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Short communication

The adenosine A_1 receptor agonist N^6-cyclopentyladenosine blocks the disruptive effect of phencyclidine on prepulse inhibition of the acoustic startle response in the rat

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Abstract

Systemic administration of the NMDA receptor antagonist phencyclidine (PCP; 4 mg/kg) produced a profound reduction in prepulse inhibition of the acoustic startle response in rats. Pre-treatment with the selective adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) blocked (0.5 mg/kg) or attenuated (0.1 and 0.2 mg/kg) the disruptive effect of PCP on prepulse inhibition. These findings suggest that adenosine may regulate the inhibitory effect of NMDA receptor blockade on prepulse inhibition, and raise the possibility that adenosine may be a potentially useful target for anti-psychotic medication. Further, 0.5 mg/kg CPA by itself was without effect on prepulse inhibition but did decrease startle amplitude, raising the possibility that adenosine, acting via A_1 receptors, may be a component of the neurochemical substrate that modulates the acoustic startle response.

Keywords: Acoustic startle response; NMDA receptor antagonist; Phencyclidine; Prepulse inhibition

1. Introduction

The non-competitive NMDA receptor antagonist phencyclidine (PCP) and related compounds reduce prepulse inhibition of the acoustic startle response in rodents (Mansbach and Geyer, 1989; Bakshi and Geyer, 1995; Al-Amin and Schwarzkopf, 1996). Prepulse inhibition refers to the reduction in startle amplitude when a startle-inducing stimulus is preceded by a weaker stimulus that is not, in itself, sufficient to elicit startle. Schizophrenic and schizotypal patients show reduced prepulse inhibition as compared to control subjects (Braff et al., 1992; Cadenhead et al., 1993) and prepulse inhibition has been used as an operational definition of sensorimotor gating, or the filtering of exteroceptive stimuli, which is thought to be impaired in schizophrenics (Geyer et al., 1990; Braff et al., 1992; Swerdlow and Geyer, 1998). Identifying compounds that ameliorate the PCP-induced deficits in prepulse inhibition might therefore provide insight into possible therapeutic strategies for treating schizophrenia.

The endogenous neurotransmitter adenosine has received considerable attention of late as a possible target for the development of anti-psychotic medication, primarily due to the fact that adenosine is co-localized with dopamine and has been shown to have anti-dopaminergic properties (Ferré, 1997). Four types of adenosine receptors have been identified in the central nervous system: A_1, A_{2A}, A_{2B}, and A_3 (Fredholm, 1995). The adenosine A_1 and A_{2A} receptor subtypes have received the most attention, as these receptors have been shown to regulate dopaminergic neurotransmission, and interact with dopamine D_1 and D_2 receptors, respectively (Ferré, 1997).

Selective adenosine A_1 and A_2 receptor agonists also antagonize the behavioural and neurobiological effects of PCP and other NMDA receptor antagonists. For instance, selective A_1 receptor agonists block the discriminative stimulus properties of PCP (Brown and Welch, 1982) and selective A_1 and A_2 receptor agonists antagonize the locomotor stimulatory effect of PCP and dizocilpine (Fraser et al., 1997; Rimondini et al., 1997). Similarly, selective A_1 and A_2 receptor agonists prevent the EEG effects of dizocilpine (Popoli et al., 1997). To date, there have been no studies that have examined the effects of selective

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adenosine receptor agonists on the disruptive effect of PCP on prepulse inhibition. However, Koch and Hauber (1998) have argued that adenosine A_1 receptor activation may be part of the mechanism regulating prepulse inhibition. The purpose of the present experiment was, therefore, to determine whether the selective adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) would ameliorate the disruptive effect of PCP on prepulse inhibition.

2. Materials and methods

2.1. Subjects

A total of 18 male Wistar rats (Charles River, Quebec) weighing 250–275 g at the start of the experiment, were housed in individual hanging wire mesh cages in a temperature and light controlled environment, with lights on–off at 0700–1900 h. Rats had ad libitum access to water and standard Purina lab chow pellets throughout the experiment.

2.2. Drugs

PCP (Health and Welfare Canada) and CPA (RBI) were dissolved in physiological saline, and were administered in a volume of 1 ml/kg (PCP) or 2 ml/kg (CPA). CPA (or vehicle) was administered intraperitoneally 15 min prior to the subcutaneous administration of PCP (or vehicle). Ten minutes following the second injection, rats were placed in the startle chambers.

2.3. Prepulse inhibition test

Testing was conducted in four SR-Lab Startle Response Systems (San Diego Instruments, San Diego, CA). Each system consisted of a $37.5 \times 40.0 \times 57.5$ cm^3 isolation cabinet which housed the startle chamber. The startle chamber consisted of a 8.2 cm Plexiglas cylinder mounted on a 12.5×25.5 cm^2 Plexiglas platform with a piezoelectric accelerometer unit attached to the bottom of the platform. The accelerometer detected and transduced motion within the startle chamber and these signals were digitized, rectified, and recorded by an IBM-PC compatible computer interfaced with the startle apparatus. The computer interface assembly also controlled delivery of acoustic stimuli through a speaker mounted above the floor of the isolation cabinet. Sound levels were measured and calibrated with a Radio Shack Digital Sound Level Meter (model 33-2055) placed within each chamber. Response sensitivities were calibrated using the SR-LAB Startle Calibration System.

A test session began by placing a subject in the Plexiglas cylinder where the subject was exposed to the background noise (65 dB white noise) for 10 min. After a 10 min acclimation period, each subject was presented with three trials of the 120 dB pulse (40 ms broadband burst) and the responses from these trials were discarded. Following these three trials, each subject was presented with nine iterations of eight different types of trials: no pulse (0 dB), a startle pulse (110 dB, 40 ms broadband burst), and three prepulse intensities (70, 75, 80 dB, 20 ms broadband burst) presented alone or 100 ms preceding a startle pulse. The presentation of trial type was randomized within each of the nine iterations. The average intertrial interval was 15 s (range 10–20), and the intertrial interval was randomized across all 72 trials. The startle response was measured every 1 ms for a 250 ms period from the onset of the startle stimulus. The average startle amplitude across the 250 ms measurement period was used in the calculation of prepulse inhibition. Prepulse inhibition was defined as [100 × ((mean startle amplitude on pulse-alone trials − mean startle amplitude on prepulse + pulse trials)/mean startle amplitude on pulse-alone trials)].

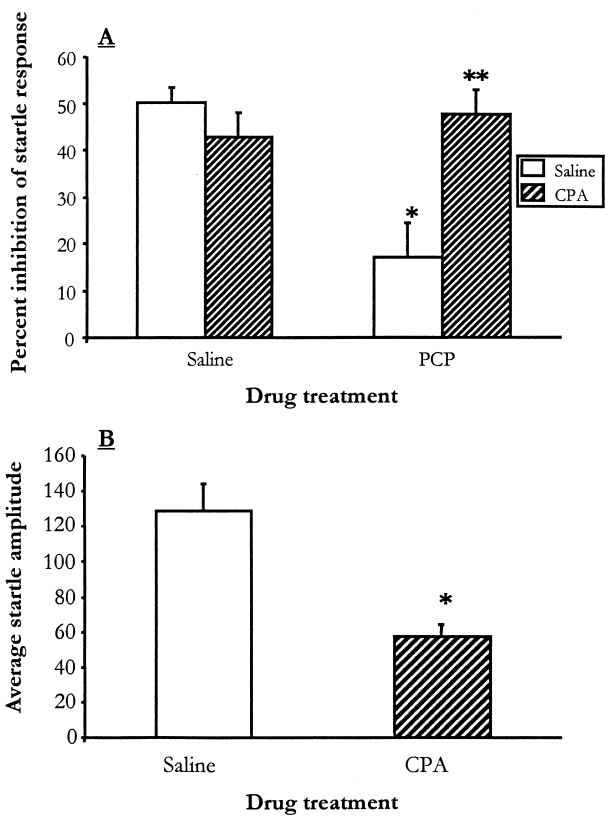

Fig. 1. (A) Prepulse inhibition of the acoustic startle response. Average (±S.E.M.) percentage prepulse inhibition of the acoustic startle response in each of the four drug treatment conditions. All rats received saline or PCP (4 mg/kg, subcutaneous) following pre-treatment (intrapertioneally) with either saline or the selective adenosine A_1 receptor agonist CPA (0.5 mg/kg). (* significantly lower than saline, $P < 0.05$; ** significantly higher than rats administered saline + PCP, $P < 0.05$). (B) Acoustic startle response. Average (±S.E.M.) startle amplitude in rats pre-treated with either saline or CPA. (* significantly lower than saline pre-treatment, $P < 0.05$).

In experiment 1, a group of rats ($N = 10$) was tested for prepulse inhibition following the administration of saline vehicle or 4.0 mg/kg PCP preceded by pre-treatment with either saline vehicle or 0.5 mg/kg CPA; this design thus yielded four drug treatment conditions: saline–saline, saline–PCP, CPA–saline, CPA–PCP. All rats received all drug combinations in a latin-square design, and there were 2 days separating each drug test. In experiment 2, a second group of rats ($N = 8$) was tested for prepulse inhibition following treatment with saline–saline, saline–PCP (4.0 mg/kg), 0.05 mg/kg CPA–PCP, 0.1 mg/kg CPA–PCP, and 0.2 mg/kg CPA–PCP; all rats received all drug combinations in a randomized order with 2 days separating each drug test.

3. Results

3.1. Experiment 1—prepulse inhibition

A $2 \times 2 \times 3$ analysis of variance (ANOVA), with drug (saline vs. PCP), pre-treatment (saline vs. CPA), and prepulse stimulus intensity (70, 75, 80 dB) as within-subjects factors revealed a significant drug × pre-treatment interac-

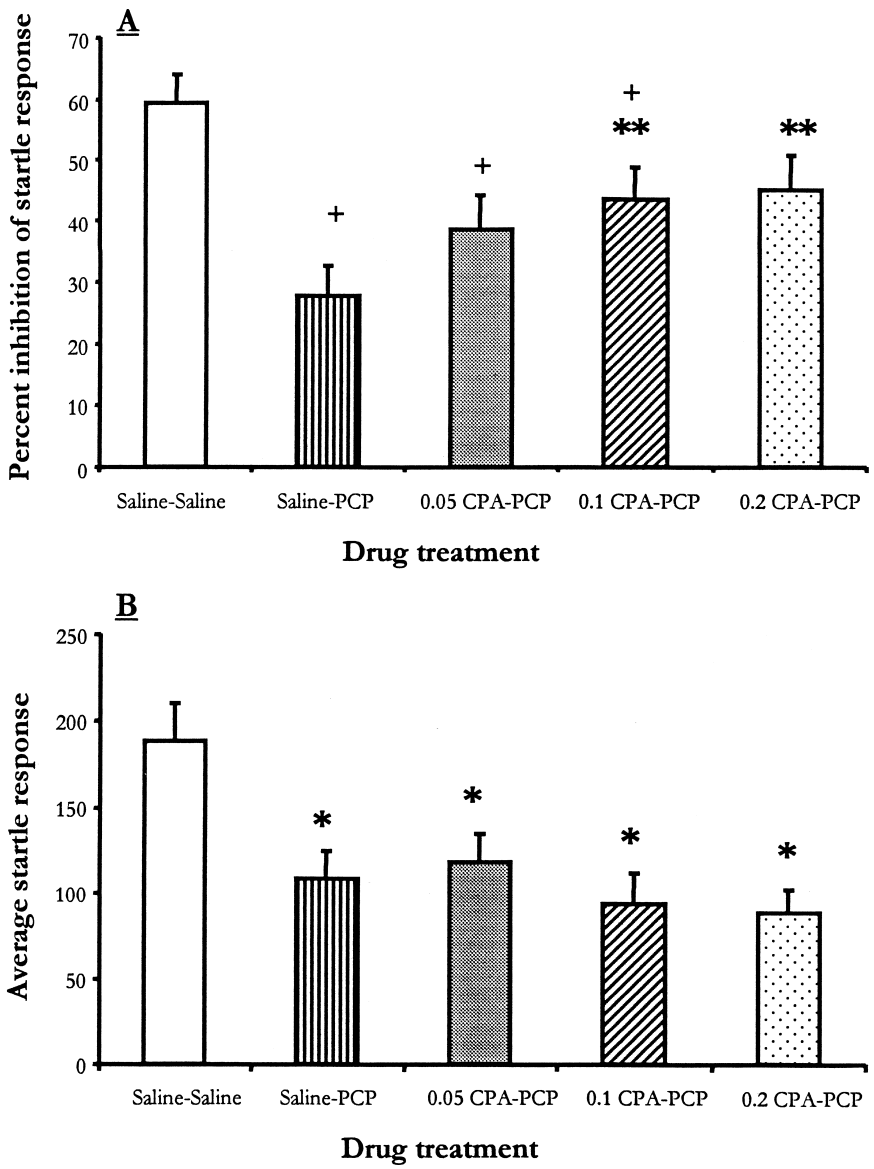

Fig. 2. (A) Prepulse inhibition of the acoustic startle response. Average (±S.E.M.) percentage prepulse inhibition of the acoustic startle response in each of the five drug treatment conditions. All rats received saline or PCP (4 mg/kg, subcutaneous) following pre-treatment (intrapertioneally) with either saline or the selective adenosine A_1 receptor agonist CPA ($^{+}$ significantly lower than saline, $P < 0.05$; ** significantly higher than rats administered saline + PCP, $P < 0.05$). (B) Acoustic startle response. Average (±S.E.M.) startle amplitude under each of the drug conditions (* significantly lower than saline pre-treatment, $P < 0.05$).

tion, $F_{1,9} = 5.79$, $P < 0.05$. As shown in Fig. 1A, post-hoc analysis revealed that PCP significantly reduced prepulse inhibition as compared to saline treatment, and this effect was reversed by pre-treatment with CPA; CPA by itself did not significantly affect prepulse inhibition.

3.2. Experiment 1—startle

A two-way ANOVA, with drug (saline vs. PCP) and pre-treatment (saline vs. CPA) as the within-subjects factors revealed a significant main effect of pre-treatment on startle response, $F_{1,9} = 15.96$, $P < 0.05$. As shown in Fig. 1B, pre-treatment with CPA resulted in a significant reduction in response to the startle-inducing stimulus.

3.3. Experiment 2—prepulse inhibition

A 5×3 ANOVA, with drug treatment and prepulse stimulus intensity as the within-subjects factors revealed a significant main effect of drug treatment, $F_{4,28} = 5.12$, $P < 0.05$, and a main effect of prepulse stimulus intensity, $F_{2,14} = 62.36$, $P < 0.05$. As shown in Fig. 2A, 4.0 mg/kg PCP produced a significant decrease in prepulse inhibition and this was attenuated by pre-treatment with 0.1 and 0.2 mg/kg CPA.

3.4. Experiment 2—startle

A One-way ANOVA, with drug treatment as the within-subjects factor, revealed a significant effect of drug treatment, $F_{4,28} = 15.74$, $P < 0.05$. As shown in Fig. 2B, PCP treatment resulted in a significant reduction in startle and this reduction was unaffected by pre-treatment with CPA.

4. Discussion

Consistent with previous reports (Mansbach and Geyer, 1989; Bakshi and Geyer, 1995), the systemic administration of the non-competitive NMDA receptor antagonist PCP resulted in a profound reduction in prepulse inhibition. Pre-treatment with the selective A_1 receptor agonist CPA resulted in a significant attenuation (0.1 and 0.2 mg/kg) or complete reversal (0.5 mg/kg) of the disruptive effect of PCP on prepulse inhibition. In light of the fact that the PCP-induced disruption of prepulse inhibition has been adopted as an animal model of the sensorimotor gating deficits observed in schizophrenics (Swerdlow and Geyer, 1998), the results of the present study indicate that selective adenosine A_1 receptor agonists may have utility as novel anti-psychotic agents.

The findings of the present study indicate that adenosine may be part of the mechanism regulating the inhibitory effect of NMDA receptor antagonism on prepulse inhibition. However, the precise mechanism by which adenosine A_1 receptor stimulation reverses the disruptive effect of PCP on prepulse inhibition remains to be elucidated. To date, the only studies that have examined the effects of adenosine agents on prepulse inhibition have examined the interaction between adenosine and dopamine. For instance, Koch and Hauber (1998) reported that the combination of sub-threshold doses of apomorphine and a nonselective adenosine antagonist resulted in a significant disruption of prepulse inhibition and that this disruption was reversed by pre-treatment with CPA (0.1–2.0 mg/kg). Koch and Hauber suggest that the regulation of prepulse inhibition involves the antagonistic interaction between adenosine A_1 receptor activation and dopamine, particularly within the nucleus accumbens.

It is possible that the ameliorative effect of CPA on the PCP-induced deficit in prepulse inhibition is mediated via an adenosine–dopamine interaction. It is known that PCP and related compounds stimulate dopamine neurotransmission, particularly within the nucleus accumbens (Carboni et al., 1989; Hertel et al., 1995; Schmidt and Fadayel, 1996). Moreover, the locomotor stimulating effect of PCP is attenuated by selective dopamine D_1 receptor blockade (Tsutsumi et al., 1995), and adenosine A_1 receptor stimulation antagonises the effects of dopamine D_1 receptor stimulation (Ferré, 1997). Thus, PCP may disrupt prepulse inhibition via stimulation of dopamine (at the D_1 receptor), which would be antagonized by selective adenosine A_1 receptor activation.

Counter to the suggestion that the disruptive effect of PCP on prepulse inhibition is mediated via mesolimbic dopamine is the observation that atypical neuroleptics such as olanzapine and clozapine are effective in ameliorating the disruptive effect of PCP on prepulse inhibition (Bakshi and Geyer, 1995; Swerdlow et al., 1996), while haloperidol or selective D_1 and D_2 receptor antagonists are ineffective in altering the disruptive effect of PCP on prepulse inhibition (Bakshi et al., 1994; Swerdlow et al., 1996). Further, Bakshi and Geyer (1998) recently investigated the effects of microinfusion of the NMDA antagonist dizocilpine into a number of limbic sites on prepulse inhibition and found that dizocilpine reduced prepulse inhibition when injected into the amygdala and dorsal hippocampus, but not into the nucleus accumbens or ventral hippocampus. However, caution must be taken in extrapolating from dizocilpine to PCP since the effects of dizocilpine and PCP on the acoustic startle response are not identical. For instance, Bakshi and Geyer (1998) found that dizocilpine increased the magnitude of the startle response when injected into a variety of limbic sites. In the present study, PCP either reduced or did not significantly affect startle amplitude and previous studies have reported variable effects of PCP on startle amplitude (Swerdlow et al., 1996; Pietraszek and Ossowska, 1998).

In the present experiment, 0.5 mg/kg CPA, by itself, produced a significant decrease in responding to the startle-inducing stimulus. Koch and Hauber (1998) also found

a reduction in startle amplitude following treatment with CPA. However, unlike in the present study, Koch and Hauber reported an enhancement of prepulse inhibition following treatment with CPA by itself. This discrepancy may be accounted for by the different doses that were used in the two studies. Koch and Hauber (1998) used a dose (1.5 mg/kg) that was three times higher than the maximum dose used in the present study. It is, therefore, possible that CPA by itself may facilitate prepulse inhibition when injected in doses higher than that used in the present study.

Although 0.5 mg/kg CPA produced a profound decrease in startle amplitude in the present study, there was no disruption of baseline prepulse inhibition at this dose. This dissociation of the effect of CPA on the startle response and prepulse inhibition has been noted for a number of other drug manipulations, including PCP (Swerdlow et al., 1996; Bakshi and Geyer, 1998; Pietraszek and Ossowska, 1998) and has led to the notion that there is a dissociation between the neurochemical/neuroanatomical substrates mediating prepulse inhibition and the acoustic startle response (Swerdlow et al., 1992).

In conclusion, the adenosine A_1 receptor agonist CPA dose-dependently reversed the disruptive effect of the psychotomimetic NMDA receptor antagonist PCP on prepulse inhibition. The minimally effective dose of CPA in attenuating the disruptive effect of PCP was 0.1 mg/kg, while a dose five times higher than this was maximally effective, completely reversing the disruptive effect of PCP on prepulse inhibition. These findings raise the possibility that stimulation of adenosine A_1 receptors may produce antipsychotic effects and thus, adenosine receptors may be a potential target for anti-psychotic medication.

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References

Al-Amin, H.A., Schwarzkopf, S.B., 1996. Effects of the PCP analog dizocilpine on sensory gating: potential relevance to clinical subtypes of schizophrenia. Biol. Psychiatry 40, 744–754.

Bakshi, V.P., Geyer, M.A., 1995. Antagonism of phencyclidine-induced deficits in prepulse inhibition by the putative atypical antipsychotic olanzapine. Psychopharmacology 122, 198–201.

Bakshi, V.P., Geyer, M.A., 1998. Multiple limbic regions mediate the disruption of prepulse inhibition produced in rats by the noncompetitive NMDA antagonist dizocilpine. J. Neurosci. 18, 8394–8401.

Bakshi, V.P., Swerlow, N.R., Geyer, M.A., 1994. Clozapine antagonizes phencyclidine-induced deficits in sensorimotor gating of the startle response. J. Pharmacol. Exp. Ther. 271, 787–794.

Braff, D.L., Grillon, C., Geyer, M.A., 1992. Gating and habituation of the startle reflex in schizophrenic patients. Arch. Gen. Psychiatry 49, 206–215.

Brown, R.G., Welch, W.M., 1982. Stereoselective antagonism of phencyclidine's discriminative properties by adenosine receptor agonists. Science 217, 1157–1159.

Cadenhead, K.S., Geyer, M.A., Braff, D.L., 1993. Impaired startle prepulse inhibition and habituation in patients with schizotypal personality disorder. Am. J. Psychiatry 150, 1862–1867.

Carboni, E., Imperato, A., Perezzani, L., Di, C.G., 1989. Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience 28, 653–661.

Ferré, S., 1997. Adenosine–dopamine interactions in the ventral striatum. Implications for the treatment of schizophrenia. Psychopharmacology 133, 107–120.

Fraser, C.M., Fisher, A., Cooke, M.J., Thompson, I.D., Stone, T.W., 1997. The involvement of adenosine receptors in the effect of dizocilpine on mice in the elevated plus-maze. Eur. Neuropsychopharmacol. 7, 267–273.

Fredholm, B.B., 1995. Purinoceptors in the nervous system. Pharmacol. Toxicol. 76, 228–239.

Geyer, M.A., Swerdlow, N.R., Mansbach, R.S., Braff, D.L., 1990. Startle response models of sensorimotor gating and habituation deficits in schizophrenia. Brain Res. Bull. 25, 485–498.

Hertel, P., Mathe, J.M., Nomikos, G.G., Iurlo, M., Mathe, A.A., Svensson, T.H., 1995. Effects of D-amphetamine and phencyclidine on behavior and extracellular concentrations of neurotensin and dopamine in the ventral striatum and the medial prefrontal cortex of the rat. Behav. Brain Res. 72, 103–114.

Koch, M., Hauber, W., 1998. Regulation of sensorimotor gating by interactions of dopamine and adenosine in the rat. Behav. Pharmacol. 9, 23–29.

Mansbach, R.S., Geyer, M.A., 1989. Effects of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat. Neuropsychopharmacology 2, 299–308.

Pietraszek, M., Ossowska, K., 1998. Chronic treatment with haloperidol diminishes the phencyclidine-induced sensorimotor gating deficit in rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 357, 466–471.

Popoli, P., Reggio, R., Pezzola, A., 1997. Adenosine A(1) and A(2) receptor agonists significantly prevent the electroencephalographic effects induced by MK-801 in rats. Eur. J. Pharmacol. 333, 143–146.

Rimondini, R., Ferre, S., Ogren, S.O., Fuxe, K., 1997. Adenosine A(2A) agonists: a potential new type of atypical antipsychotic. Neuropsychopharmacology 17, 82–91.

Schmidt, C.J., Fadayel, G.M., 1996. Regional effects of MK-801 on dopamine release: effects of competitive NMDA or 5-HT2A receptor blockade. J. Pharmacol. Exp. Ther. 277, 1541–1549.

Swerdlow, N.R., Geyer, M.A., 1998. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. Schizophr. Bull. 24, 285–301.

Swerdlow, N.R., Caine, S.B., Braff, D.L., Geyer, M.A., 1992. The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. J. Psychopharmacol. 6, 176–190.

Swerdlow, N.R., Bakshi, V., Geyer, M.A., 1996. Seroquel restores sensorimotor gating in phencyclidine-treated rats. J. Pharmacol. Exp. Ther. 279, 1290–1299.

Tsutsumi, T., Hirano, M., Matsumoto, T., Nakamura, K., Hashimoto, K., Hondo, H., Yonezawa, Y., Tsukashima, A., Nakane, H., Uchimura, H., 1995. Involvement of dopamine D1 receptors in phencyclidine-induced behavioral stimulation in rats. Clin. Neuropharmacol. 18, 64–71.