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Clozapine treatment reverses dizocilpine-induced deficits of pre-pulse inhibition of tactile startle response

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Abstract

Pre-pulse inhibition (PPI) is a phenomenon of neurobehavioral plasticity in which the motor response to a startling sensory stimulus is inhibited by a preceding sensory stimulus of a lower intensity. The current experiment used tactile startle rather than acoustic startle to determine the generality of PPI across sensory modalities. PPI is easily modeled in experimental animals and serves as a useful method for determining the neural bases for sensorimotor plasticity, which can be disturbed in sensory modulation disorders. In the current study, female Sprague–Dawley rats were tested for tactile startle PPI after an auditory pre-pulse. The glutamate NMDA receptor antagonist dizocilpine (MK-801, 0.05 mg/kg) caused a nearly total blockade of the PPI effect ($p<0.0005$). The antipsychotic drug clozapine (1.25 mg/kg, $p<0.001$ and 2.5 mg/kg $p<0.05$) significantly attenuated the dizocilpine-induced PPI impairment. Interestingly, the lower clozapine dose did not by self enhance PPI and the higher clozapine dose when given alone caused a significant ($p<0.05$) PPI impairment relative to control. Nicotine (0.2 and 0.4 mg/kg) did not significantly interact with the other treatments, though the higher nicotine dose did show a trend toward attenuating the PPI impairment caused by the high clozapine dose. These effects were replicated in a second experiment of clozapine–dizocilpine interactions without nicotine treatment. This study shows that PPI of tactile startle is dramatically impaired by blocking NMDA activation and that the prototypic atypical antipsychotic drug clozapine can correct this deficit. This may be relevant to the action of clozapine in attenuating sensory gating deficits in schizophrenia and may point to new avenues of treatment for sensory modulation disorders in which there is excessive tactile response.

Keywords: Dizocilpine; MK-801; Nicotine; Clozapine; Pre-pulse inhibition; PPI; Tactile startle

1. Introduction

Pre-pulse inhibition (PPI) is a phenomenon of sensorimotor plasticity in which the motor response to a startling sensory stimulus is inhibited by a preceding sensory stimulus of a lower intensity (Swerdlow et al., 1999). PPI in the current study was cross-modal, with an acoustic pre-pulse and a tactile startle to compare with the unimodal acoustic PPI used in the majority of studies. PPI is easily modeled in experimental animals and serves as a useful method for determining the neural bases for sensorimotor plasticity. PPI is impaired in a variety of clinical states, including most prominently schizophrenia (Braff et al., 2001; Geyer et al., 2001). Drug interaction studies can be used to help determine the neural bases of sensorimotor plasticity underlying PPI and to help develop new therapeutic treatments for people with deficits in sensorimotor adaptation.

As with any neural system, receptor systems do not act alone. There is always integration of a variety of neural systems in the bases of behavior. A variety of transmitter systems have been shown to be critically involved with PPI. It is clear that NMDA glutamate systems are keys for the neural processing underlying PPI. NMDA glutamate antagonists are useful in studying the PPI impairments relevant to schizophrenia. NMDA antagonists are psychotomimetic and produce a dramatic impairment in acoustic PPI (Depoortere et al., 1999).

Nicotinic receptors also play a key role. Nicotine can enhance acoustic PPI (Acri et al., 1994), but it potentiates NMDA antagonist-induced deficits (Levin et al., 2005). The role of nicotine seems particularly relevant, given that the great majority of people with schizophrenia smoke cigarettes (Hughes et al., 1986). Recently, Postma et al. (2006) found that nicotine significantly improved tactile PPI in both people

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with and without schizophrenia. Functional magnetic resonance imaging (fMRI) analysis showed the nicotine-induced PPI to be correlated with increased hippocampal activity.

The role of antipsychotic drugs is important to determine for two reasons: first, because these drugs are routinely given to people with schizophrenia to control hallucinations, it is important to determine their effects on other aspects of schizophrenia such as sensorimotor plasticity; second, antipsychotic drug effects on dopaminergic receptors (particularly D_2 and D_4) and serotonergic (particularly 5-HT_2) receptors can give insight into the involvement of these receptor systems in sensorimotor plasticity. Clozapine, the prototypic atypical antipsychotic drug has antagonistic effects at a number of receptor systems, notably dopamine and serotonin (Schotte et al., 1993; Seeman, 2002). Clozapine has been found in Sprague–Dawley rats to reverse the acoustic PPI deficit caused by the dopamine agonist apomorphine (Swerdlow and Geyer, 1993; Swerdlow et al., 1998).

Nearly all of the earlier work with PPI has determined modulation of acoustic startle; however, the PPI effect is also seen in other sensory modalities, including touch (Keith et al., 1991; Mansbach and Geyer, 1989) and like inhibition of acoustic startle is impaired in schizophrenia (Postma et al., 2006). Tactile PPI is particularly important for determining the generality of neurotransmitter system involvement in sensorimotor plasticity seen with one modality and with two. It may be the case that the change in startle response to an auditory stimulus after an auditory pre-pulse may be idiosyncratic to the repeated stimulus of the same sensory neural pathways. The cross-modal approach avoids this particular problem.

In schizophrenia there are documented impairments in unimodal auditory PPI, which are attenuated by clozapine (Geyer et al., 2001; Le Pen and Moreau, 2002; Oranje et al., 2002). These have been related to abnormalities in sensory gating in general. A variety of studies with experimental animals show that clozapine reverses PPI-disruptive effects of NMDA antagonists in models of schizophrenia (Andreasen et al., 2006; Ballmaier et al., 2001; Bubenikova et al., 2005; Linn et al., 2003; Lipina et al., 2005; Swerdlow et al., 1996). However, there is little research determining the generality or specificity of sensory gating across modalities. Differential pharmacological effects in unimodal auditory PPI vs. cross-modal auditory–tactile PPI would indicate the involvement of differential neural systems in bases of these different measures of sensorimotor plasticity. Therapeutic treatments that counteract one modality of sensory gating but not another may be less effective in relieving dysfunction than those, which address the problem across sensory modalities.

The current study determined the interactions of nicotine and clozapine in the face of tactile PPI impairments caused by the glutamate NMDA antagonist dizocilpine (MK-801). Dizocilpine was used to mimic the PPI impairment seen in schizophrenia. Clozapine was used to test the effect of this prototypic antipsychotic drug. Nicotine was used because the great majority of people with schizophrenia smoke cigarettes and thus take nicotine along with their antipsychotic medication (Hughes et al., 1986). Previously, we found nicotine co-treatment with clozapine to be necessary to alleviate the dizocilpine-induced PPI impairments in the startle caused by auditory stimuli (Levin et al., 2005). The current study evaluated the differences in these interactions with regard to suppression of tactile startle.

2. Methods

2.1. Subjects

Female Sprague–Dawley rats ($N=12$ for the first experiment and another $N=12$ for the second experiment) were tested for PPI of startle to a tactile air puff stimulus after an auditory pre-pulse. Acute interactions among the glutamate NMDA receptor antagonist dizocilpine, the antipsychotic drug clozapine and nicotine with regard to PPI were investigated. The drug treatment was given in a repeated measures counterbalanced design which would have tested behavioral response in all phases of the estrous cycle. The protocols were approved by the Duke University Animal Care and Use Committee.

2.2. Tactile pre-pulse inhibition equipment

Tactile startle reflex amplitude and pre-pulse inhibition were measured in the Startle Response System made by San Diego Instruments (SDI). The piezoelectric disk attached to the bottom of each Plexiglas constraining tube (9 cm inside diameter) to measure startle responses was calibrated using the SDI calibrator. The data were collected for 100 ms at a rate of 1 mHz. A speaker was housed within the chamber directly above the constraining tube, and the air puff was delivered through the top of the tube. The air puff was controlled at 4 psi with a standard gas regulator, and the sound intensity of the speaker in each chamber was calibrated using a digital sound level meter (Extech Instruments). The background noise was 65 dB white noise. Animals were inserted into the constraining tubes and were placed in the sound attenuating startle chambers for each test session.

2.3. Tactile pre-pulse inhibition procedure

After the animals were placed in the chambers, there was a 5 minute acclimation period. Trials had either a startling tactile stimulus alone or with a pre-startle acoustic stimulus of any of three intensities. The data from startle only trials in all blocks was used to calculate the PPI%. Block 1 consisted of 6 startle only trials (tactile air puff stimulus). Block 2 comprised 48 trials: 12 startle only trials and 36 auditory pre-pulse plus tactile startle trials. Within the pre-pulse trials there were 3 pre-pulse levels: 68, 71, and 77 dB pure tone. The trials were presented in random order with the inter-trial duration ranging from 10 to 20 s. Block 3 had an additional 5 tactile startle only trials. Each auditory stimulus had a 2 ms rise/fall time with a 20 ms duration. The pre-pulse/startle delay was 100 ms onset to onset. The startle alone data from all trials without a pre-pulse including all the session blocks were included in the analysis. The percent pre-pulse inhibition was calculated by dividing the peak amplitude startle response which occurred after the pre-pulse by the peak amplitude startle response which was not preceded by a pre-pulse subtracting this proportion from 1 and multiplying it by 100. The entire test period lasted approximately 34 min.

2.4. Drug administration

The drug treatments were injected subcutaneously in a volume of 1 ml/kg of the normal saline vehicle alone or in combination 10 min before the acclimation period. Saline alone injections were used as the control condition. The study examined nicotine–dizocilpine–clozapine interactions: nicotine (0, 0.2 and 0.4 mg/kg), dizocilpine (0 and 0.05 mg/kg) and clozapine (0, 1.25 and 2.5 mg/kg). These doses were selected because they were previously found to cause significantly interactive effects in our previous study with PPI to acoustic startle (Levin et al., 2005). The 0.05 mg/kg dose of dizocilpine caused a robust impairment of acoustic PPI, which was attenuated, by a combination of nicotine and clozapine in the dose ranges tested here. The 18 drug doses and combinations in the first experiment and the six doses and combinations in the second experiment were administered in a repeated measures counterbalanced order as listed below. There was at least an interval of 2 days between doses. Given that females received the drug treatments in different orders and that on any given day each animal received a different dose combination in the first experiment and all the dose combinations were represented in the second experiment, estrus cycle synchronicity would not be confounded with drug dose combination.

Nicotine	Dizocilpine	Clozapine
0	0	0
0	0	1.25
0	0	2.5
0	0.05	0
0	0.05	1.25
0	0.05	2.5
0.2	0	0
0.2	0	1.25
0.2	0	2.5
0.2	0.05	0
0.2	0.05	1.25
0.2	0.05	2.5
0.4	0	0
0.4	0	1.25
0.4	0	2.5
0.4	0.05	0
0.4	0.05	1.25
0.4	0.05	2.5

A follow-up experiment was run in a separate set of rats ($N=12$) to verify the critical dose combinations of clozapine (0, 1.25 and 2.5 mg/kg) and dizocilpine (0 and 0.05 mg/kg) with fewer total doses given, a total of only six total combinations in a repeated measures counterbalance design.

Dizocilpine	Clozapine
0	0
0	1.25
0	2.5
0.05	0
0.05	1.25
0.05	2.5

2.5. Data analysis

The percent inhibition of tactile startle response of the three acoustic pre-pulse intensities was determined for each of the drug combinations. In the main study (Exp. 1) the interactions of the drug effects were tested in a multi-factorial repeated measures design. All rats received all of the drug doses and combinations with different counterbalanced orders used. Analysis of variance (ANOVA) for repeated measures was used to assess the startle response and inhibition with the pre-pulse. The factors for the analysis were dizocilpine, nicotine, clozapine and pre-pulse intensity. Significant interactions were followed up by tests of the simple main effects comparing the effects of the individual drugs and combinations (Keppel, 1982). A p-value of 0.05 was used as the threshold for significance. The follow-up study (Exp. 2) was conducted to verify that the same differences in response to the clozapine and dizocilpine doses were seen in a separate set of rats that were given fewer drug doses (6 in Exp. 2 vs. 18 in Exp. 1). Since specific comparisons were tested on the basis of the results of Exp. 1, planned comparisons were made within the ANOVA.

3. Results

3.1. Experiment 1

3.1.1. Startle alone

The main effect of dizocilpine was significant ($F(1,11)=14.42$, $p<0.05$) with an overall increased startle without pre-pulse. However, there was a differential effect of dizocilpine depending on the clozapine co-treatment (see below). The main effect of clozapine was also significant ($F(2,22)=12.14$, $p<0.0005$). Significant reduction in startle was seen with both the 1.25 mg/kg ($p<0.0001$) and 2.5 mg/kg ($p<0.0001$) clozapine doses. The dizocilpine × clozapine interaction was significant ($F(2,22)=9.01$, $p<0.005$). Both dizocilpine and clozapine caused significant reductions in startle but they were less than additive. Dizocilpine when given alone caused a significant decrease in startle amplitude ($p<0.01$). Clozapine either at the 1.25 mg/kg dose ($p<0.0001$) or 2.5 mg/kg ($p<0.0001$) dose significantly decreased startle response. However the addition of clozapine to dizocilpine did not significantly add to the response seen with dizocilpine alone.

Nicotine also decreased startle without pre-pulse with both the 0.2 mg/kg ($p<0.05$) and 0.4 mg/kg ($p<0.005$) nicotine doses being effective (Fig. 3). The three-way interaction of dizocilpine × clozapine × nicotine was not significant.

3.1.2. Pre-pulse inhibition

There was a significant overall main effect ($F(2,22)=41.00$, $p<0.0005$) of pre-pulse intensity in the entire study with significant increase in percent PPI with 77 dB pre-pulse relative to 68 dB ($p<0.0005$) and 71 dB ($p<0.0005$), which were not different from each other. Under vehicle injection conditions the PPI% rose from 24.2 ± 8.0% with the 68 dB pre-pulse to 33.2 ± 5.7% with the 71 dB pre-pulse to 50.1 ± 4.6% with the 77 dB

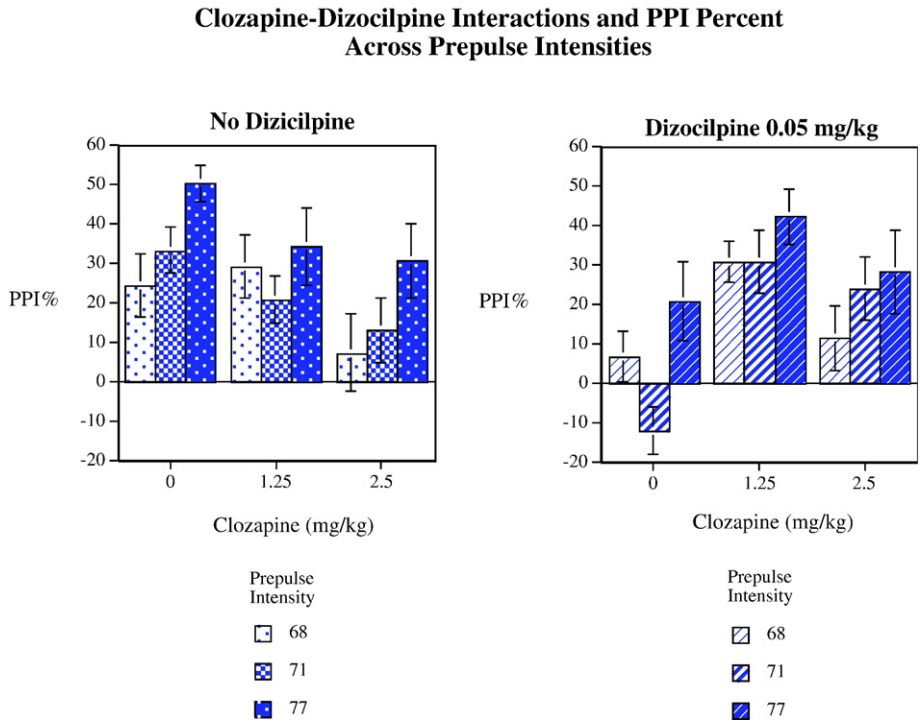

Fig. 1. Percent PPI with 68, 71 and 77 dB pre-pulse over a 65 dB background white noise level clozapine interactions with dizocilpine ($N=12$, mean±sem).

pre-pulse. Inhibition after the 77 dB pre-pulse was significantly greater than after either the 68 dB ($p<0.005$) or the 71 dB ($p<0.05$) pre-pulse. Reaction after the two lower intensities also did not significantly differ from each other under vehicle injection conditions. There were no significant interactions of PPI intensity. Performance of the rats with the different pre-pulse intensities for the clozapine and dizocilpine treatments is presented in Fig. 1.

The main effect of dizocilpine was significant ($F(1,11)=8.33$, $p<0.025$). The glutamate NMDA receptor antagonist dizocilpine (MK-801) when given alone at a dose of 0.05 mg/kg caused a nearly total blockade of PPI of tactile startle

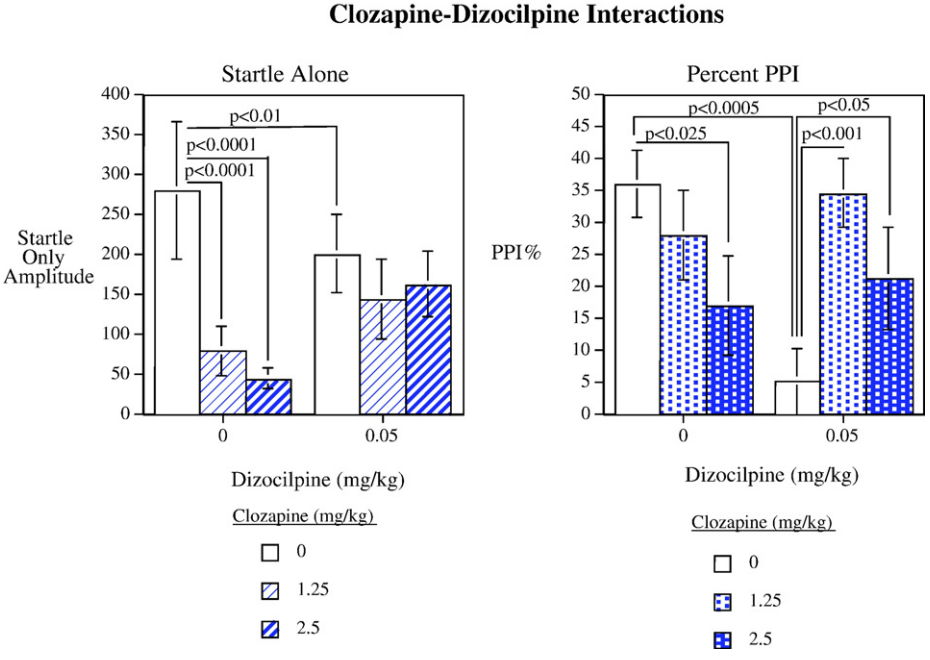

Fig. 2. Startle response without pre-pulse and percent PPI (average of response with 68, 71 and 77 dB pre-pulse over a 65 dB background white noise level): clozapine interactions with dizocilpine ($N=12$, mean±sem).

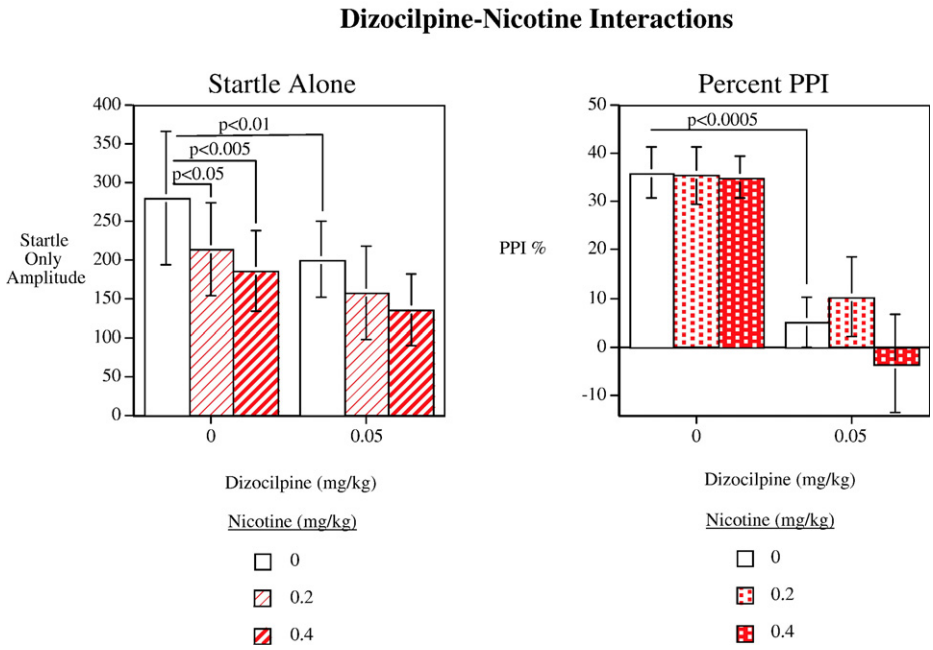

Fig. 3. Startle response without pre-pulse and percent PPI (average of response with 68, 71 and 77 dB pre-pulse over a 65 dB background white noise level): nicotine interactions with dizocilpine (N=12, mean±sem).

(p<0.0001) as shown in Fig. 2. PPI decreased from more than 35% to less than 5%.

The dizocilpine×clozapine interaction was significant (F(1,11)=9.81, p<0.001). Co-administration of the antipsychotic drug clozapine at doses of 1.25 mg/kg (p<0.001) and 2.5 mg/kg (p<0.05) significantly attenuated the dizocilpine-induced PPI impairment (Fig. 2). Clozapine when given alone did not increase PPI. In fact, the higher clozapine dose (2.5 mg/kg) when given alone without dizocilpine caused a significant (p<0.05) PPI impairment relative to control and the lower clozapine dose showed no signs of improving PPI in the absence of dizocilpine (Fig. 2). The 1.25 mg/kg clozapine dose restored PPI to nearly control levels of approximately 35%.

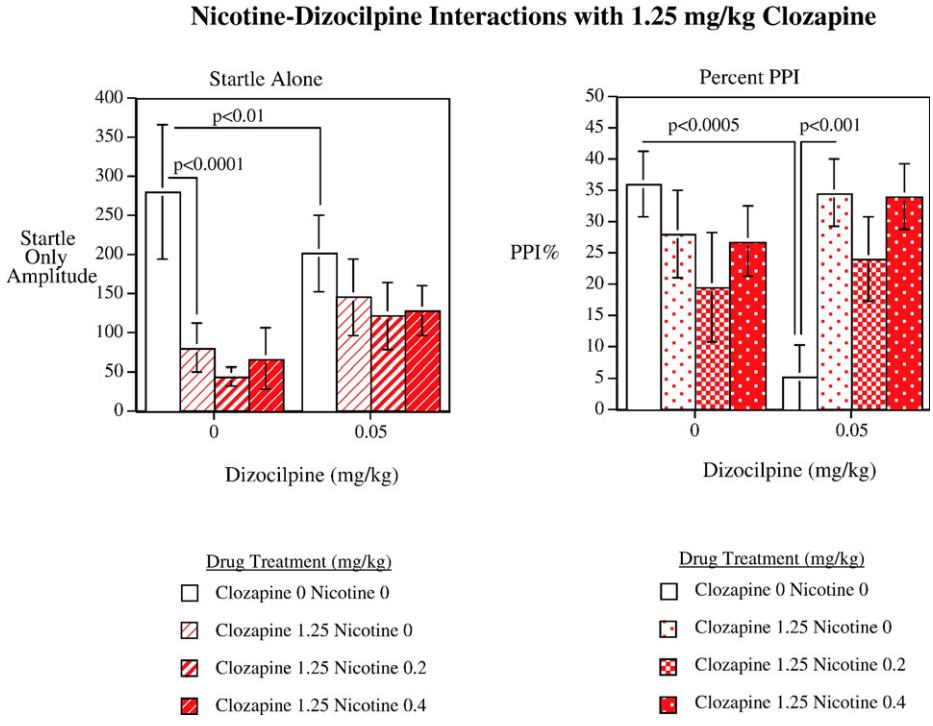

Fig. 4. Startle response without pre-pulse and percent PPI (average of response with 68, 71 and 77 dB pre-pulse over a 65 dB background white noise level): nicotine interactions with dizocilpine and 1.25 mg/kg clozapine (N=12, mean±sem).

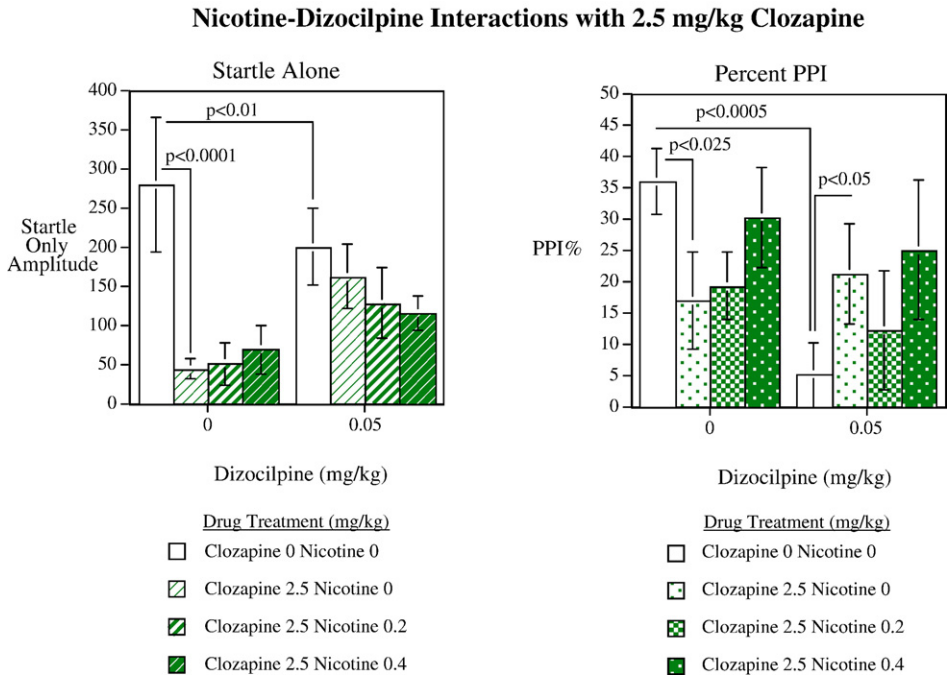

Fig. 5. Startle response without pre-pulse and percent PPI (average of response with 68, 71 and 77 dB pre-pulse over a 65 dB background white noise level): nicotine interactions with dizocilpine and 2.5 mg/kg clozapine (N=12, mean±sem).

Nicotine (0.2 and 0.4 mg/kg) did not significantly interact with dizocilpine (Fig. 3). Neither dose significantly attenuated the dizocilpine-induced impairment. There was some indication that the higher nicotine dose attenuated the PPI impairment caused by the high clozapine dose, but this was not significant (Fig. 4). Nicotine co-administration was not seen to significantly affect the clozapine-induced reversal of the PPI impairment caused by dizocilpine (Figs. 4 and 5). The three- and four-way interactions of dizocilpine, clozapine, nicotine and pre-pulse stimulus intensity were not significant.

3.2. Experiment 2

In the follow-up study, similar effects were found with dizocilpine-induced impairment and its reversal with clozapine co-administration in a different set of animals with fewer total

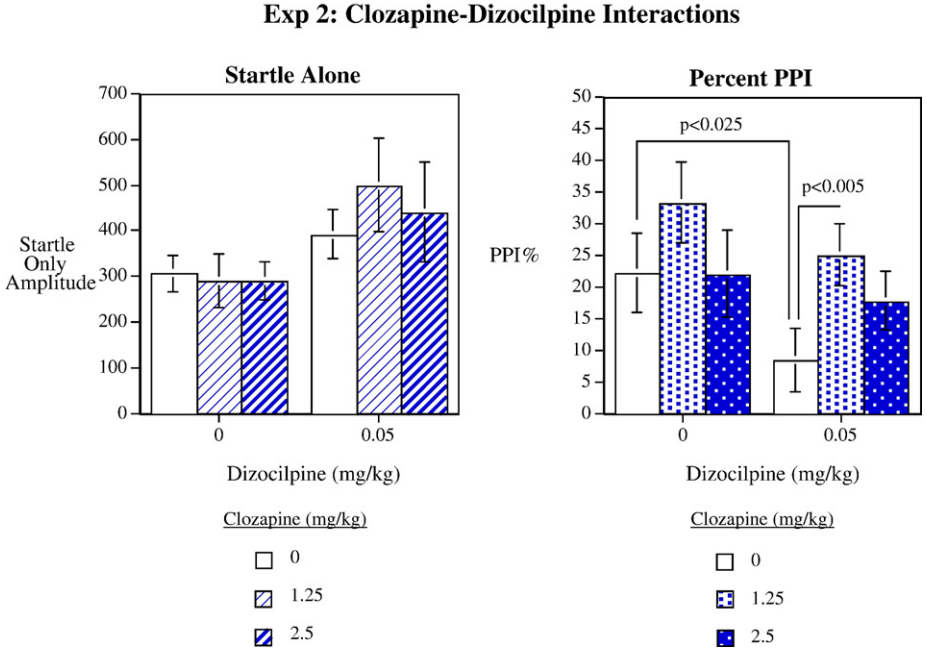

Fig. 6. Experiment 2: startle response without pre-pulse and percent PPI (average of response with 68, 71 and 77 dB pre-pulse over a 65 dB background white noise level): clozapine interactions with dizocilpine (N=12, mean±sem).

drug doses given because nicotine was not included in the study (Fig. 6). Replicating the first study, the 0.05 mg/kg dizocilpine dose caused a significant ($F(1,22)=7.48$, $p<0.025$) impairment in tactile PPI relative to the control condition with 22.2±6.2% PPI after the saline vehicle injection compared with 8.4±4.9% PPI after dizocilpine. As seen previously, this dizocilpine-induced PPI impairment was significantly ($F(1,22)=10.78$, $p<0.005$) attenuated by the 1.25 mg/kg dose of clozapine with a rise to 33.2±6.4% with the combination of dizocilpine and 1.25 mg/kg of clozapine. Also as in the first study the higher 2.5 mg/kg clozapine dose was less effective than the 1.25 mg/kg dose with only a nearly significant ($F(1,22)=3.42$, $p<0.08$) attenuation of the dizocilpine-induced PPI impairment. There were a few differences in this smaller study with fewer drug doses administered because of the elimination of t = nicotine from the experiment. The effect of clozapine decreasing response to startle alone was not seen and in a possibly related finding, clozapine when given alone no decrease in PPI% was seen. Also, dizocilpine caused an overall significant increase in response to startle alone ($F(1,11)=7.64$, $p<0.025$), which was not seen in Exp. 1. These differences may be related to the fewer total times these drugs were given (18 conditions in Exp. 1 vs. 6 conditions in Exp. 2) or the elimination of inter-current nicotine administration from Exp. 1 to Exp. 2. Despite these differences in the smaller Exp. 2, it did replicate the principal findings of Exp. 1, that dizocilpine impaired auditory pre-pulse inhibition of tactile startle response and that clozapine co-treatment reversed this effect. Even the dose-effect function of the lower 1.25 mg/kg clozapine being more clearly effective than 2.5 mg/kg of clozapine was a common finding in the two experiments.

4. Discussion

Auditory pre-pulses significantly clearly attenuated tactile startle response caused by a sudden air puff to the back of the rats in the current study. This fit into the same type of PPI effect documented in a wide variety of studies using unimodal auditory pre-pulse and auditory startle (Swerdlow et al., 1999). Others have also shown that the PPI effect is not limited to attenuating auditory startle; it is also seen in other sensory modalities, including touch (Keith et al., 1991; Mansbach and Geyer, 1989). Replicating earlier results with unimodal PPI (Depoortere et al., 1999; Levin et al., 2005), the current bimodal study showed that the glutamate NMDA receptor antagonist dizocilpine (MK-801, 0.05 mg/kg) caused a nearly total blockade of the PPI effect. This NMDA blockade-induced impairment was significantly attenuated by the antipsychotic drug clozapine. The more effective dose was 1.25 mg/kg of clozapine. The higher clozapine dose (2.5 mg/kg) also significantly attenuated the dizocilpine-induced PPI impairment; although, this effect was less robust. This was not a mere adding of effects. The clozapine attenuation of dizocilpine-induced PPI impairment was not accompanied by a finding that clozapine by itself increased PPI. The higher clozapine dose without dizocilpine caused a significant PPI impairment relative to control and the lower clozapine dose did not have a significant effect on PPI without dizocilpine.

Nicotine (0.2 and 0.4 mg/kg) did not significantly interact with the other treatments, though the higher nicotine dose did show a trend toward attenuating the PPI impairment caused by the high clozapine dose. This stands in contrast to the effects with unimodal PPI. Previously in unimodal PPI studies it was found that nicotine can enhance acoustic PPI (Acri et al., 1994), but potentiates NMDA antagonist-induced deficits (Levin et al., 2005).

Startle alone was in some cases affected by drug treatment, raising the question of how this may have affected the interpretation of PPI% effects. A critical point of the study is that clozapine reverses the tactile PPI% impairment caused by dizocilpine. The combination of clozapine with dizocilpine significantly increased tactile PPI% even thought it did not detectibly affect dizocilpine effects on tactile startle only response. Nicotine at the doses tested did not significantly influence either the dizocilpine-induced reduction of startle only response or the dizocilpine reduction of PPI%. The only case where the reduction of startle only response may influence the interpretation of the drug effects on PPI% is the case of clozapine without dizocilpine where there was a reduction in startle alone response. The clozapine-induced reduction in PPI% when given without dizocilpine may be related to a floor effect presented by this reduction in startle alone. Nevertheless, clozapine was effective in reducing the dizocilpine-induced tactile PPI% impairment. In addition, in the second study clozapine was not found to significantly affect startle alone performance and yet it still effectively reduced the NMDA blockade.

The results from the current study using cross-modal PPI had some important similarities but also differences with our earlier study of the same dizocilpine, clozapine and nicotine interactions using unimodal auditory PPI (Levin et al., 2005). Similar to the current study with cross-modal PPI, with unimodal PPI 0.05 mg/kg of dizocilpine dramatically impaired percent PPI. In contrast to the current results with cross-modal PPI, with unimodal PPI clozapine at 1.25 and 2.5 mg/kg was not effective in attenuating the dizocilpine-induced deficit in PPI%. We previously found that the addition of nicotine co-treatment with clozapine was required to attenuate the dizocilpine-induced unimodal PPI deficit. This addition of nicotine was not required in the current study of cross-modal PPI.

PPI is substantially impaired in people with schizophrenia (Braff et al., 2001; Geyer et al., 2001; Le Pen and Moreau, 2002; Oranje et al., 2002). Clinical studies have found that the antipsychotic drug clozapine effectively improves the unimodal auditory PPI impairments in patients with schizophrenia (Kumari and Sharma, 2002). However, given that the great majority of people with schizophrenia use tobacco, the necessity of nicotine as a co-treatment for this clinical effect remains unclear. In rats, it has been shown that clozapine by itself is effective in attenuating unimodal auditory PPI impairments due to NMDA glutamate blockade (Andreasen et al., 2006; Ballmaier et al., 2001; Bubenikova et al., 2005; Geyer et al., 2001; Linn et al., 2003; Lipina et al., 2005; Swerdlow et al., 1996), although there are some contrary results that clozapine by itself does not significantly attenuate unimodal auditory PPI impairments caused by dizocilpine (Bast et al., 2000; Hoffman et al., 1993; Levin et al., 2005). Bast

et al. showed that dizocilpine (0.1 mg/kg) impaired unimodal acoustic PPI and that this effect was not reversed by clozapine at a dose of 5 mg/kg (Bast et al., 2000).

The nature of the drug effects seen on PPI needs to be interpreted in light of the drug effects on startle alone without pre-pulse. The drug effects on startle alone and pre-pulse inhibition of startle were different in several ways. Dizocilpine significantly decreased both startle alone and percent PPI but the effect was much more robust with PPI. In contrast, clozapine which also decreased startle alone and PPI% had a more potent effect on startle with the lower 1.25 mg/kg dose causing a pronounced inhibition of startle alone but less of an effect on PPI. Nicotine caused a clearly significant reduction in startle alone with no detectable effect on PPI. The drug interactions were more clearly seen with PPI than startle. Clozapine at both doses significantly attenuated the PPI impairment caused by dizocilpine, whereas no such effect was seen with startle alone (Fig. 2). In addition, the clozapine effect reversing the dizocilpine-induced PPI impairment was replicated in experiment 2, whereas the clozapine effect on startle alone was not (Fig. 6).

The use of the cross-modal PPI paradigm provides important resolution of interpretative issues. For example when the warning stimulus is processed through the same sensory system as the startling stimulus it is not easily known if the PPI effect is modulated through adaptation of the pure sensory processing or later effects. The processing of the pre-pulse through one sensory system and the startle through another sensory system provides the added information that the PPI effect is mediated downstream of the immediate processing of the sensory system affected by the pre-pulse.

The current result shows the effectiveness of clozapine in reversing the bimodal auditory–tactile PPI impairment caused by dizocilpine. With inhibition of tactile startle by an auditory pre-pulse, clozapine treatment by itself was sufficient to significantly attenuate the impairment caused by the NMDA antagonist dizocilpine. In our previous study (Levin et al., 2005) as well as that from another studies (Bast et al., 2000; Hoffman et al., 1993) clozapine alone was not sufficient to reverse the dizocilpine-induced PPI impairment. Clozapine plus nicotine was necessary for significant attenuation of the unimodal auditory PPI impairment caused by the NMDA antagonist dizocilpine (Levin et al., 2005). However, others have found clozapine by itself to attenuate dizocilpine-induced unimodal auditory PPI impairments by a higher clozapine (Bakshi et al., 1994). Which of the multiple mechanisms of clozapine are necessary for its therapeutic effects in restoring tactile PPI remains to be discovered by further study. Such work is important for the development of treatment by disorders of hyper-responsive startle such as with sensory modulation disorder.

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