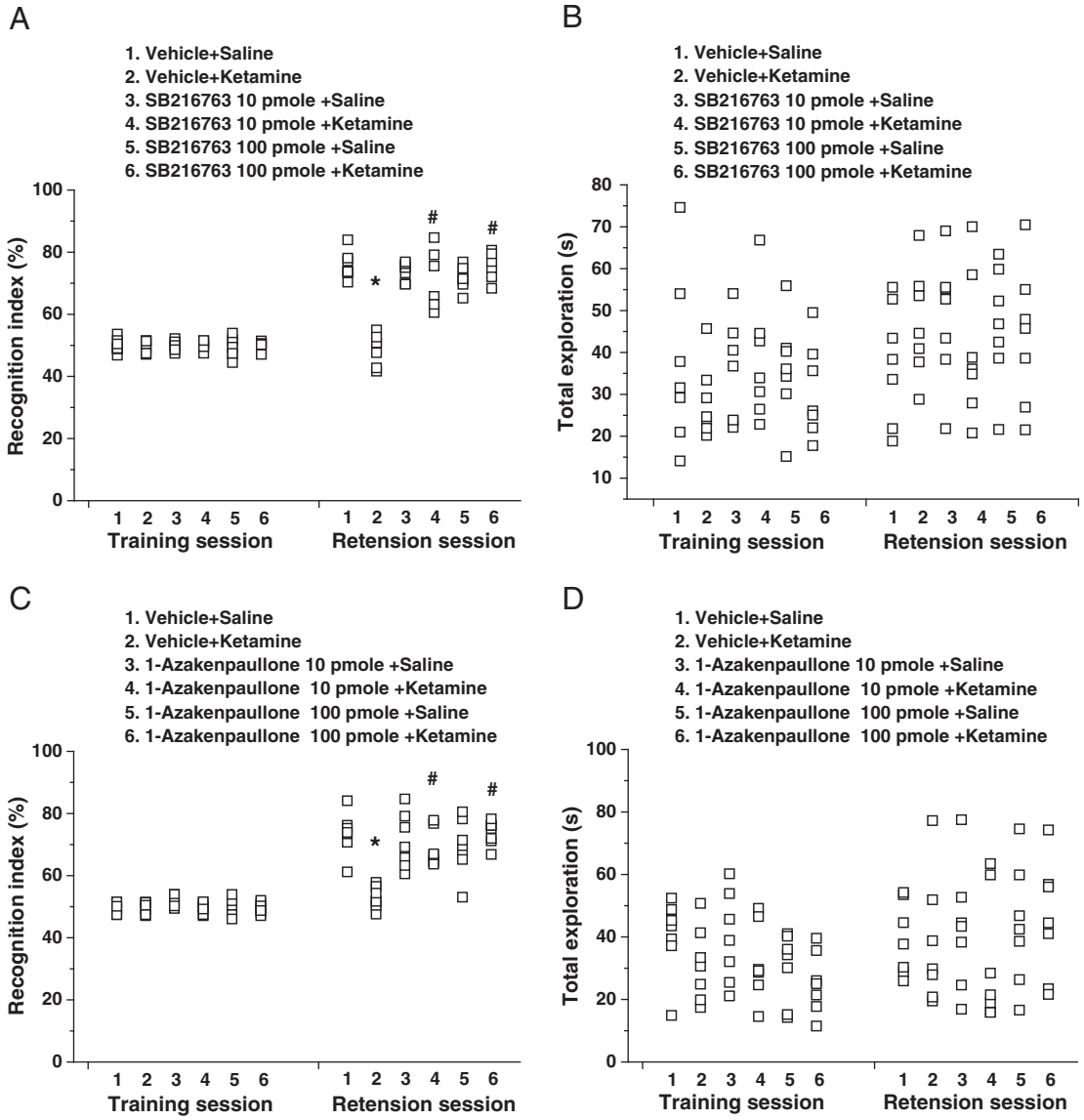

Fig. 4. Effects of SB216763 and 1-azakenpaullone on ketamine-induced cognitive deficit in the novel object recognition test. Mice were pretreated with vehicle, SB216763 (10 or 100 pmol, i.c.v.) (A, B) or 1-azakenpaullone (10 or 100 pmol, i.c.v.) (C, D) 5 min prior to administration of ketamine (30 mg/kg, i.p.) or saline. The training session and retention session were performed 20 min and 24 h later, respectively. The amount of time spent exploring the novel object and total exploring time were measured. The preference index was calculated as described in Materials and methods. All values are expressed as the mean ± SEM (n = 7). Data were analyzed by two-way ANOVA. *P<0.01, compared with the vehicle/saline, #P<0.01, vs. vehicle/ketamine (post hoc Student–Newman–Keuls test).

and β and interfere with other kinases, albeit with lower potency. Development of specific inhibitors for GSK-3β or selective blockade of GSK-3β expression by an antisense oilgonucleotide or siRNA may clarify the specific role of GSK-3β in the ketamine-induced psychomimetic activity.

Role of funding source

This work was supported by a grant from National Scientific Council (NSC), Taiwan (NSC 96-2628-B-320-001-MY3). The NSC had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Ming-Huan Chan designed the study and participated for writing the first draft of the manuscript. Pao-Hsiang Chiu managed the literature searches and undertook the experiments and statistical analysis. Chia-Yu Lin undertook the experiments and statistical analysis. Hwei-Hsien Chen designed the study, wrote the protocol, and participated for writing the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

None to declare.

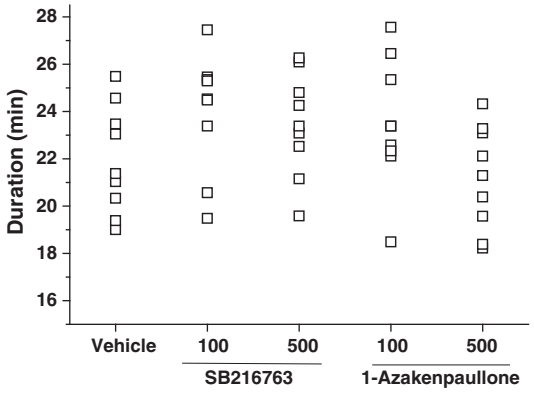

Fig. 5. Effects of SB216763 and 1-azakenpaullone on ketamine-induced loss of righting reflex. Mice were pretreated with vehicle, SB216763 (100 and 500 pmol, i.c.v.) or 1-azakenpaullone (100 and 500 pmol, i.c.v.) 5 min prior to administration of ketamine (100 mg/kg, i.p.). The latency to loss of righting reflex was recorded. All values are expressed as the mean ± SEM (n = 9). Data were analyzed by one-way ANOVA.

Acknowledgments

We thank Mr. Jen-Hou Sou, who kindly provided technical assistance and Dr. Robert S. Aronstam, Ph.D. for editing this paper. This work was supported by a grant from the National Scientific Council, Taiwan (NSC 96-2320-B-320-006-MY3).

References

Abdul-Monim, Z., Reynolds, G.P., Neill, J.C., 2003. The atypical antipsychotic ziprasidone, but not haloperidol, improves phencyclidine-induced cognitive deficits in a reversal learning task in the rat. J. Psychopharmacol. 17 (1), 57–65.

Adler, C.M., Goldberg, T.E., Malhotra, A.K., Pickar, D., Breier, A., 1998. Effects of ketamine on thought disorder, working memory, and semantic memory in healthy volunteers. Biol. Psychiatry 43 (11), 811–816.

Beaulieu, J.M., Gainetdinov, R.R., Caron, M.G., 2009. Akt/GSK3 signaling in the action of psychotropic drugs. Annu. Rev. Pharmacol. Toxicol. 49, 327–347.

Beurel, E., Song, L., Jope, R.S., 2011. Inhibition of glycogen synthase kinase-3 is necessary for the rapid antidepressant effect of ketamine in mice. Mol. Psychiatry 16 (11), 1068–1070.

Breier, A., Malhotra, A.K., Pinals, D.A., Weisenfeld, N.I., Pickar, D., 1997. Association of ketamine-induced psychosis with focal activation of the prefrontal cortex in healthy volunteers. Am. J. Psychiatry 154 (6), 805–811.

Brody, S.A., Conquet, F., Geyer, M.A., 2004. Effect of antipsychotic treatment on the prepulse inhibition deficit of mGluR5 knockout mice. Psychopharmacology (Berl.) 172 (2), 187–195.

Bubenikova-Valesova, V., Horacek, J., Vrajova, M., Hoschl, C., 2008. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. Neurosci. Biobehav. Rev. 32 (5), 1014–1023.

Chan, M.H., Chiu, P.H., Sou, J.H., Chen, H.H., 2008. Attenuation of ketamine-evoked behavioral responses by mGluR5 positive modulators in mice. Psychopharmacology (Berl.) 198 (1), 141–148.

Chen, G., Huang, L.D., Jiang, Y.M., Manji, H.K., 1999. The mood-stabilizing agent valproate inhibits the activity of glycogen synthase kinase-3. J. Neurochem. 72 (3), 1327–1330.

Chen, P., Gu, Z., Liu, W., Yan, Z., 2007. Glycogen synthase kinase 3 regulates N-methyl-D-aspartate receptor channel trafficking and function in cortical neurons. Mol. Pharmacol. 72 (1), 40–51.

Coghlan, M.P., Culbert, A.A., Cross, D.A., Corcoran, S.L., Yates, J.W., Pearce, N.J., Rausch, O.L., Murphy, G.J., Carter, P.S., Roxbee Cox, L., Mills, D., Brown, M.J., Haigh, D., Ward, R.W., Smith, D.G., Murray, K.J., Reith, A.D., Holder, J.C., 2000. Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription. Chem. Biol. 7 (10), 793–803.

Dunn, M.J., Killcross, S., 2007. Clozapine, SCH 23390 and alpha-flupenthixol but not haloperidol attenuate acute phencyclidine-induced disruption of conditional discrimination performance. Psychopharmacology (Berl.) 190 (4), 403–414.

Elyaman, W., Terro, F., Wong, N.S., Hugon, J., 2002. In vivo activation and nuclear translocation of phosphorylated glycogen synthase kinase-3beta in neuronal apoptosis: links to tau phosphorylation. Eur. J. Neurosci. 15 (4), 651–660.

Emamian, E.S., Hall, D., Birnbaum, M.J., Karayiorgou, M., Gogos, J.A., 2004. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. Nat. Genet. 36 (2), 131–137.

Freyberg, Z., Ferrando, S.J., Javitch, J.A., 2010. Roles of the Akt/GSK-3 and Wnt signaling pathways in schizophrenia and antipsychotic drug action. Am. J. Psychiatry 167 (4), 388–396.

Ghasemi, M., Raza, M., Dehpour, A.R., 2010a. NMDA receptor antagonists augment antidepressant-like effects of lithium in the mouse forced swimming test. J. Psychopharmacol. 24 (4), 585–594.

Ghasemi, M., Shafaroodi, H., Nazarbeiki, S., Meskar, H., Heydarpour, P., Ghasemi, A., Talab, S.S., Ziai, P., Bahremand, A., Dehpour, A.R., 2010b. Voltage-dependent calcium channel and NMDA receptor antagonists augment anticonvulsant effects of lithium chloride on pentylenetetrazole-induced clonic seizures in mice. Epilepsy Behav. 18 (3), 171–178.

Gleason, S.D., Shannon, H.E., 1997. Blockade of phencyclidine-induced hyperlocomotion by olanzapine, clozapine and serotonin receptor subtype selective antagonists in mice. Psychopharmacology (Berl.) 129 (1), 79–84.

Grimes, C.A., Jope, R.S., 2001. The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. Prog. Neurobiol. 65 (4), 391–426.

Hashimoto, R., Fujimaki, K., Jeong, M.R., Christ, L., Chuang, D.M., 2003. Lithium-induced inhibition of Src tyrosine kinase in rat cerebral cortical neurons: a role in neuroprotection against N-methyl-D-aspartate receptor-mediated excitotoxicity. FEBS Lett. 538 (1–3), 145–148.

Idris, N.F., Repeto, P., Neill, J.C., Large, C.H., 2005. Investigation of the effects of lamotrigine and clozapine in improving reversal-learning impairments induced by acute phencyclidine and D-amphetamine in the rat. Psychopharmacology (Berl.) 179 (2), 336–348.

Irifune, M., Sato, T., Kamata, Y., Nishikawa, T., Dohi, T., Kawahara, M., 2000. Evidence for GABA(A) receptor agonistic properties of ketamine: convulsive and anesthetic behavioral models in mice. Anesth. Analg. 91 (1), 230–236.

Javitt, D.C., 2007. Glutamate and schizophrenia: phencyclidine, N-methyl-D-aspartate receptors, and dopamine–glutamate interactions. Int. Rev. Neurobiol. 78, 69–108.

Jonathan Ryves, W., Dalton, E.C., Harwood, A.J., Williams, R.S., 2005. GSK-3 activity in neocortical cells is inhibited by lithium but not carbamazepine or valproic acid. Bipolar Disord. 7 (3), 260–265.

Kapfhamer, D., Berger, K.H., Hopf, F.W., Seif, T., Kharazia, V., Bonci, A., Heberlein, U., 2010. Protein phosphatase 2a and glycogen synthase kinase 3 signaling modulate prepulse inhibition of the acoustic startle response by altering cortical M-Type potassium channel activity. J. Neurosci. 30 (26), 8830–8840.

Klein, P.S., Melton, D.A., 1996. A molecular mechanism for the effect of lithium on development. Proc. Natl. Acad. Sci. U. S. A. 93 (16), 8455–8459.

Kozlovsky, N., Amar, S., Belmaker, R.H., Agam, G., 2006. Psychotropic drugs affect Ser9-phosphorylated GSK-3 beta protein levels in rodent frontal cortex. Int. J. Neuropsychopharmacol. 9 (3), 337–342.

Kozlovsky, N., Belmaker, R.H., Agam, G., 2000. Low GSK-3beta immunoreactivity in postmortem frontal cortex of schizophrenic patients. Am. J. Psychiatry 157 (5), 831–833.

Krystal, J.H., Karper, L.P., Seibyl, J.P., Freeman, G.K., Delaney, R., Bremner, J.D., Heninger, G.R., Bowers Jr., M.B., Charney, D.S., 1994. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch. Gen. Psychiatry 51 (3), 199–214.

Kunick, C., Lauenroth, K., Leost, M., Meijer, L., Lemcke, T., 2004. 1-Azakenpaullone is a selective inhibitor of glycogen synthase kinase-3 beta. Bioorg. Med. Chem. Lett. 14 (2), 413–416.

Lahti, A.C., Weiler, M.A., Tamara Michaelidis, B.A., Parwani, A., Tamminga, C.A., 2001. Effects of ketamine in normal and schizophrenic volunteers. Neuropsychopharmacology 25 (4), 455–467.

Lei, G., Xia, Y., Johnson, K.M., 2008. The role of Akt-GSK-3beta signaling and synaptic strength in phencyclidine-induced neurodegeneration. Neuropsychopharmacology 33 (6), 1343–1353.

Li, X., Zhu, W., Roh, M.S., Friedman, A.B., Rosborough, K., Jope, R.S., 2004. In vivo regulation of glycogen synthase kinase-3beta (GSK3beta) by serotonergic activity in mouse brain. Neuropsychopharmacology 29 (8), 1426–1431.

Li, Y.C., Xi, D., Roman, J., Huang, Y.Q., Gao, W.J., 2009. Activation of glycogen synthase kinase-3 beta is required for hyperdopamine and D2 receptor-mediated inhibition of synaptic NMDA receptor function in the rat prefrontal cortex. J. Neurosci. 29 (49), 15551–15563.

Liu, F., Gong, X., Zhang, G., Marquis, K., Reinhart, P., Andree, T.H., 2005. The inhibition of glycogen synthase kinase 3beta by a metabotropic glutamate receptor 5 mediated pathway confers neuroprotection to Abeta peptides. J. Neurochem. 95 (5), 1363–1372.

Liu, J., Moghaddam, B., 1995. Regulation of glutamate efflux by excitatory amino acid receptors: evidence for tonic inhibitory and phasic excitatory regulation. J. Pharmacol. Exp. Ther. 274 (3), 1209–1215.

Lovestone, S., Killick, R., Di Forti, M., Murray, R., 2007. Schizophrenia as a GSK-3 dysregulation disorder. Trends Neurosci. 30 (4), 142–149.

Ma, J., Zhang, G.Y., 2003. Lithium reduced N-methyl-D-aspartate receptor subunit 2A tyrosine phosphorylation and its interactions with Src and Fyn mediated by PSD-95 in rat hippocampus following cerebral ischemia. Neurosci. Lett. 348 (3), 185–189.

Moghaddam, B., Adams, B., Verma, A., Daly, D., 1997. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J. Neurosci. 17 (8), 2921–2927.

Newcomer, J.W., Farber, N.B., Jevtovic-Todorovic, V., Selke, G., Melson, A.K., Hershey, T., Craft, S., Olney, J.W., 1999. Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. Neuropsychopharmacology 20 (2), 106–118.

Ong, J.C., Brody, S.A., Large, C.H., Geyer, M.A., 2005. An investigation of the efficacy of mood stabilizers in rodent models of prepulse inhibition. J. Pharmacol. Exp. Ther. 315 (3), 1163–1171.

Peineau, S., Taghibiglou, C., Bradley, C., Wong, T.P., Liu, L., Lu, J., Lo, E., Wu, D., Saule, E., Bouschet, T., Matthews, P., Isaac, J.T., Bortolotto, Z.A., Wang, Y.T., Collingridge, G.L., 2007. LTP inhibits LTD in the hippocampus via regulation of GSK3beta. Neuron 53 (5), 703–717.

Roh, M.S., Seo, M.S., Kim, Y., Kim, S.H., Jeon, W.J., Ahn, Y.M., Kang, U.G., Juhnn, Y.S., Kim, Y.S., 2007. Haloperidol and clozapine differentially regulate signals upstream of glycogen synthase kinase 3 in the rat frontal cortex. Exp. Mol. Med. 39 (3), 353–360.

Seeman, P., Kapur, S., 2003. Anesthetics inhibit high-affinity states of dopamine D2 and other G-linked receptors. Synapse 50 (1), 35–40.

Seeman, P., Ko, F., Tallerico, T., 2005. Dopamine receptor contribution to the action of PCP, LSD and ketamine psychotomimetics. Mol. Psychiatry 10 (9), 877–883.

Shang, Y., Wu, Y., Yao, S., Wang, X., Feng, D., Yang, W., 2007. Protective effect of erythropoietin against ketamine-induced apoptosis in cultured rat cortical neurons: involvement of PI3K/Akt and GSK-3 beta pathway. Apoptosis 12 (12), 2187–2195.

Smith, G.S., Schloesser, R., Brodie, J.D., Dewey, S.L., Logan, J., Vitkun, S.A., Simkowitz, P., Hurley, A., Cooper, T., Volkow, N.D., Cancro, R., 1998. Glutamate modulation of dopamine measured in vivo with positron emission tomography (PET) and 11C-raclopride in normal human subjects. Neuropsychopharmacology 18 (1), 18–25.

Solt, K., Forman, S.A., 2007. Correlating the clinical actions and molecular mechanisms of general anesthetics. Curr. Opin. Anaesthesiol. 20 (4), 300–306.

Sou, J.H., Chan, M.H., Chen, H.H., 2006. Ketamine, but not propofol, anaesthesia is regulated by metabotropic glutamate 5 receptors. Br. J. Anaesth. 96 (5), 597–601.

Stambolic, V., Ruel, L., Woodgett, J.R., 1996. Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. Curr. Biol. 6 (12), 1664–1668.

Sutton, L.P., Rushlow, W.J., 2011. Regulation of Akt and Wnt signaling by the group II metabotropic glutamate receptor antagonist LY341495 and agonist LY379268. J. Neurochem. 117 (6), 973–983.

Svenningsson, P., Tzavara, E.T., Carruthers, R., Rachleff, I., Wattler, S., Nehls, M., McKinzie, D.L., Fienberg, A.A., Nomikos, G.G., Greengard, P., 2003. Diverse

psychotomimetics act through a common signaling pathway. Science 302 (5649), 1412–1415.
Takadera, T., Ishida, A., Ohyashiki, T., 2006. Ketamine-induced apoptosis in cultured rat cortical neurons. Toxicol. Appl. Pharmacol. 210 (1–2), 100–107.
Umeda, K., Suemaru, K., Todo, N., Egashira, N., Mishima, K., Iwasaki, K., Fujiwara, M., Araki, H., 2006. Effects of mood stabilizers on the disruption of prepulse inhibition induced by apomorphine or dizocilpine in mice. Eur. J. Pharmacol. 553 (1–3), 157–162.
Wei, J., Liu, W., Yan, Z., 2010. Regulation of AMPA receptor trafficking and function by glycogen synthase kinase 3. J. Biol. Chem. 285 (34), 26369–26376.
Zhu, L.Q., Wang, S.H., Liu, D., Yin, Y.Y., Tian, Q., Wang, X.C., Wang, Q., Chen, J.G., Wang, J.Z., 2007. Activation of glycogen synthase kinase-3 inhibits long-term potentiation with synapse-associated impairments. J. Neurosci. 27 (45), 12211–12220.