

Abnormally persistent latent inhibition induced by lesions to the nucleus accumbens core, basolateral amygdala and orbitofrontal cortex is reversed by clozapine but not by haloperidol

Daniela Schiller¹, Lee Zuckerman, Ina Weiner^{*}

Department of Psychology, Tel-Aviv University, P.O.B. 39040, Ramat Aviv, Tel-Aviv 69978, Israel

Received 15 October 2004; revised 12 December 2004; accepted 10 March 2005

Abstract

Latent inhibition (LI) is the proactive interference of inconsequential preexposure to a stimulus with its ability to signal significant events, and disrupted LI is considered to model positive symptoms of schizophrenia. We have recently shown that lesions of the nucleus accumbens core (NACc), basolateral amygdala (BLA) and orbitofrontal cortex (OFC) produce abnormally persistent LI, and suggested that this phenomenon may model negative symptoms. Here we tested whether NACc, BLA and OFC lesion-induced persistent LI would be reversed by the atypical antipsychotic drug (APD) clozapine but not by the typical APD haloperidol. Because clozapine's action is likely reflecting its 5HT_{2A} receptor antagonism, we also tested whether NACc lesion-induced persistent LI would be reversed by the selective 5HT_{2A} antagonist M100907. LI was measured in a conditioned emotional response procedure by comparing suppression of drinking in response to a tone in rats receiving 0 (nonpreexposed) or 40 tone presentations (preexposed) followed by five tone-shock pairings. Under these conditions, control rats did not show LI but all lesioned rats persisted in exhibiting LI, and this was reversed by clozapine but not by haloperidol. In addition, M100907 reversed NACc lesion-induced persistent LI. These two novel phenomena, abnormally persistent LI and its selective reversal by an atypical APD, suggest a novel index of schizophrenia relevant behavioral abnormality and of atypical antipsychotic activity in the LI model. The identification of brain regions whose damage leads to persistent LI in the rat may provide valuable cues on dysfunctional brain circuits involved in negative symptoms and in the action of atypical APDs.

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Keywords: Latent inhibition; Nucleus accumbens core; Basolateral amygdala; Orbitofrontal cortex; Antipsychotic drugs; Schizophrenia

1. Introduction

Latent inhibition (LI) is the phenomenon whereby repeated nonreinforced stimulus preexposure retards organisms' capacity to generate a conditioned response to this stimulus when it is subsequently paired with reinforcement. During the last two decades, LI has been

established as a leading model of schizophrenia and antipsychotic drug (APD) action, based on two sets of data: (1) LI is disrupted in amphetamine-treated rats and normal humans, in high-schizotypal humans, and in the acute stages of schizophrenia. (2) APDs reverse loss of LI in amphetamine-treated rats and in schizophrenia patients, and when given on their own produce LI in rats and humans under parametric conditions that disrupt LI in no-drug state (e.g., low number of preexposures, high number of conditioning trials; for reviews, see Gray et al., 1991; Moser et al., 2000; Weiner, 1990, 2003; Weiner and Feldon, 1997). Although LI potentiation fulfills

^{*} Corresponding author. Tel.: +972 3 6409733; fax: +972 3 6409518.
E-mail address: weiner@post.tau.ac.il (I. Weiner).

¹ Present address: Center for Neural Science, New York University, 4 Washington Place, Room 809, New York 10003, NY.

the criteria for predictive validity because it is obtained with a variety of typical and atypical APDs differing in their in vivo and in vitro pharmacology and is selective and specific for this class of drugs (e.g., Dunn et al., 1993; Killcross et al., 1994; Shadach et al., 2000; Trimble et al., 1997; Weiner and Feldon, 1987; Weiner et al., 1996a, 1997, 2003a,b), it does not differentiate between typical and atypical APDs, limiting considerably the utility of the LI model as a tool for the detection of novel atypical APDs with potentially superior clinical efficacy.

We have recently shown that typical and atypical APDs exert a differential effect on LI, which becomes manifested when these drugs are tested under parametric conditions that produce LI in no-drug animals (Shadach et al., 2000; Weiner et al., 2003a). Under such conditions, the atypical APDs clozapine and risperidone, but not the typical APD haloperidol, *disrupted* LI. We further showed that this dissociation between the two classes of drugs occurred when they were administered in the preexposure stage. While these results strengthen the predictive validity of the LI model because they allow the discrimination between typical and atypical APDs, they raise a question regarding the “therapeutic” relevance of APD-induced LI disruption. Thus, while the capacity of APDs to potentiate LI, i.e., to normalize disrupted LI, is congruent with a beneficial or therapeutic action of APDs, because disrupted LI is considered to model a cognitive impairment in schizophrenia, the capacity of atypical APDs to disrupt LI is seemingly incongruent with such an action.

This incongruity might be resolved when considering an additional body of findings on LI, namely, that certain pharmacological and lesion manipulations, rather than disrupting LI, produce an abnormally persistent LI (Weiner, 2003). More specifically, rats receiving systemic administration of the *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801, or sustaining lesions to the dorsal hippocampus, the core subregion of the nucleus accumbens (NACc), the basolateral amygdala (BLA), or the orbitofrontal cortex (OFC), have intact LI under conditions that yield LI in controls and moreover, *persist* in exhibiting LI under conditions that disrupt LI in controls (Gaisler-Salomon and Weiner, 2003; Holt and Maren, 1999; Schiller and Weiner, 2004; unpublished observations). We suggested that under such conditions, the capacity of atypical APDs to disrupt LI would become “therapeutic”, because normalization of abnormally persistent LI requires that it would be disrupted. In support of this notion we showed that the atypical APD clozapine but not the typical APD haloperidol, given in preexposure, reversed the abnormally persistent LI produced by MK-801 (Gaisler-Salomon and Weiner, 2003).

The present experiments tested whether the same differentiation between the effects of clozapine and haloperidol would be obtained with persistent LI following

lesions to the NACc, BLA and OFC. More specifically, we expected that with parameters of preexposure and conditioning that would disrupt LI in controls NACc (Experiment 1), BLA (Experiment 2) and OFC (Experiment 3) lesioned rats receiving vehicle or haloperidol in preexposure would show LI, whereas those receiving clozapine would not show LI like sham controls. In addition, because the capacity of clozapine is likely reflecting its 5HT_{2A} receptor antagonism (Shadach et al., 2000; Weiner, 2003; Weiner et al., 2003a), we tested in NACc lesioned rats whether the selective 5HT_{2A} antagonist M100907, like clozapine, would reverse persistent LI (Experiment 4). LI was measured in a thirst motivated conditioned emotional response procedure with parameters of preexposure and conditioning which have been shown by us repeatedly to disrupt LI in normal rats, and are conventionally used in our laboratory to demonstrate persistent LI namely, 40 preexposures and 5 conditioning trials (Gaisler-Salomon and Weiner, 2003; Shadach et al., 2000; Schiller and Weiner, 2004; Weiner et al., 1997, 2003a).

2. Materials and methods

2.1. Subjects

Male Wistar rats (Sackler Faculty of Medicine, Tel-Aviv University, Israel) approximately 4 months old and weighing 350–480 g, were housed four to a cage under reversed cycle lighting (lights on: 07:00–19:00) with ad lib access to food and water except for the duration of the LI experiments (see below). All experimental protocols conformed to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University, Israel; and to the guidelines of the NIH (animal welfare assurance number A5010-01, expires on 11/30/06). All efforts were made to minimize the number of animals used and their suffering.

2.2. Surgery

Rats were given an i.p. injection of diazepam (0.6 mg/kg) and 5 min later were anaesthetized with i.p. injection of avertin (0.01 ml/g). They were placed in a stereotaxic frame and an incision was made into the scalp to expose the skull. The vertical coordinates of bregma and lambda were measured in order to align them in same (level head) plane. A small square of bone was removed over the spots where the cannula or electrode would later enter. NACc excitotoxic lesions were made by bilateral infusion of 0.25 μ l NMDA (0.12 M; Sigma Chemicals, Israel) dissolved in phosphate buffer saline (PBS; pH adjusted to 7.4 with 1 N NaOH), using two injection sites in each hemisphere: 2.2 mm and 1.6 mm anterior to bregma, 1.9 mm lateral to the midline and 6.7 mm ventral to dura.

OFC excitotoxic lesions were made by bilateral infusions of 0.3 μ l NMDA dissolved in PBS, at the following coordinates: 3.2 mm anterior to bregma, 2.4 mm lateral to the midline, 5.5 mm ventral to the skull. Infusions were at a flow rate of 0.1 μ l/min and made through the tip of a Hamilton syringe (26 G cannula) using a manually driven pump (Kopf, microinjection unit, model 5000) mounted onto the stereotaxic frame. Following injections the cannula remained at the injection site for a period of 5 min to allow complete diffusion of the neurotoxin. Sham-operated controls underwent the same surgical procedure, but received injections of a corresponding dose of PBS alone. BLA electrolytic lesions were made by passing a 0.5 mA, 15 s current via a 0.3 mm electrode, insulated except for the tip. A constant current DC source was used. The coordinates were: 2.8 mm posterior to bregma, 5 mm lateral to the midline, 8.5 mm ventral to the skull. Sham-operated controls underwent the same surgical procedure, but the electrodes were inserted 6.5 mm ventral to the skull and no current was passed. We used an electrolytic BLA lesion, because we found that such lesions produce the same effects on LI like excitotoxic but without impairing fear conditioning in the nonpreexposed group (Schiller and Weiner, 2004; Weiner et al., 1996b; unpublished observations). At the end of surgery, the hole in the bone was covered by sterispon, and the scalp incisions were sutured by Michel clips. Following surgery rats were returned to their home cages and allowed 14 days of recovery before the initiation of behavioral testing.

2.3. Apparatus and procedure

Rats were tested in Campden Instruments rodent test chambers (Model 410) with a retractable bottle, each enclosed in a ventilated sound-attenuating chest. When the bottle was not present, a metal lid covered the hole. Licks were detected by a Campden Instruments drinkometer (Model 453). The preexposed to-be-conditioned stimulus was a 10 s, 80 dB, 2.8 kHz tone produced by a Sonalert module (Model SC 628). Shock was supplied through the floor by a Campden Instruments shock generator (model 521/C) and a shock scrambler (model 521/S) set at 0.5 mA intensity and 1 s duration. Equipment programming and data recording were computer controlled.

Prior to the beginning of each experiment, rats were handled for about 2 min daily for 5 days. A 23 h water restriction schedule was initiated simultaneously with handling and continued throughout the LI experiment. On the next 5 days, rats were trained to drink in the experimental chamber for 15 min/day. Water in the test apparatus was given in addition to the daily ration of 1 h given in the home cages. The LI procedure was conducted on days 11–14 and consisted of four stages given 24 h apart:

Preexposure. With the bottle removed, the preexposed (PE) rats received 40 tone presentations with an inter-stimulus interval of 40 s. The nonpreexposed (NPE) rats were confined to the chamber for an identical period of time without receiving the tone.

Conditioning. With the bottle removed, each rat received five tone-shock pairings given 5 min apart. The shock immediately followed tone termination. The first tone-shock pairing was given 5 min after the start of the session. After the last pairing, rats were left in the experimental chamber for an additional 5 min.

Retraining. Rats were given a 15 min drinking session as in initial training. Data of rats that failed to complete 600 licks were dropped from the analysis.

Test. Each rat was placed in the chamber and allowed to drink from the bottle. When the rat completed 75 licks the tone was presented for 5 min. The following times were recorded: time to first lick, time to complete licks 1–50, time to complete licks 51–75 (before tone onset, A period) and time to complete licks 76–100 (after tone onset, B period). Times to complete licks 76–100 were logarithmically transformed to allow parametric analysis of variance (ANOVA). Longer log times indicate stronger suppression of drinking. LI is defined as significantly shorter log times to complete licks 76–100 of the PE as compared to NPE rats.

2.4. Drug administration

All drugs were administered IP in a volume of 1 ml/kg. Haloperidol, prepared from an ampoule containing 5 mg haloperidol in 1 ml solvent containing 6 mg lactic acid (Janssen, Belgium) and diluted with saline, was administered at a dose of 0.1 mg/kg 60 min prior to the preexposure stage. Clozapine (Novartis, Switzerland) and M100907 (Sanofi-Aventis, USA), dissolved in 1 N acetic acid (1.5 ml/10 mg) and diluted with saline, were administered at a dose of 5 mg/kg and 0.3 mg/kg, respectively, 30 min prior to the preexposure stage. The doses of haloperidol and clozapine were the same as those we used for demonstrating their differential preexposure-based effects on LI in normal and MK-801-treated rats (Gaisler-Salomon and Weiner, 2003; Shadach et al., 2000), and the dose of M100907 was chosen on the basis of relevant results in the literature (Hitchcock et al., 1997; Moser et al., 1996). No-drug controls received the corresponding vehicle.

2.5. Histology

After the completion of behavioral testing, rats were anaesthetized with an overdose of pentobarbital (60 mg/ml) and perfused intracardially with physiological saline, followed by 10% formalin. Their brains were removed from the skulls and stored in 10% formalin for at least 24 h before being sectioned in the coronal plane

at 50 μm thickness. Every second section was mounted and stained with cresyl violet for histological examination. Verification of placements used the atlas of Paxinos and Watson (1998).

2.6. Experimental design

Experiments 1–3 included eight experimental groups in a 2×4 design with main factors of preexposure (NPE, PE) and condition of lesion and drug administration (sham-vehicle, lesion-vehicle, lesion-clozapine, lesion-haloperidol). Experiment 4 included eight groups in a $2 \times 2 \times 2$ design with main factors of preexposure (NPE, PE), lesion (sham, NACc) and drug (vehicle, M100907). We did not administer the APDs to sham rats, because with the parameters used here (40 preexposures and 5 conditioning trials), we showed that both APDs have no effect when given at the preexposure stage (they cannot disrupt disrupted LI; Gaisler-Salomon and Weiner, 2003; Shadach et al., 2000; Weiner et al., 2003a). Because the effects of M100907 were never tested under these conditions, this drug was administered to shams.

2.7. Statistical analysis

Times to complete licks 51–75 (prior to tone onset) and mean log times to complete licks 76–100 (after tone onset) were analyzed using a two- (Experiments 1–3) or three- (Experiment 4) way ANOVAs. In cases of significant interactions, post-hoc two-tailed *t* tests based on the error term of the ANOVA were used to assess the difference between NPE and PE groups within each condition.

3. Results

3.1. Experiment 1 – The effects of 0.1 mg/kg haloperidol and 5 mg/kg clozapine on NACc lesion-induced persistent LI

3.1.1. Histology

The NACc lesions were consistent among rats in the lesion group and generally similar to the lesion shown in Fig. 1A (bottom panel). Reconstruction of the maximal (gray) and minimal (black) extents of the NACc lesions is presented in Fig. 1B. Histological analysis of the sections in NMDA-injected rats confirmed the presence of a bilateral lesion. Gliosis and cell poor areas typically surrounded the anterior commissure and the cannulae tracks. The lesions extended rostrocaudally approximately from 2.2 to 0.7 mm rostral to bregma, and mediolaterally maximally from 1.2 to 2.4 mm lateral to the midline. The horizontal location of the lesion was typically at about the level of the anterior commissure. Occasionally, the affected area extended rostrally into

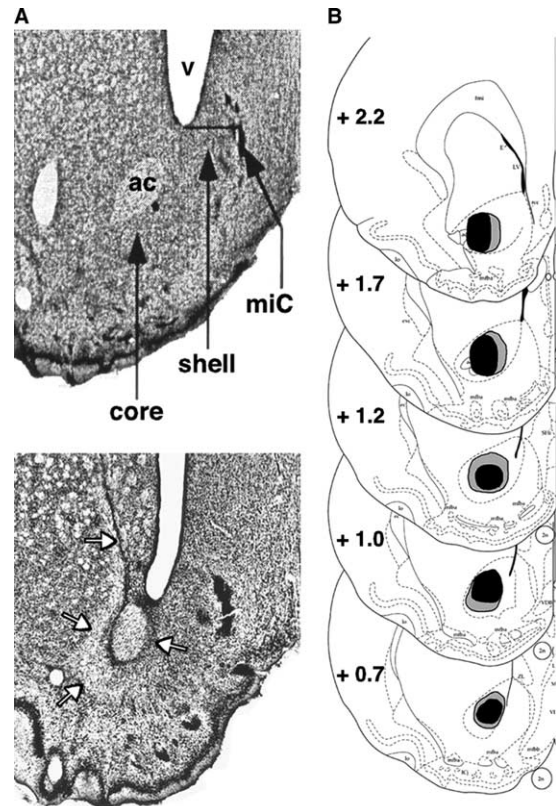


Fig. 1. Photomicrographs and a reconstruction of the region of damage in NACc lesioned rats. (A) Photomicrographs of coronal sections taken through the NACc of the left hemisphere of representative sham (top) and NACc lesioned (bottom) rats. Arrows mark gliosis and cell free area in the NACc and along the injection track. Abbreviations: ac – anterior commissure; miC – main island of Calleja; V – lateral ventricle. (B) Reconstruction of the minimal (black) and maximal (gray) extents of the NACc lesions. Coordinates of the coronal sections are indicated with reference to Bregma according to the stereotaxic atlas of Paxinos and Watson (1998).

the anterior olfactory nucleus and caudally into the bed nucleus of the stria terminalis. The shell of the NAC appeared undamaged. Data of one NACc rat for which there was no discernable damage to the NACc in one hemisphere, and of two NACc rats for which the damage was ventral to the NACc were excluded from statistical analysis. There was no discernable damage in any of the sham-lesioned control rats. Thus, the final analysis included 43 rats (12 sham, 31 NACc; *n* per group = 5–6).

3.1.2. Behavior

Table 1 presents the mean times (in sec) to complete licks 50–75 (A period) and licks 75–100 (B period) in the eight experimental groups. The eight experimental groups did not differ in their times to complete licks 51–75 (all *p*'s > 0.1; overall mean A period = 6.63 s). Fig. 2 presents the mean log times to complete licks 76–100 of the preexposed and nonpreexposed groups in the sham-vehicle, NACc-vehicle, NACc-clozapine

Table 1
Mean times (in seconds) to complete licks 50–75 (A period) and licks 75–100 (B period) in Experiment 1

	A period	B period
Sham-vehicle		
PE (n = 6)	6.9 ± 0.4	229.6 ± 27.9
NPE (n = 6)	9.1 ± 1.5	206.4 ± 25.9
NACc-vehicle		
PE (n = 5)	6.3 ± 0.6	13.8 ± 3.6
NPE (n = 5)	6.3 ± 0.3	259.6 ± 24.9
NACc-clozapine		
PE (n = 6)	6.4 ± 0.3	226.6 ± 29.0
NPE (n = 5)	5.6 ± 0.2	252.9 ± 27.2
NACc-haloperidol		
PE (n = 5)	6.0 ± 0.1	18.0 ± 3.6
NPE (n = 5)	6.5 ± 0.4	222.9 ± 19.5

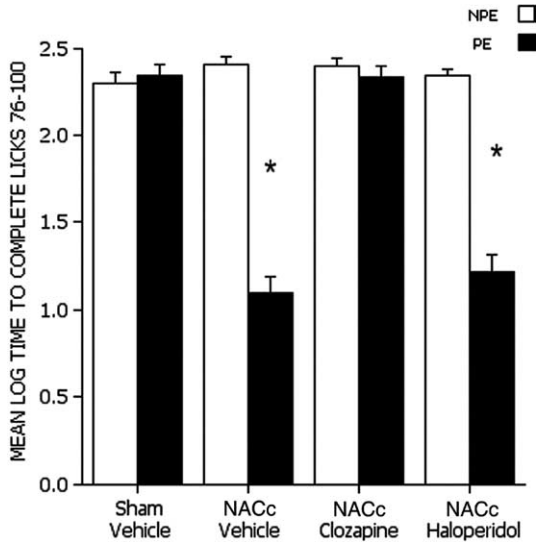


Fig. 2. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the nonpreexposed (NPE) and preexposed (PE) rats in the sham-vehicle, NACc-vehicle, NACc-clozapine and NACc-haloperidol conditions. Asterisk indicate a significant difference between the NPE and PE groups, namely, presence of LI.

and NACc-haloperidol conditions. As can be seen, there was no LI in vehicle-injected sham rats, whereas vehicle-injected NACc rats showed LI. NACc rats injected with haloperidol continued to exhibit LI, but those injected with clozapine did not show LI, similarly to sham rats. This was supported by ANOVA which yielded a significant main effect of preexposure, $F(1,35) = 167.60$, $p < 0.0001$, and condition, $F(3,35) = 49.80$, $p < 0.0001$, and a significant preexposure × condition interaction, $F(3,35) = 56.27$, $p < 0.0001$. Post-hoc comparisons confirmed the existence of LI in the NACc-vehicle, $t(35) = 13.40$, $p < 0.001$, and NACc-haloperidol, $t(35) = 11.33$, $p < 0.001$, conditions, but not in the sham-vehicle and the NACc-clozapine conditions, $t(35) = 0.34$, NS, and $t(35) = 0.60$, NS, respectively.

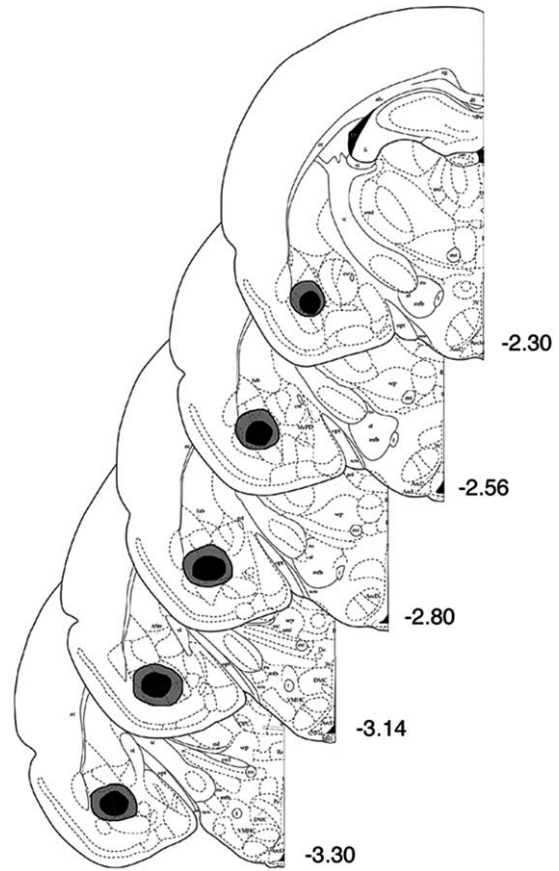


Fig. 3. Reconstruction of the minimal (black) and maximal (gray) extents of the BLA lesions. Coordinates of the coronal sections are indicated with reference to Bregma according to the stereotaxic atlas of Paxinos and Watson (1998).

3.2. Experiment 2 – The effects of 0.1 mg/kg haloperidol and 5 mg/kg clozapine on BLA lesion-induced persistent LI

3.2.1. Histology

Reconstruction of the maximal (gray) and minimal (black) extents of the BLA lesions is presented in Fig. 3. In most rats, the lesions extended rostrocaudally approximately from 2.3 to 3.3 mm caudal to bregma, and mediolaterally maximally from 4.2 to 5.6 mm lateral to the midline. The horizontal location of the lesion was typically at about the level of the bottom of the external capsule. Data of four BLA rats for which there was no discernable damage to the BLA in one hemisphere, and of two BLA rats for which the damage was medial to the BLA were excluded from statistical analysis. There was no discernable damage in any sham-lesioned control rats. Thus, the final analysis included 58 rats (16 sham, 42 BLA; n per group = 6–8).

3.2.2. Behavior

Table 2 presents the mean times (in sec) to complete licks 50–75 (A period) and licks 75–100 (B period) in

Table 2

Mean times (in seconds) to complete licks 50–75 (A period) and licks 75–100 (B period) in Experiment 2

	A period	B period
Sham-vehicle		
PE ($n = 8$)	10.7 ± 1.7	165.6 ± 41.8
NPE ($n = 8$)	7.4 ± 0.5	103.8 ± 31.5
BLA-vehicle		
PE ($n = 6$)	10.4 ± 1.1	53.6 ± 18.2
NPE ($n = 6$)	9.3 ± 1.2	142.5 ± 41.9
BLA-clozapine		
PE ($n = 8$)	9.0 ± 1.1	129.7 ± 37.4
NPE ($n = 8$)	9.6 ± 1.8	151.3 ± 43.8
BLA-haloperidol		
PE ($n = 7$)	8.9 ± 0.6	44.4 ± 10.9
NPE ($n = 7$)	11.7 ± 3.3	162.5 ± 49.0

the eight experimental groups. The six experimental groups did not differ in their times to complete licks 51–75 (all p 's > 0.1; overall mean A period = 9.63 s). Fig. 4 presents the mean log times to complete licks 76–100 of the preexposed and nonpreexposed groups in the sham-vehicle, BLA-vehicle, BLA-clozapine and BLA-haloperidol conditions. As can be seen, there was no LI in vehicle-injected sham rats, whereas vehicle-injected BLA rats showed LI. BLA rats injected with haloperidol continued to exhibit LI, but those injected with clozapine did not show LI, similarly to sham rats. This was supported by ANOVA which yielded a significant main effect of preexposure, $F(1,50) = 4.20$, $p < 0.05$, and a significant preexposure × condition interaction, $F(3,50) = 3.27$, $p < 0.05$. Post-hoc comparisons con-

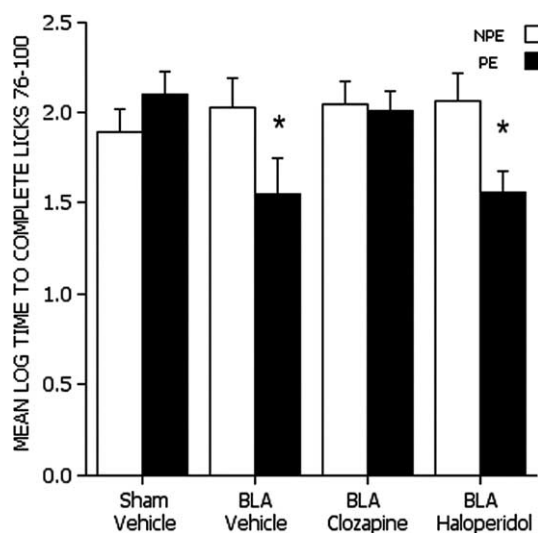


Fig. 4. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the nonpreexposed (NPE) and preexposed (PE) rats in the sham-vehicle, BLA-vehicle, BLA-clozapine and BLA-haloperidol conditions. Asterisks indicate a significant difference between the NPE and PE groups, namely, presence of LI.

firmed the existence of LI in the BLA-vehicle, $t(50) = 2.24$, $p < 0.05$, and BLA-haloperidol, $t(50) = 2.56$, $p < 0.05$, conditions, but not in the sham-vehicle and the BLA-clozapine conditions, $t(50) = 0.15$, NS, and $t(50) = 0.17$, NS, respectively.

3.3. Experiment 3 – The effects of 0.1 mg/kg haloperidol and 5 mg/kg clozapine on OFC lesion-induced persistent LI

3.3.1. Histology

The OFC lesions were consistent among rats in the lesion group and generally similar to the lesion shown in Fig. 5A (right panel). Reconstruction of the maximal (gray) and minimal (black) extents of the OFC lesions is presented in Fig. 5B. Histological analysis of the sections in NMDA-injected rats confirmed the presence of a bilateral lesion. Gliosis and cell poor areas were typically found in the ventral and lateral orbital cortex and occasionally extended to the dorsolateral orbital and agranular insular cortex. The lesions extended rostrocaudally approximately from 4.7 to 2.7 mm rostral to bregma, and mediolaterally maximally from 0.6 to 3.4 mm lateral to the midline. The horizontal location of the lesion was typically at about the level of the dorsal bank of the rhinal fissure. Data of one OFC rat for which there was no discernable damage to the OFC in one hemisphere were excluded from statistical analysis. There was no discernable damage in any of the sham-lesioned control rats. Thus, the final analysis included forty-seven rats (12 sham, 35 OFC; n per group = 5–6).

3.3.2. Behavior

Table 3 presents the mean times (in sec) to complete licks 50–75 (A period) and licks 75–100 (B period) in the eight experimental groups. The eight experimental groups did not differ in their times to complete licks 51–75 (all p 's > 0.1; overall mean A period = 11.06 s). Fig. 6 presents the mean log times to complete licks 76–100 of the preexposed and nonpreexposed groups in the sham-vehicle, OFC-vehicle, OFC-clozapine and OFC-haloperidol conditions. As can be seen, there was no LI in vehicle-injected sham rats, whereas vehicle-injected OFC rats showed LI. OFC rats injected with haloperidol continued to exhibit LI, but those injected with clozapine did not show LI, similarly to sham rats. These observations were supported by ANOVA which yielded a significant main effect of preexposure, $F(1,39) = 12.05$, $p < 0.01$, and a significant preexposure × condition interaction, $F(3,39) = 2.90$, $p < 0.05$. Post-hoc comparisons confirmed the existence of LI in the OFC-vehicle, $t(39) = 3.12$, $p < 0.01$, and OFC-haloperidol, $t(39) = 3.22$, $p < 0.01$, conditions, but not in the sham-vehicle and the OFC-clozapine conditions, $t(39) = 0.29$, and $t(39) = 0.24$, NS, respectively.

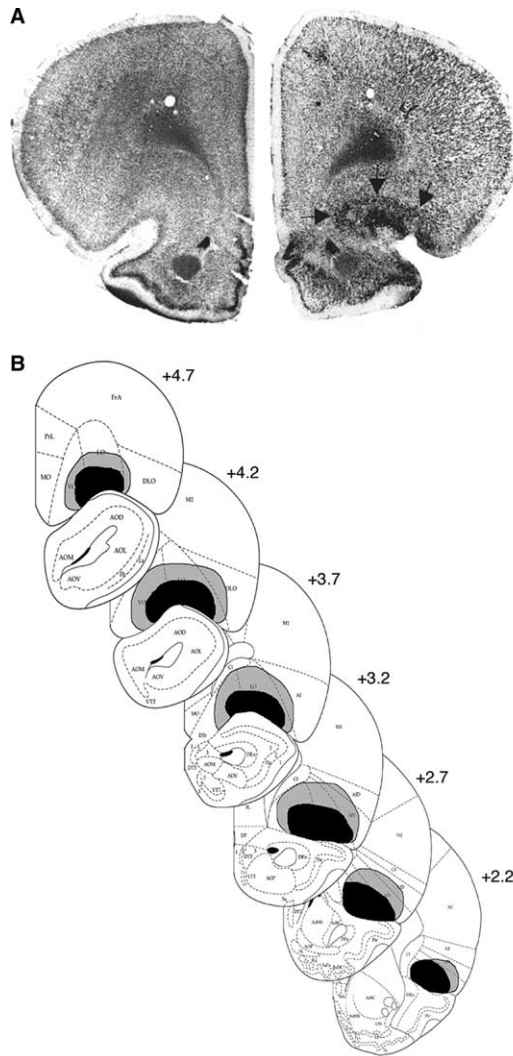


Fig. 5. Photomicrographs and a reconstruction of the region of damage in OFC lesioned rats. (A) Photomicrographs of coronal sections taken through the OFC in representative sham (left) and OFC lesioned (right) rats. Arrows denote lesion borders. (B) Reconstruction of the minimal (black) and maximal (gray) extents of the OFC lesions. Coordinates of the coronal sections are indicated with reference to Bregma according to the stereotaxic atlas of Paxinos and Watson (1998).

3.4. Experiment 4 – The effects of 0.3 mg/kg M100907 on NACc lesion-induced persistent LI

3.4.1. Histology

The lesions were very similar to those seen in Experiment 1. Data of three NACc rats for which there was no discernable damage to the NACc in one hemisphere were excluded from statistical analysis. There was no discernable damage in any sham-lesioned control rats. Thus, the final analysis included 41 rats (20 sham, 21 NAC; n per group = 5–6).

3.4.2. Behavior

Table 4 presents the mean times (in sec) to complete licks 50–75 (A period) and licks 75–100 (B period) in

Table 3

Mean times (in seconds) to complete licks 50–75 (A period) and licks 75–100 (B period) in Experiment 3

	A period	B period
Sham-vehicle		
PE ($n = 6$)	11.3 ± 2.2	204.7 ± 60.3
NPE ($n = 6$)	12.2 ± 3.2	159.3 ± 45.4
OFC-vehicle		
PE ($n = 6$)	10.0 ± 2.1	61.3 ± 47.8
NPE ($n = 6$)	10.4 ± 2.5	206.9 ± 48.2
OFC-clozapine		
PE ($n = 6$)	10.9 ± 2.9	192.4 ± 50.2
NPE ($n = 6$)	11.0 ± 3.3	196.4 ± 46.7
OFC-haloperidol		
PE ($n = 6$)	10.2 ± 3.3	40.9 ± 20.0
NPE ($n = 5$)	12.5 ± 4.2	214.4 ± 39.5

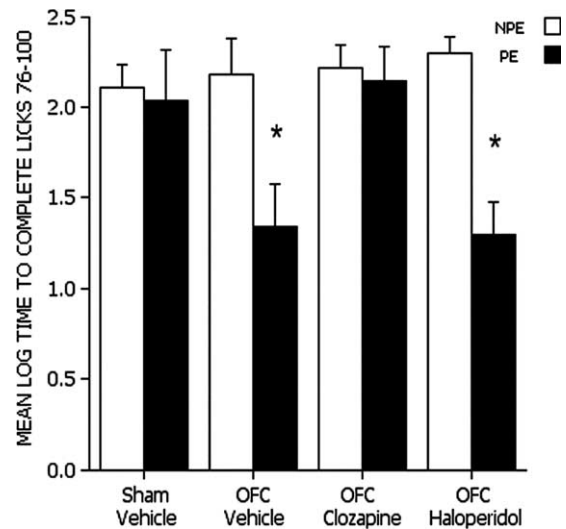


Fig. 6. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the nonpreexposed (NPE) and preexposed (PE) rats in the sham-vehicle, OFC-vehicle, OFC-clozapine and OFC-haloperidol conditions. Asterisks indicate a significant difference between the NPE and PE groups, namely, presence of LI.

Table 4

Mean times (in seconds) to complete licks 50–75 (A period) and licks 75–100 (B period) in Experiment 4

	A period	B period
Sham-vehicle		
PE ($n = 5$)	9.9 ± 1.2	205.3 ± 58.1
NPE ($n = 5$)	9.8 ± 2.4	266.6 ± 33.4
Sham-M100907		
PE ($n = 5$)	10.0 ± 1.5	208.8 ± 57.2
NPE ($n = 5$)	6.9 ± 0.3	262.0 ± 38.0
NACc-vehicle		
PE ($n = 5$)	12.5 ± 5.4	35.9 ± 6.6
NPE ($n = 5$)	8.9 ± 1.4	245.0 ± 34.1
NACc-M100907		
PE ($n = 5$)	17.6 ± 8.4	236.8 ± 38.7
NPE ($n = 6$)	8.8 ± 1.6	192.9 ± 48.3

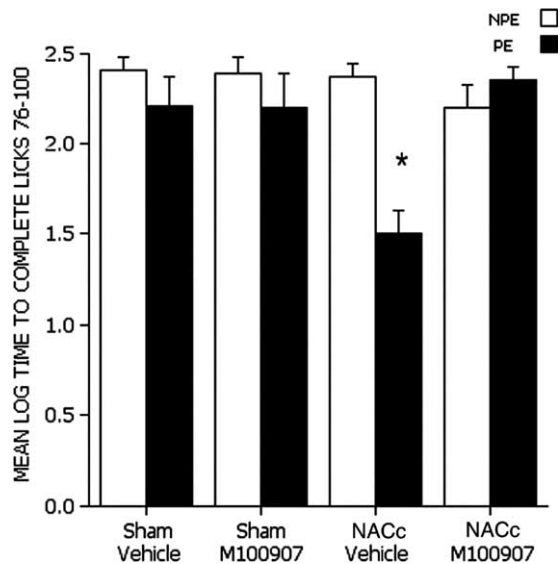


Fig. 7. Means and standard errors of the log times to complete licks 76–100 (after tone onset) of the nonpreexposed (NPE) and preexposed (PE) rats in the sham-vehicle, sham-M100907, NACc-vehicle and NACc-M100907 groups. Asterisks indicate a significant difference between the NPE and PE groups, namely, presence of LI.

the eight experimental groups. The eight experimental groups did not differ in their times to complete licks 51–75 (all p 's > 0.1; overall mean A period = 10.55 s). Fig. 7 presents the mean log times to complete licks 76–100 of the preexposed and nonpreexposed groups in the sham-vehicle, sham-M100907, NACc-vehicle and NACc-M100907 conditions. As can be seen, there was no LI in sham rats injected with vehicle or M100907. NACc rats injected with vehicle showed LI, but those injected with M100907 did not show LI, similarly to sham rats. This was supported by ANOVA which yielded significant main effects of preexposure, $F(1,33) = 10.14$, $p < 0.01$, and lesion, $F(1,33) = 4.99$, $p < 0.05$, as well as significant preexposure \times drug, $F(1,33) = 8.66$, $p < 0.01$, and preexposure \times lesion \times drug, $F(1,33) = 8.23$, $p < 0.01$, interactions. Post-hoc comparisons confirmed the existence of LI in the NACc-vehicle condition, $t(33) = 4.93$, $p < 0.001$, but not in the sham-vehicle, sham-M100907 and NACc-M100907 conditions, $t(33) = 1.15$, NS, $t(33) = 1.08$, NS, and $t(33) = 0.88$, NS, respectively.

4. Discussion

The present study confirms our recent findings (Schiller and Weiner, 2004, submitted) that rats sustaining NACc, BLA or OFC lesions, exhibit LI under conditions that disrupt LI in sham controls. More specifically, sham-operated rats that received 40 tone preexposures followed by five tone-shock conditioning trials, showed levels of suppression comparable to those of their nonpreexposed counterparts, i.e., did not show LI, but rats with NACc,

BLA, or OFC lesions, exhibited LI in spite of repeated pairings of the stimulus with shock. Importantly, all three lesions led to the emergence of LI exclusively via reducing conditioning in the preexposed group while having no effect on conditioning in the nonpreexposed group. Thus, the lesions did not impair the capacity to acquire the stimulus-no event contingency (in the lesioned PE groups) or the stimulus-reinforcement contingency (in the lesioned NPE groups). Rather, they specifically impaired rats' capacity to shift responding from the stimulus-no event association to the stimulus-reinforcement association under conditions triggering such a shift in controls. In other words, lesioned rats perseverated in ignoring the preexposed stimulus under conditions in which normal rats switched to treating it as relevant.

As predicted, persistent LI in NACc, BLA and OFC lesioned rats was reversed (disrupted) by clozapine but not by haloperidol administered in preexposure, so that clozapine-treated lesioned rats had no LI like sham controls. The latter effect was seen also in NACc lesioned rats administered with M100907. Although clozapine has a wide receptor profile (Arnt and Skarsfeldt, 1998; Leysen et al., 1993; Meltzer, 1989), the preexposure-based disruption of LI is likely to reflect its 5HT2A antagonism, because such disruption is not produced by the DA2 blocker haloperidol, but is produced by the mixed DA2/5HT2A antagonist risperidone (Weiner et al., 2003a) as well as by the selective 5HT2A receptor antagonists ritanserin (Shadach et al., 2000) and M100907 (the present study; Zuckerman et al., 2001). This is in line with the extant data indicating that serotonergic manipulations exert their effects on LI via the preexposure stage (for review, see Moser et al., 2000; Weiner, 2003). It is worth pointing out in this context that since NACc, BLA and OFC lesion-induced LI persistence is apparently due to impaired response flexibility in conditioning, reversal of lesions-induced persistent LI by clozapine cannot be due to a direct interaction between its 5HT2A antagonism and the underlying lesion-induced dysfunction, but rather is likely to reflect interactions within the complex fore-brain circuitry that modulates LI (Weiner, 2003). That is, persistent LI induced by these lesions may depend on mechanisms that differ from but interact with mechanisms through which drugs such as clozapine and M100907 exert their effects.

The lack of effect of haloperidol should be treated with more caution. At the dose used here (0.1 mg/kg) haloperidol is a selective D2 blocker (Leysen et al., 1993; Schotte et al., 1996), and its lack of effect is therefore consistent with findings that DA mechanisms are not involved in preexposure (Weiner, 2003). However, given that at higher doses haloperidol loses such selectivity, and in fact, at doses ranging between 1.5 and 2.8 mg/kg becomes a 5HT2A antagonist (Leysen et al., 1993), there remains a possibility that it would act like clozapine. If this were

the case, then persistent LI would provide a tool for dissociating typical and atypical APDs as a function of their relative affinity for 5HT_{2A} receptors and by corollary, their 5HT₂/D₂ affinity ratio.

As pointed out in the Introduction, a differential pre-exposure-based effect of haloperidol and clozapine at the doses used here was previously demonstrated by us in rats treated with MK-801 that, like lesioned rats here, persisted in exhibiting LI under conditions that disrupted LI in controls (Gaisler-Salomon and Weiner, 2003), as well as in intact rats under parameters that yielded LI (Shadach et al., 2000; Weiner et al., 2003a). The present results show that these two classes of APDs can also be dissociated on the background of a NACc, BLA and OFC lesion-induced persistent LI, although this is restricted to the specific doses tested. The latter implies that persistent LI induced by a variety of pharmacological and lesion manipulations and normal LI share common processes that are responsive to clozapine. These are apparently the processes related to the acquisition of the stimulus-no event association in the preexposure stage, which are sensitive to serotonergic manipulations.

The present results have two central implications. The first concerns the behavioral indices of antipsychotic activity in the LI model. The conventional index of antipsychotic activity in the LI model, shared by typical and atypical APDs, has been potentiation of weak LI. The present data complement previous results (Gaisler-Salomon and Weiner, 2003; Lipina et al., 2005; Shadach et al., 2000; Weiner et al., 2003a) in indicating that the LI model may provide an additional behavioral index, disruption of strong LI, that may have predictive validity for atypical antipsychotic action that is distinct from the typical (LI potentiating) antipsychotic action. Moreover, the capacity of atypical APDs to reverse abnormally persistent LI endows APD-induced LI disruption with “therapeutic” relevance, because disruption of abnormally persistent LI normalizes animals’ behavior. Finally, the fact that NACc, BLA and OFC lesion-induced persistent LI was selectively reversed by clozapine, supports the relevance of this phenomenon to negative symptoms, because it is commonly asserted that an animal model which is sensitive to atypical but not typical APDs may have predictive validity for the latter condition/s (Arnt and Skarsfeldt, 1998; Brunello et al., 1995; Kinon and Lieberman, 1996), in line with clinical data suggesting higher efficacy of atypical APDs against negative symptoms (e.g., Meltzer and Sumiyoshi, 2003; Moller, 2003; but see Geddes et al., 2000; Kapur and Remington, 2001). Clearly, these implications are restricted to the specific doses used to date to demonstrate the preexposure-based dissociation between haloperidol and atypical APDs. Thorough dose-response studies are needed in order to establish whether such a dissociation is reliable, and to ascertain that the disruptive effect is 5HT mediated. Likewise, further studies are needed to

test additional typical and atypical compounds in the persistent LI model in order to validate its capacity to differentiate between the two classes of APDs.

The second implication concerns the relevance of abnormally persistent LI to the LI model of schizophrenia. While most clinical reports have been consistent in indicating that LI disruption is associated with acute schizophrenia (Baruch et al., 1988; Gray et al., 1992, 1995; Rascle et al., 2001; Vaitl and Lipp, 1997; Vaitl et al., 2002), the fact that LI is intact and indeed may be restored to normal in chronic schizophrenia patients (Gray et al., 1995), coupled with reports on intact LI also in acute patients (Swerdlow et al., 1996; Williams et al., 1998), has remained puzzling and has been taken to weaken the strength of the LI model. The findings in rats that certain pharmacological and lesion manipulations, rather than disrupting LI, produce an abnormally persistent LI (Holt and Maren, 1999; Gaisler-Salomon and Weiner, 2003; Schiller and Weiner, 2004), indicate that abnormality of LI is not exclusively manifested as a loss of this phenomenon but also as its abnormal persistence, raising the possibility that “normal” LI seen in chronic schizophrenia patients is actually “persistent” LI. The direct relevance of abnormally persistent LI to the clinical condition derives from results of two recent studies, which have demonstrated excessively strong LI in schizophrenia patients, and showed that this abnormality was positively correlated with the severity of negative symptoms (Cohen et al., 2003; Rascle et al., 2001). These findings support the possibility that persistent LI in the rat may model a cognitive deficit associated with negative symptoms of schizophrenia (Weiner, 2003; Weiner and Feldon, 1997; Weiner and Joel, 2002). In this context, the present results imply that the NACc, the BLA and the OFC may be components of a circuitry whose dysfunction plays a role in negative symptoms, consistent with the prominent role given to these areas in numerous accounts of neuropathology of schizophrenia (Anderson et al., 2002; Baaré et al., 1999; Carlsson and Carlsson, 1990; Gray et al., 1991; Gur et al., 2000; Haber and Fudge, 1997; O’Donnell and Grace, 1998; Sanfilippo et al., 2000; Swerdlow and Koob, 1987). Relatedly, our results suggest that enhanced LI seen in patients with negative symptoms may be underlied by dysfunction of these same brain regions. Further studies in rats using procedures enabling a selective disconnection of selected brain regions are needed to reveal essential interactions between the NACc, the BLA, and the OFC in LI.

To date, the study of LI has focused on disrupted LI and its reversal by APDs as a correlate of a cognitive dysfunction associated with positive symptoms of schizophrenia and an index of typical antipsychotic activity, respectively. The present study reported two phenomena, abnormally persistent LI and its selective reversal by an atypical APD, which were suggested to provide a correlate of a cognitive dysfunction associated with

negative symptoms of schizophrenia and atypical antipsychotic activity, respectively. If confirmed in dose-response studies and with additional APDs, and given that schizophrenia patients can exhibit persistent LI, the present findings could provide a basis for an animal model that would provide valuable cues on dysfunctional brain circuits involved in negative symptoms of schizophrenia and the action of extant and novel atypical APDs.

Acknowledgements

We thank Novartis (Switzerland), Janssen (Belgium) and Sanofi-Aventis (USA) for their generous gifts of clozapine, haloperidol, and M100907, respectively. This research was supported in part by the Wolf Foundation award to D.S.

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