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Title: Lamotrigine blocks the initiation and expression of repeated high-dose methamphetamine-induced prepulse inhibition deficit in rats

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Keywords
Prepulse inhibition, Lamotrigine, Methamphetamine, Schizophrenia
Abstract

Our group developed a new psychostimulant animal model reflecting some clinical aspects of schizophrenia better than the conventional model does. In this model, long-lasting prepulse inhibition (PPI) deficit at the basement state is induced via repeated administration of methamphetamine (METH, 2.5 mg/kg) without challenge injection of this psychostimulant. This study elucidates the effects of lamotrigine (LTG, 30 mg/kg) on the initiation and expression of a steady-state PPI deficit induced by the repeated METH administration. We assessed the effect of coadministration of LTG and METH on the initiation of PPI deficit. The LTG was injected 120 min after each METH injection for 5 times on every alternate day and for an additional 5 times every day, amounting to a total of 10 times. After 11–13 days of the withdrawal period, we measured PPI using the SR-LAB system. Using other animals after 20 min of LTG injection, we subsequently examined the effect of a single injection of LTG on the expression of PPI deficit caused by the repeated METH administration. The LTG blocked the initiation of PPI deficit induced by the repeated METH administration at 68 dB of prepulse intensity, but had no effect on the startle amplitude. The LTG prevented the initiation and expression of neuroplastic PPI deficit detected at the baseline state without any METH challenge injection, which was induced by the repeated administration of this psychostimulant. Results suggest that LTG is useful for blocking progressive deterioration of neurocognitive function and recovering the neurocognitive deficit in schizophrenia.
Introduction

Progressive pathophysiological changes are putatively associated with schizophrenia [25]. These changes include progressive cognitive dysfunction, brain atrophy, or switch from treatment responsiveness to treatment resistance for dopamine (DA) D2 receptor antagonist [26, 31, 35]. Identifying methods to prevent development of pathophysiological changes in schizophrenia or to enable recovery from it is important.

Prepulse inhibition (PPI) is a phenomenon by which moderate-intensity prepulse stimuli attenuate startle responses to subsequent intense stimuli. This phenomenon is considered to represent an operational measure of sensorimotor gating [12]. Actually, PPI reflects automatic pre-attentive and preconscious sensorimotor inhibition, which serves the function of avoiding behavioral interference [23]. As proposed by Braff et al. (1999), PPI deficits might correlate more strongly with cognitive abnormalities and thought disorder than with schizophrenic positive and negative symptoms [6]. The PPI is an indicator of cognitive dysfunction [11]; has been reported to be abnormally low in schizophrenia patients. Therefore, methods that can prevent development of a PPI deficit or promote recovery from PPI deficit might be new clinical treatment strategies targeted at improving cognitive function in schizophrenia.

Chronic abuse of methamphetamine (METH), a drug which increases extracellular DA levels by releasing cytoplasmic DA [5, 29], leads to the development of positive schizophrenia-like symptoms, such as hallucinations and delusions, but rarely induces the so-called negative symptoms, such as flattened affect and social withdrawal. The METH-induced psychosis can therefore be used as a model for treatment-responsive schizophrenia [16] because positive symptoms are known to respond well to typical antipsychotics [34], which mainly antagonize DA D2 receptors.

An acute injection of a phencyclidine-like drug, ketamine [2, 22], which non-competitively blocks N-methyl-d-aspartate (NMDA) receptors, induces not only positive schizophrenia-like symptoms but also negative symptoms and cognitive dysfunction. Psychosis induced by an NMDA receptor antagonist can be used as a model for treatment-resistant schizophrenia that does not
respond well to typical antipsychotics [9, 20]; schizophrenia of this type responds well to clozapine, an atypical antipsychotic [17, 18].

Based on the models mentioned above, our group [1] recently developed a new psychostimulant model that reflects the clinical features of progressive pathophysiology in schizophrenia. In this model, METH was repeatedly administered to induce PPI deficit and express this deficit at a baseline state without any challenge injection of this psychostimulant. In the PPI deficit model used in this study, we excluded acute effects of METH by any challenge injection of METH because our group intends to model the pathophysiology of schizophrenia but not the abuse of METH. The PPI deficit in schizophrenia is not induced and expressed by an acute injection of this psychostimulant. In this model, METH-induced delayed increase in extracellular glutamate levels in the medial prefrontal cortex (mPFC) plays an important role in the initiation of repeated METH-induced PPI deficit.

Clinically, lamotrigine (LTG) is known as an anticonvulsant [19] and mood stabilizer [10]. A clinical trial demonstrated that administration of LTG along with stable clozapine exhibited a beneficial effect on the psychopathological symptoms of clozapine treatment-resistant schizophrenic patients [38]. Furthermore, LTG has been shown to prevent the disruption of PPI induced by an acute injection of ketamine, an NMDA receptor antagonist, in mice [7].

This study analyzed the effects of LTG on the initiation phase and expression phase of repeated METH-induced neuroplastic PPI deficit detected at the baseline state without any challenge injection of this psychostimulant. In the initiation experiment, no drugs were present when PPI was measured, but LTG was given before each dose of METH during the chronic treatment phase. This experiment measures the ability of LTG to prevent the effects of chronic METH on PPI. In the expression experiment, LTG was present only during the final test session after chronic treatment with METH alone. This experiment determines whether LTG can reverse an established deficit that was induced by chronic METH use.

Materials and Methods
Six-week-old male Sprague–Dawley rats (SLC, Inc., Japan) weighing 160–180 g at the start of the experiment were housed individually in plastic cages (30 × 25 × 18 cm) with a wire mesh top and sawdust bedding. The animal house was maintained under controlled conditions of light (6:30–18:30), temperature (24°C), and humidity (50%). The animals were provided a standard laboratory diet and tap water and handled daily for at least 3 days before the start of the experiment. A total of 98 rats were used. Each rat weighed 330–380 g (10-week-old) when PPI was tested. This study was conducted in accordance with the guidelines for the care and use of laboratory animals of the Hokkaido University Graduate School of Medicine and the National Institute of Health (NIH) guidelines on animal care.

For this study, METH (Dainippon Sumitomo Pharma Co., Ltd., Japan) was dissolved in sterile physiological saline and injected subcutaneously at a volume of 1.0 ml/kg; LTG (gift from GlaxoSmithKline, U.K.) was dissolved in 10 ml of distilled water with 10 drops of 0.1 N HCl and was injected intraperitoneally at a volume of 4.0 ml/kg. Vehicles for METH and LTG were saline (1 ml/kg) and approximately 0.002 N HCl in distilled water (4 ml/kg), respectively.

This study used dosages of METH (2.5 mg/kg) and LTG (30 mg/kg) that were chosen after considering the results obtained in previous studies [1, 7, 13].

In experiment 1, the effects of LTG (30 mg/kg) on the initiation of PPI deficit induced by the repeated administration of METH (2.5 mg/kg) were analyzed. Vehicle or LTG (30 mg/kg) was injected 120 min after the administration of METH (2.5 mg/kg) or an equivalent amount of saline for 5 times on every alternate day, and for an additional 5 times every day, amounting to a total of 10 times (Fig. 1). The PPI was measured without the administration of the acute challenging drug after an 11–13-day withdrawal period from the last treatment. The current design was based on preliminary studies showing that this is the protocol giving the most robust effects. For that reason, this protocol was modified from that of our previous study [1]. Each group included 11–12 animals.
In experiment 2, the effects of LTG (30 mg/kg) on the expression of PPI deficit induced by the repeated administration of METH (2.5 mg/kg) were analyzed using animals other than those in Experiment 1. METH (2.5 mg/kg) or an equivalent amount of saline was injected 5 times on every alternate day, and an additional 5 times every day, amounting to a total of 10 times (Fig. 1). At 11–13-day withdrawal period from the last METH treatment, PPI was measured at 20 min after a single injection of LTG (30 mg/kg) or vehicle. Each group included 13–14 animals.

The startle response was assessed using SR-LAB systems (San Diego Instruments Inc., San Diego, California), as described previously [32]. Rats were placed in the startle apparatus and allowed to accustom themselves to the equipment for 10 min in the presence of a 65-dB white noise background. Subsequently, the rats were presented with a series of five startle-pulse-alone trials to habituate them to the startle response. This series of stimuli was followed by 45 trials consisting of no pulse (0 dB), a startle pulse (120 dB, 40 ms) alone, or prepulses of three intensities (68 dB, 71 dB, and 77 dB; 20 ms) presented with or 100 ms before a startle pulse. To exclude the possibility that a particular trial is accidentally clustered, the session was composed of pseudo-randomized trials. The time between the trials was 10–20 s. The startle responses were measured every 1 ms over a 100-ms period from the onset of the startle stimulus. The value of PPI% was calculated using the following formula: PPI = 100 − ([P + S] / S) × 100, where “P + S” is the mean response amplitude for prepulse plus startle trials and “S” is the mean response amplitude for the startle pulse alone trials.

Data from PPI and startle amplitude in all startle trials were analyzed using a repeated measures three-way analysis of variance (ANOVA) (METH x LTG x Intensities) followed by Duncan’s post-hoc test to determine significant differences between the different groups at a significance level of $p < 0.05$.

Results

Results show that LTG (30 mg/kg) blocked the initiation of repeated METH (2.5 mg/kg) administration-induced PPI deficit at 68 dB prepulse intensity (Fig. 2). A repeated measures three-way ANOVA showed significant effects of
METH (2.5 mg/kg) \([F(1, 41) = 6.77, p < 0.05]\), prepulse intensity \([F(2, 82) = 250.16, p < 0.01]\), METH (2.5 mg/kg) × prepulse intensity interaction \([F(2, 82) = 3.84, p < 0.05]\), and METH (2.5 mg/kg) × LTG (30 mg/kg) × prepulse intensity interaction \([F(2, 82) = 6.37, p < 0.05]\). However, no significant effect of LTG (30 mg/kg) \([F(1,41) = 3.39, p = 0.07]\), METH (2.5 mg/kg) × LTG (30 mg/kg) interaction \([F(1,41) = 2.41, p = 0.13]\), or LTG (30 mg/kg) × prepulse intensity interaction \([F(2, 82) = 0.17, p = 0.75]\) was found. Duncan’s post-hoc test revealed that LTG (30 mg/kg) blocked the initiation of repeated administration of METH (2.5 mg/kg)-induced PPI deficit at 68 dB of prepulse intensity \((p < 0.01)\).

No significant effect of METH or LTG was found on the startle amplitude (Fig. 2). A repeated measures three-way ANOVA showed a significant effect of prepulse intensity \([F(3, 123) = 68.14, p < 0.01]\), but no significant effect of METH (2.5 mg/kg) \([F(1, 41) = 0.02, p = 0.89]\), LTG (30 mg/kg) \([F(1,41) = 0.29, p = 0.60]\), METH (2.5 mg/kg) × LTG (30 mg/kg) interaction \([F(1,41) = 0.59, p = 0.46]\), METH (2.5 mg/kg) × prepulse intensity interaction \([F(3, 123) = 1.74, p = 0.16]\), LTG (30 mg/kg) × prepulse intensity interaction \([F(3, 123) = 0.88, p = 0.45]\), or METH (2.5 mg/kg) × LTG (30 mg/kg) × prepulse intensity interaction \([F(3, 123) =1.51, p = 0.22]\) was found. Administration of LTG (30 mg/kg) blocked the expression of repeated METH (2.5 mg/kg) administration-induced PPI deficit at 68 dB of the prepulse intensity (Fig. 3). The LTG (30 mg/kg) increased PPI at 77 dB of the prepulse intensity. A repeated measures three-way ANOVA indicated significant effects of LTG (30 mg/kg) \([F(1, 49) = 9.49, p < 0.01]\), prepulse intensity \([F(2, 98) = 238.53, p < 0.01]\), METH (2.5 mg/kg) × prepulse intensity interaction \([F(2, 98) = 4.18, p < 0.05]\), and METH (2.5 mg/kg) × LTG (30 mg/kg) × prepulse intensity interaction \([F(2, 98) = 4.23, p < 0.05]\). However, no significant effect of METH (2.5 mg/kg) \([F(1, 49) = 0.001, p = 0.98]\), METH (2.5 mg/kg) × LTG (30 mg/kg) interaction \([F(1,49) = 0.74, p = 0.40]\), or LTG (30 mg/kg) × prepulse intensity interaction \([F(2, 98) = 0.10, p = 0.90]\) was found. Duncan’s post-hoc test revealed that LTG (30 mg/kg) blocked the expression of repeated METH (2.5 mg/kg) administration-induced PPI deficit at 68 dB of prepulse intensity \((p < 0.01)\) and increased PPI at 77 dB of the prepulse intensity \((p < 0.05)\).
No significant effect of METH or LTG was found on the startle amplitude (Fig. 3). A repeated measures three-way ANOVA showed a significant effect of prepulse intensity \( [F(3, 147) = 55.91, p < 0.01] \), but no significant effect was found for METH (2.5 mg/kg) \( [F(1, 49) = 0.24, p = 0.63] \), LTG (30 mg/kg) \( [F(1, 49) = 0.88, p = 0.35] \), METH (2.5 mg/kg) × LTG (30 mg/kg) interaction \( [F(1, 49) = 0.90, p = 0.35] \), METH (2.5 mg/kg) × prepulse intensity interaction \( [F(3, 147) = 1.51, p = 0.21] \), LTG (30 mg/kg) × prepulse intensity interaction \( [F(3, 147) = 0.75, p = 0.53] \), or METH (2.5 mg/kg) × LTG (30 mg/kg) × prepulse intensity interaction \( [F(3, 147) = 1.60, p = 0.19] \).

**Discussion**

In fact, LTG has been considered to act via a reduction in glutamate release by exerting an inhibitory effect on sodium channels, thereby reducing neurotransmitter exocytosis [24, 39]. PPI involves the release of various neurotransmitters such as dopamine, glutamate, serotonin, gamma-aminobutyric acid (GABA), and neuropeptide, which form the biological basis of sensorimotor gating [11]. Countless pharmacological and lesion studies have greatly improved our understanding of the neuronal circuits involved in PPI [36]. The circuits involved with PPI deficit are composed of multiple forebrain regions, including the amygdala, dorsal hippocampus, and mPFC [3]. The mPFC plays a crucial role in the regulation of PPI [30].

Our previous study revealed that 2.5 mg/kg, but not 1.0 mg/kg of METH induced increases in glutamate levels in the mPFC [1] and the nucleus accumbens [14, 15], suggesting that repeatedly increased glutamate release in the neuronal circuit, which comprises the mPFC and nucleus accumbens, is related to the METH-induced long-lasting PPI deficit. Furthermore our group [1] found that several drugs such as olanzapine and risperidone, which block METH-induced delayed increase in extracellular glutamate levels in mPFC, prevented the initiation of PPI deficit induced by the repeated administration of METH. Furthermore, LTG has been shown to reduce glutamate release in vitro [8] [27]. Considering these findings, we can speculate that LTG might exert its effects on the initiations of PPI deficit by reducing glutamatergic release in neural regions such as mPFC during the repeated administration of METH.
In this study, PPI was measured after an 11–13-day withdrawal period from the last METH administration. Consequently, a steady-state PPI but not the acute METH-induced one was measured. At 68 dB, LTG recovered the expression of PPI deficit that was induced by repeated administration of METH. Repeated doses of METH reduce the mRNA expression of the NMDA subunit NR1 to induce NMDA receptor dysfunction [33]. These neurocognitive and neurochemical changes are considered to be long-lasting deficits but they are not considered acute effects of METH because these changes are detected after sufficient withdrawal from repeated administration of this psychostimulant. Therefore, expression of the neuroplastic PPI deficit induced by the repeated administration of METH might reflect the state underlying hypofunction of NMDA receptors [15]. The PPI deficit is induced acutely by hypofunction of NMDA receptor-mediated glutamatergic neurotransmission by a single administration of NMDA receptor antagonists [4]. The LTG reverses PPI deficit induced by ketamine, an NMDA receptor antagonist, but not amphetamine [7]. Glutamate tonically stimulates GABAergic neurotransmission via NMDA receptor [21]. Therefore, LTG might recover the NMDA receptor hypofunction-induced PPI deficit by increasing GABAergic release.

Progressive cognitive dysfunction [28] might be the chronic cause of illness or treatment resistance in schizophrenia [25, 26]. An NMDA receptor blockade-mediated PPI deficit has been considered to model cognitive dysfunction of schizophrenia [7]. Patients in the lowest quartile of PPI are significantly more impaired in terms of the specific functional measure [37]. It has been suggested that, in addition to advocating treatment for hallucination or delusion, it is important to treat longitudinal progressive cognitive changes in schizophrenia. Actually, LTG might prevent the progression of cognitive deficit and recover cognitive dysfunction in schizophrenia patients.

Major limitations of this study are: 1) METH only induces significant PPI deficits at 68 dB prepulse trials, and 2) high variation was found in the METH group despite the large group size. Accordingly, further studies will be necessary to confirm these results and elucidate the mechanism of action. Nevertheless, three-way ANOVA revealed the clear interaction of METH × LTG × prepulse intensity for both initiation and expression experiments, which
supports the significance of METH-induced PPI deficits at only 68 dB prepulse trials. In addition, acute effect of METH with LTG on PPI should be examined in rats in future studies for comparison with the results obtained by Brody et al. in mice [7].

In summary, LTG blocked both the initiation and expression of PPI deficit induced by repeated administration of METH (2.5 mg/kg). These results suggest that LTG is a useful drug to block or recover the progressive deterioration of neurocognitive function in schizophrenia.

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Author disclosure information
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References


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**Figure legends**

Figure 1. Schematic representation of the time-line of the experiments (d= days).

Figure 2. Effect of LTG (30 mg/kg) on the initiation of PPI deficit induced by repeated administration of METH (2.5 mg/kg). #, $p < 0.01$, METH + Vehicle vs. Saline + Vehicle; a, $p < 0.01$, METH + Vehicle vs. METH + LTG; b, $p < 0.05$, METH + Vehicle vs. METH + LTG

Figure 3. Effect of LTG (30 mg/kg) on the expression of PPI induced by the repeated administration of METH (2.5 mg/kg). *, $p < 0.05$, METH/Vehicle vs. Saline/Vehicle; Saline/LTG vs. Saline/Vehicle; a, $p < 0.01$, METH/Vehicle vs. METH/LTG