

Effects of haloperidol and clozapine on sensorimotor gating deficits induced by 5-hydroxytryptamine depletion in the brain

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1 Evidence is increasing for a role of brain 5-hydroxytryptamine (5-HT) systems in schizophrenia. We previously showed that brain 5-HT depletion causes disruption of prepulse inhibition, a measure of sensorimotor gating that is deficient in schizophrenia. Antipsychotic treatment has been reported to reverse these deficits in patients with schizophrenia. The present study was designed to investigate the ability of antipsychotic drugs to reverse prepulse inhibition deficits caused by lesions of the brain 5-HT system in rats.

2 In male Sprague–Dawley rats, selected parts of the brain 5-HT systems were lesioned by micro-injection of the 5-HT neurotoxin 5,7-dihydroxytryptamine into the dorsal raphe nucleus (DRN) or median raphe nucleus (MRN). The effects of antipsychotic drugs on lesion-induced changes in prepulse inhibition were examined 2 weeks after the surgery.

3 There was significant disruption of prepulse inhibition in the MRN-lesioned group compared to sham-operated controls. This deficiency in prepulse inhibition was restored by clozapine (1 and 5 mg kg⁻¹) treatment, and by treatment with a relatively high dose of haloperidol (0.25 mg kg⁻¹). There was no significant effect of the DRN lesions on prepulse inhibition compared with sham-operated controls.

4 These results indicate that 5-HT depletion in MRN-innervated brain structures leads to disruption of prepulse inhibition. Treatment with both antipsychotic drugs, haloperidol and clozapine, significantly increased prepulse inhibition in these animals back to the level seen in sham-operated controls. The present findings highlight the importance of the 5-HT systems in cognitive models of schizophrenia.

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Abbreviations: ANOVA, analysis of variance; 5,7-DHT, 5,7-dihydroxytryptamine; DRN, dorsal raphe nucleus; MRN, median raphe nucleus; PP, prepulse intensity; s.e.m., standard error of the mean

Introduction

Schizophrenia is a chronic and severe psychiatric illness characterized by hallucinations, delusions, cognitive impairment and thought disorders (Breier, 1999; Millan, 2000; Rowley *et al.*, 2000). Despite many years of research, mechanisms underlying the development of schizophrenia remain unclear. One established hypothesis of schizophrenia involves overactivity in the mesolimbic/mesocortical dopaminergic systems (Carlsson & Lindqvist, 1963; Spanagel & Weiss, 1999). However, there is emerging evidence to suggest that dysfunction of the dopamine system may be due to pathologic changes in other neurotransmitter systems, particularly 5-hydroxytryptamine (5-HT) (Harrison, 1999; Dean, 2000). Post-mortem studies have shown significant alterations in 5-HT systems in schizophrenia. For example, the density of 5-HT_{2A} receptors was reduced in the frontal cortex of subjects with schizophrenia (Dean & Hayes, 1996), while density of 5-HT_{1A} receptors was increased in this region (Bantick *et al.*, 2001).

In order to characterize the role of brain 5-HT activity in schizophrenia, animal behavioural models are used. Prepulse

inhibition of acoustic startle is a model of sensorimotor gating and sensory information processing (Geyer *et al.*, 1990; Swerdlow & Geyer, 1998). In schizophrenia and other neuropsychiatric disorders, there is a deficiency in prepulse inhibition, which may lead to sensory flooding and cognitive fragmentation (Geyer *et al.*, 1990; Swerdlow *et al.*, 1994). In the prepulse inhibition model, a sudden, relatively intense stimulus, such as a loud tone, will induce a startle response. A sensory input will normally initiate a short-term inhibition of responses to a subsequent stimulus. Therefore, when a startle-producing stimulus is preceded by a weak prepulse, the reflex response is significantly reduced, that is, prepulse inhibition (Wiley, 1994; Geyer & Swerdlow, 1998).

Prepulse inhibition is modulated by several brain systems, such as the mesolimbic dopaminergic system (Geyer *et al.*, 1990). In addition, 5-HT in the brain plays an important role in the regulation of prepulse inhibition (Davis *et al.*, 1980; Geyer *et al.*, 1990; Fletcher *et al.*, 2001; Prinssen *et al.*, 2002). Treatment with the serotonin 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) or serotonin releasers, such as 3,4-methylenedioxyamphetamine (MDMA) and fenfluramine, disrupted prepulse inhibition in

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rats (for a review, see Geyer *et al.*, 2001). Most 5-HT projections in the forebrain originate in the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN) (Azmitia & Whitaker-Azmitia, 1995). The DRN projects to the frontal cortex, ventral hippocampus and striatal regions (Adell & Myers, 1995; McQuade & Sharp, 1997; Mokler *et al.*, 1998), while the MRN projects to the dorsal hippocampus and cingulate cortex (Mokler *et al.*, 1998; Thomas *et al.*, 2000). In a previous study, we showed that 5-HT projections arising from these two distinct raphe nuclei are differentially involved in the regulation of prepulse inhibition (Kusljic *et al.*, 2003). Rats with MRN lesions were found to display marked and significant disruption of prepulse inhibition, whereas disruption of prepulse inhibition in the DRN-lesioned rats was smaller and dependent on prepulse intensity (PP) (Kusljic *et al.*, 2003).

Clinically effective antipsychotic drugs display antagonist activity at dopamine D₂-like receptors in the brain (Seeman, 1987; Ellenbroek *et al.*, 1991). Compared to typical antipsychotic drugs, such as haloperidol, atypicals, such as clozapine, display a relatively low potency at dopamine D₂-like receptors, but high affinity for a number of 5-HT receptor subtypes, muscarinic (M1–M3), alpha adrenergic and histaminergic H₁ receptors (O'Dell *et al.*, 1990; Barnes & Sharp, 1999). In rats, typical and atypical antipsychotic drugs have usually been investigated for their ability to antagonize drug-induced prepulse inhibition deficits. For example, the deficits in prepulse inhibition produced by NMDA receptor antagonists appeared to be more sensitive to clozapine-like atypical antipsychotic drugs than to typical antipsychotic drugs (for a review see (Geyer *et al.*, 2001). Furthermore, atypical antipsychotic drugs seem to be more potent in restoring the sensorimotor gating deficits seen in schizophrenia patients than typical antipsychotic drugs (Geyer *et al.*, 2001; Kumari & Sharma, 2002; Oranje *et al.*, 2002).

The aim of the present study was to investigate the ability of antipsychotic drugs to reverse deficits in prepulse inhibition observed in rats with DRN lesions or MRN lesions. After 5,7-dihydroxytryptamine (5,7-DHT) lesions of the DRN or MRN, rats were tested for prepulse inhibition with or without treatment with the typical antipsychotic, haloperidol, or the atypical antipsychotic drug, clozapine. Haloperidol and clozapine were used as a representative example of clinically effective antipsychotic drugs.

Methods

Animals

Experiments were carried out on 28 male Sprague–Dawley rats (Department of Pathology, University of Melbourne, Australia), weighing 250–300 g at the time of the surgery. The rats were housed under standard conditions in groups of 2–3, with free access to food and water. They were maintained on a 12:12 h light/dark cycle (lights on at 0700 h) at a constant temperature of 21°C. The experimental protocol and surgical procedures were approved by the Animal Experimentation Ethics Committee of the University of Melbourne, Australia.

Surgery

Rats were pretreated with 20 mg kg⁻¹ of desipramine, 30 min prior to lesions, to prevent destruction of noradrenergic

neurons by 5,7-DHT (Jonsson, 1980), and anaesthetized with sodium pentobarbitone (60 mg/kg intraperitoneally (i.p.), Rhone Merieux, QLD, Australia). The rat was mounted in a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA, U.S.A.) with the incisor bar set at -3.3 mm (Paxinos & Watson, 1998). The stereotaxic surgery was performed as described previously (Kusljic *et al.*, 2003). Briefly, a measure of 1 µl of 5 µg µl⁻¹ of 5,7-DHT was microinjected by hand, using a micrometer, over a period of 2 min. With bregma as zero and the stereotaxic arm at 25°, the coordinates were as follows: for the DRN: -8.0 mm posterior, +2.9 mm lateral and -6.8 mm ventral of bregma; for the MRN: -8.0 mm posterior, +3.7 mm lateral and -8.8 mm ventral of bregma. Sham-operated controls, comprising an equal number of DRN or MRN injections, underwent the same surgical procedure and received an equal volume of vehicle solution containing ascorbic acid. The animals were subcutaneously (s.c.) administered with 5 mg kg⁻¹ of carprofen (Heriot AgVet, Rowville, VIC, Australia), a nonsteroidal, anti-inflammatory analgesic, to reduce postoperative inflammation and discomfort. Rats were placed on a heat pad until they recovered from anaesthesia. After surgery, rats were allowed to recover for 2 weeks, during which they were handled and health checks were made 2–3 times a week.

Prepulse inhibition of the acoustic startle reflex and experimental design

Prepulse inhibition experiments were performed starting 2 weeks after the surgery. The study included 10 DRN-lesioned rats, 10 MRN-lesioned rats and eight sham-lesioned rats. To determine the ability of antipsychotic drugs to reverse the disruption of prepulse inhibition caused by 5-hydroxytryptaminergic lesions, rats were pretreated with saline, haloperidol or clozapine (i.p.) 15 min before being placed in the automated chambers. Two doses of haloperidol (0.05, 0.25 mg kg⁻¹) and two doses of clozapine (1, 5 mg kg⁻¹) were used. These doses were chosen on the basis of our preliminary dose–response experiments and literature (Keith *et al.*, 1991; Swerdlow *et al.*, 1991; Swerdlow & Geyer, 1993; Bakshi *et al.*, 1994). Thus, each rat was tested for prepulse inhibition five times with 3–4 days interval, with the sequence of treatments being randomized.

Prepulse inhibition testing was carried out using six automated startle chambers (SR-LAB, San Diego Instruments, San Diego, CA, U.S.A.) consisting of clear Plexiglas cylinders, 9 cm in diameter, resting on a platform inside a ventilated, sound-attenuated and illuminated chamber. A speaker, mounted 24 cm above the cylinder, produced both continuous background noise of 70 dB and acoustic stimuli. Whole-body startle responses of the animal in response to acoustic stimuli caused vibrations of the Plexiglas cylinder, which were then converted to quantitative responses by a piezoelectric accelerometer unit attached underneath the platform. Percentage prepulse inhibition was calculated as $100 \times (\text{response to pulse-alone trials} - \text{response to prepulse + pulse trials}) / (\text{response to pulse-alone trials})$ (Geyer & Swerdlow, 1998).

A single prepulse inhibition session lasted for about 45 min and consisted of high- and low-intensity stimulus combinations with a continuous background noise of 70 dB. The session started and ended with a block of 10 pulse-alone trials. These blocks, together with 20 randomly presented pulse-alone

trials during the prepulse inhibition protocol, were used to calculate basal startle reactivity and startle habituation. Prepulse inhibition was assessed by random presentation of 115 dB pulses and 10 of each of prepulse-2, -4, -8, -12 and -16 and 10 of 'no-stim'. For example, prepulse-8 (PP8) is a 20 ms prepulse of 8 dB above the background noise, that is, 78 dB, followed 100 ms later by a 40 ms 115 dB pulse (van den Buuse & Eikelis, 2001; van den Buuse *et al.*, 2004).

Measurement of 5-HT concentrations

At the end of the experiments, rats were killed by decapitation and brains were removed from the skull. Brain structures were dissected on a cold plate and the following tissue samples were weighed and stored until biochemical assays were carried out: frontal cortex, striatum, hypothalamus, dorsal hippocampus, ventral hippocampus and cerebellum (Gispén *et al.*, 1972). The pattern of 5-HT depletion after microinjection of 5,7-DHT was determined using high-performance liquid chromatography (HPLC). HPLC was carried out as described previously (Kusljic *et al.*, 2005). Briefly, the tissue samples were homogenized in 500 μ l of 0.1 M perchloric acid by ultrasonication and centrifuged at 15,500 $\times g$ for 5 min. A 50 μ l aliquot of the supernatant was injected into the HPLC system to determine the content of 5-HT (ng mg⁻¹ tissue of wet weight). Each run was 8 min and the retention time for 5-HT was 2.6 min.

Drugs and solutions

Haloperidol (Serenace[®], 5 mg ampoules, Searle Laboratories, Crows Nest, NSW, Australia) was diluted to the required dose in 0.9% saline. Clozapine (Sigma Chemical Co., St Louis, MO, U.S.A.) was dissolved in 0.1 N HCl, diluted with saline and pH adjusted to as close to neutrality as possible. Desipramine HCl (Sigma) was dissolved in distilled water to 20 mg ml⁻¹ and injected i.p. All treatments were administered in an injection volume of 1 ml kg⁻¹ of body weight. The neurotoxin 5,7-DHT (Sigma) was dissolved in 0.1% ascorbic acid (BDH Chemicals, Kilsyth, VIC, Australia) in saline to prevent oxidation of the neurotoxin.

Data analysis

Data were expressed as the mean \pm the standard error of the mean (s.e.m.). Statistical analysis was performed using the statistical software package SYSTAT 9.0 (SPSS Inc., Chicago,

IL, U.S.A.). All data were analysed with analysis of variance (ANOVA), with repeated measures where appropriate. In the prepulse inhibition experiments, factors were group (sham, DRN-lesioned or MRN-lesioned), drug (five treatments) and habituation (four blocks of 10 startle responses) or group (sham, DRN-lesioned or MRN-lesioned), drug (five treatments) and prepulse (five different PPs), where drug, habituation and prepulse were repeated-measures factors. A main effect of prepulse was seen in all experiments and is not reported in detail here, except if an interaction with another statistical factor was found.

For HPLC measurements, one-way ANOVA was used, followed by Bonferroni-corrected *t*-test comparison. A '*P*'-value of <0.05 was considered to be statistically significant.

Results

Effect of raphe lesions on brain 5-HT concentrations

Micro-injection of 5,7-DHT into the DRN or MRN caused a significant reduction in 5-HT concentration in brain regions innervated by these nuclei (Table 1). 5-HT concentrations in the frontal cortex and striatum were significantly different between the groups ($F_{2,25} = 242.7$, $P < 0.001$ and $F_{2,25} = 531.5$, $P < 0.001$, respectively). *Post-hoc* analysis with Bonferroni-corrected *t*-test showed significantly lower concentrations of 5-HT in the frontal cortex (71% depletion) and striatum (69% depletion) of DRN-lesioned rats compared to either of the other groups (Table 1). 5-HT concentration of the hypothalamus was also significantly different between the groups ($F_{2,25} = 304.6$, $P < 0.001$), with significantly lower concentrations of 5-HT in both DRN-lesioned (64% depletion) and MRN-lesioned rats (71% depletion) compared to controls (Table 1). 5-HT concentration in the dorsal hippocampus was also significantly reduced ($F_{2,25} = 267.8$, $P < 0.001$) in both DRN- and MRN-lesioned rats. However, depletion in 5-HT concentration was greater in MRN-lesioned rats (73% depletion) than in DRN-lesioned rats (34% depletion). In contrast, significant depletion of 5-HT in the ventral hippocampus ($F_{2,25} = 221.7$, $P < 0.001$) was more pronounced in DRN-lesioned rats (76% depletion) than in MRN-lesioned rats (33% depletion). 5-HT concentrations in cerebellum were significantly reduced ($F_{2,25} = 128.6$, $P < 0.001$) both in DRN-lesioned (37%) and in MRN-lesioned rats (50%) when compared to sham-operated controls.

Table 1 5-HT content of dissected brain regions of rats with 5,7-DHT induced lesions of the DRN or MRN when compared with sham-operated controls

Brain region	Sham (n = 8)	DRN-lesioned (n = 10)	MRN-lesioned (n = 10)
Frontal cortex	0.75 \pm 0.03	0.22 \pm 0.02*	0.74 \pm 0.02
Striatum	0.85 \pm 0.02	0.27 \pm 0.01*	0.85 \pm 0.01
Hypothalamus	2.19 \pm 0.05	0.80 \pm 0.04*	0.63 \pm 0.05*
Dorsal hippocampus	1.23 \pm 0.02	0.82 \pm 0.03*	0.33 \pm 0.02*
Ventral hippocampus	1.32 \pm 0.04	0.32 \pm 0.04*	0.89 \pm 0.02*
Cerebellum	0.64 \pm 0.02	0.40 \pm 0.01*	0.32 \pm 0.01*

Data values represent mean \pm s.e.m. tissue 5-HT concentration expressed as ng mg⁻¹ of tissue wet weight obtained from six brain areas of sham-operated, DRN-lesioned and MRN-lesioned rats. Differences between the groups were analysed by ANOVA followed by Bonferroni-corrected *t*-test comparison, * $P < 0.001$.

Effect of 5,7-DHT lesions on baseline startle habituation and prepulse inhibition

Startle amplitude (four blocks of 10 trials) was different between the groups (Figure 1a). When analyzing combined data after saline treatment from all three groups, ANOVA indicated a strong trend for a main effect of group on startle amplitude ($F_{2,25} = 3.4$, $P = 0.051$). While MRN-lesioned rats tended to display higher startle responses compared to controls (main effect of group $F_{1,16} = 3.8$, $P = 0.068$), there was no effect in DRN-lesioned rats. There was significant habituation of the startle response (main effect of block $F_{3,75} = 6.9$, $P < 0.001$), but the lack of a group \times habituation interaction indicated no effect of the lesions on startle habituation (Figure 1a).

Prepulse inhibition experiments showed that in all groups an increase in PP led to a proportional reduction of the startle response and consequently to a proportional degree of percentage inhibition (Figure 1b). Thus, there was a significant main effect of PP ($F_{4,100} = 127.9$, $P < 0.001$). When comparing

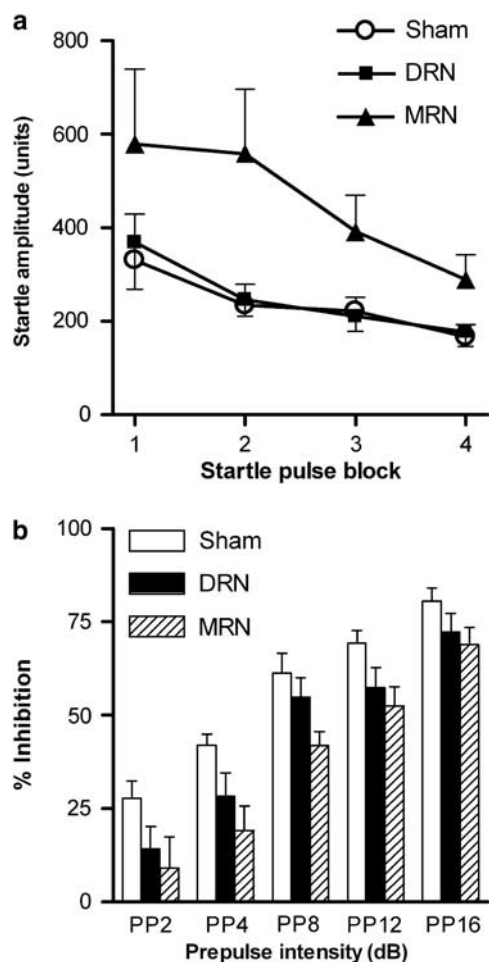


Figure 1 Effect of sham surgery ($n = 8$) or 5,7-DHT lesions of the DRN ($n = 10$) or MRN ($n = 10$) on baseline startle habituation (a) and baseline prepulse inhibition (b). Data are expressed as mean startle amplitudes (arbitrary units) \pm s.e.m. for each of the four blocks of 10 115-dB pulses and as % inhibition \pm s.e.m., respectively. ANOVA indicated a main effect of lesion when comparing prepulse inhibition data from sham-operated and MRN-lesioned rats ($P = 0.007$).

all three groups, there was a significant main effect of group ($F_{2,25} = 3.9$, $P = 0.034$) on baseline prepulse inhibition. Further ANOVAs were conducted on data from DRN-lesioned rats and sham-operated controls and on data from MRN-lesioned rats and sham-operated controls. In the MRN-lesioned group, prepulse inhibition was significantly disrupted and this effect was not dependent on the PP (main effect of group $F_{1,16} = 9.4$, $P = 0.007$) (Figure 1b). Further analysis revealed that prepulse inhibition was significantly reduced in MRN-lesioned rats at PP4, PP8 and PP12 (Figure 1b). There were no significant differences between the DRN-lesioned rats and sham-operated controls.

Effect of antipsychotic drugs on startle habituation

ANOVA of startle data from all three lesion groups and all treatments revealed significant main effects of group ($F_{2,25} = 3.9$, $P = 0.033$), drug ($F_{4,100} = 10.9$, $P < 0.001$) and block ($F_{3,75} = 19.3$, $P < 0.001$) and an interaction of drug \times block ($F_{12,300} = 1.8$, $P = 0.043$).

When comparing habituation data from DRN-lesioned rats and sham-operated controls, there were significant main effects of Drug ($F_{4,64} = 8.0$, $P < 0.001$) and block ($F_{3,48} = 21.8$, $P < 0.001$). Similarly, when comparing habituation data from MRN-lesioned rats and sham-operated controls, there were significant main effects of group ($F_{1,16} = 5.1$, $P = 0.038$), drug ($F_{4,64} = 6.9$, $P < 0.001$) and block ($F_{3,48} = 9.9$, $P < 0.001$).

In sham-operated controls, startle amplitude was reduced after treatment with haloperidol or clozapine (Figure 2a and d). ANOVA of combined drug data from this group indicated a significant main effect of drug ($F_{4,28} = 5.6$, $P = 0.002$) and significant habituation ($F_{3,21} = 9.8$, $P < 0.001$). Pairwise analysis of each drug treatment *versus* saline treatment revealed a significant main effect of 0.25 mg kg⁻¹ of haloperidol ($F_{1,7} = 9.6$, $P = 0.018$) and 5 mg kg⁻¹ of clozapine ($F_{1,7} = 7.9$, $P = 0.026$).

In DRN-lesioned rats, clozapine treatment reduced startle amplitude (Figure 2e). ANOVA of combined drug data from the DRN-lesioned group revealed a significant main effect of drug ($F_{4,36} = 4.8$, $P = 0.003$) and significant habituation ($F_{3,27} = 14.2$, $P < 0.001$). Further pairwise analysis indicated a significant main effect of 5 mg kg⁻¹ of clozapine ($F_{1,9} = 15.1$, $P = 0.004$) when compared to saline treatment.

In MRN-lesioned rats, startle amplitude was reduced after treatment with haloperidol or clozapine (Figure 2c and f). Again, ANOVA of combined drug data in this group revealed a significant main effect of drug ($F_{4,36} = 4.5$, $P = 0.005$) and a significant habituation ($F_{3,27} = 6.5$, $P = 0.002$). Further pairwise analysis indicated a significant main effect of 0.25 mg kg⁻¹ of haloperidol ($F_{1,9} = 6.9$, $P = 0.027$) and of 5 mg kg⁻¹ of clozapine ($F_{1,9} = 10.6$, $P = 0.01$) when compared to saline treatment.

Effects of antipsychotic drugs on prepulse inhibition

ANOVA of prepulse inhibition data from all three lesion groups and all treatments revealed significant main effects of group ($F_{2,25} = 3.5$, $P = 0.046$), prepulse ($F_{4,100} = 440.8$, $P < 0.001$), and an interaction of group \times prepulse ($F_{8,100} = 2.9$, $P = 0.006$) and of drug \times prepulse ($F_{16,400} = 1.7$, $P = 0.039$).

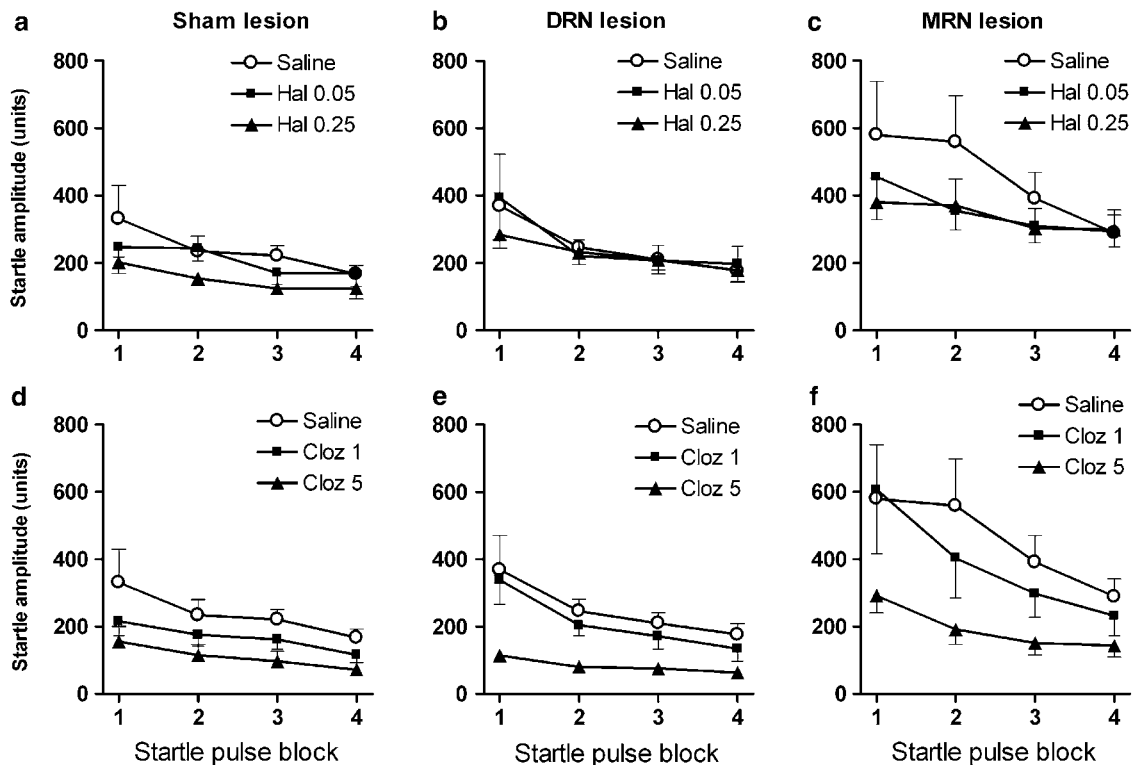


Figure 2 Effect of antipsychotic drugs on startle amplitude and startle habituation in sham-operated (a and d, $n = 8$), DRN-lesioned (b and e, $n = 10$) and MRN-lesioned rats (c and f, $n = 10$). Rats were treated with haloperidol (Hal 0.05 and Hal 0.25, top panels) or clozapine (Cloz 1 and Cloz 5, bottom panels). Data are expressed as mean startle amplitudes (arbitrary units) \pm s.e.m. for each of the four blocks of 10 115-dB pulses.

When comparing prepulse inhibition data from DRN-lesioned rats and sham-operated controls, there was no significant main effect of group, drug or any of interactions. By contrast, when comparing prepulse inhibition data from MRN-lesioned rats and sham-operated controls for all drug treatments, there was a significant main effect of group ($F_{1,16} = 10.1$, $P = 0.006$) and a group \times prepulse interaction ($F_{4,64} = 5.7$, $P = 0.001$).

Analysis of prepulse inhibition data in the individual experimental groups showed that, in the sham-operated group, there was no effect of treatment with antipsychotic drugs on prepulse inhibition when compared to saline treatment (Figure 3a and d, Table 2). Similarly, there was no significant effect of treatment with antipsychotic drugs in the DRN-lesioned rats (Figure 3b and e, Table 2). In the MRN-lesioned group (Figure 3c and f, Table 2), there was a significant drug \times prepulse interaction after treatment with 0.25 mg kg⁻¹ haloperidol ($F_{4,36} = 3.1$, $P = 0.028$), reflecting higher prepulse inhibition values in these lesioned animals after haloperidol treatment, depending on the PP. However, analysis of prepulse inhibition at individual PPs only reached trend level (PP4: $P = 0.127$, PP8: $P = 0.145$, PP12: $P = 0.085$, PP16: $P = 0.085$).

In the MRN-lesioned group, similar to haloperidol treatment, there was a significant drug \times prepulse interaction after treatment with 5 mg kg⁻¹ clozapine ($F_{4,36} = 2.7$, $P = 0.046$), reflecting an increase in prepulse inhibition after this treatment, depending on the PP. Analysis of data of individual PPs revealed a strong trend for a significant increase in prepulse inhibition at PP8 ($P = 0.053$) and significant effects of clozapine treatment at PP12 ($P = 0.011$) and PP16 ($P = 0.042$).

Discussion and conclusions

Sensorimotor gating models, such as prepulse inhibition of the acoustic startle response (Geyer & Markou, 1995; Koch, 2000), provide a rare opportunity for cross-species explorations into information processing and attentional deficits in schizophrenia. Deficits in both prepulse inhibition of startle and in startle habituation are characteristics of the illness (Geyer & Braff, 1987). The experiments in the present study were designed to assess the effectiveness of treatment with haloperidol and clozapine in normalizing deficits in sensorimotor gating produced by raphe lesions. We confirmed earlier results (Kusljic *et al.*, 2003) that MRN lesions, caused a disruption of prepulse inhibition. The main finding of this study was that treatment with clozapine and haloperidol increased prepulse inhibition in rats with MRN lesions but not in rats with DRN lesions or controls. Microinjection of 5,7-DHT into the raphe nuclei reduced 5-HT concentrations in anatomically restricted regions, reflecting the distribution of 5-HT terminals arising from the DRN or MRN.

The major pathways thought to be involved in the acoustic startle response are located in the lower brainstem, and the pontine reticular nucleus has been shown to be a critical sensorimotor interface in the pathway that mediates the startle response (Davis *et al.*, 1982; Koch & Schnitzler, 1997). Several studies have indicated that a reduction in brain 5-HT levels is associated with an increased sensitivity to various sensory stimuli (Davis & Sheard, 1974; Davis *et al.*, 1980). Rats with MRN lesions tended to have increased startle responses compared to sham-operated controls. These findings suggest

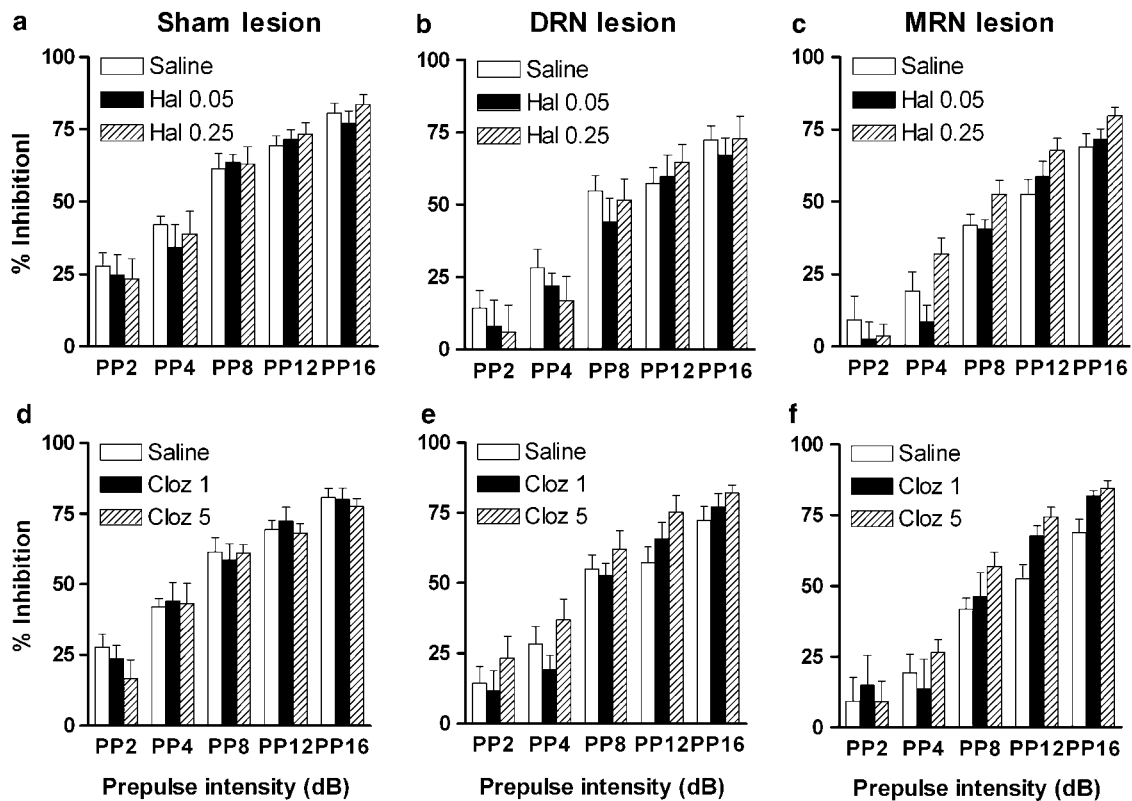


Figure 3 Effect of antipsychotic drugs on prepulse inhibition of the acoustic startle. Prepulse inhibition is expressed as % inhibition \pm s.e.m. for sham-operated (panels a and d, $n=8$), DRN-lesioned (panels b and e, $n=10$) and MRN-lesioned rats (panels c and f, $n=10$) at different PPs. Animals were treated with haloperidol (Hal 0.05 and Hal 0.25, top panels) or clozapine (Cloz 1 and Cloz 5, bottom panels). ANOVA indicated that treatment with clozapine and 0.25 mg kg⁻¹ haloperidol was able to reverse disruption in prepulse inhibition in MRN-lesioned rats compared to sham-operated controls.

that 5-HT in brain structures innervated by the MRN, such as dorsal hippocampus and cingulate cortex, may be involved in modulation of normal responses to startle stimuli.

In contrast to startle, prepulse inhibition is modulated by a neuronal circuit consisting of cortico-limbic brain structures in which the nucleus accumbens plays an important role (Geyer *et al.*, 1990; Koch & Schnitzler, 1997; Swerdlow & Geyer, 1998). Using a different prepulse inhibition paradigm, robust disruption of prepulse inhibition has also been found in rats with combined 5,7-DHT lesions of the DRN and MRN or after treatment with a tryptophan hydroxylase inhibitor (Fletcher *et al.*, 2001; Prinssen *et al.*, 2002). Our findings confirm that the normal regulation of prepulse inhibition depends on intact 5-HT transmission in brain structures receiving predominant 5-hydroxytryptaminergic input from the MRN, such as dorsal hippocampus and cingulate cortex.

Models of disruption of prepulse inhibition in schizophrenia have been tested with antipsychotic drugs in order to assess the validity of these models for the identification of clinical treatments for schizophrenia. Animal studies have shown that treatment with antipsychotic drugs, such as haloperidol and clozapine, is able to reverse prepulse inhibition deficits induced by excessive central dopaminergic stimulation following apomorphine treatment (Swerdlow *et al.*, 1991; Swerdlow & Geyer, 1993). However, haloperidol failed to restore prepulse inhibition deficits produced by NMDA-receptor antagonists such as phencyclidine and dizocilpine (Keith *et al.*, 1991). In contrast, treatment with atypical antipsychotic drugs, such as clozapine and ziprasidone, was able to antagonize NMDA-receptor

antagonist-induced deficits in prepulse inhibition (Bakshi *et al.*, 1994; Bakshi & Geyer, 1995; Mansbach *et al.*, 2001).

In this study, we demonstrated that treatment with both antipsychotic drugs, haloperidol and clozapine, significantly increased prepulse inhibition in rats with lesions of the MRN, effectively bringing prepulse inhibition in these animals back to the level seen in sham-operated controls. Thus, we were able to restore prepulse inhibition deficits produced by a 5-HT depletion in the brain. However, the exact mechanism by which clozapine and haloperidol affect these sensorimotor gating deficits is far from clear. The antipsychotic profile of clozapine appears to reside in its broad pattern of interaction with various dopamine and 5-HT receptor subtypes (Millan, 2000). Meltzer *et al.* have proposed a model in which affinity for 5-HT₂ receptors relative to dopamine D₂ receptor affinity is a crucial component differentiating typical from atypical antipsychotic drugs (Meltzer, 1989; Meltzer *et al.*, 1989). However, according to the other school of thought, all antipsychotic drugs, including both typical and atypical, act by blocking dopamine D₂ receptors (Kapur & Seeman, 2001). Animal data and *in vitro* data showed that a rapid dissociation from the D₂ receptor at a molecular level, not high affinity at the 5-HT₂ receptor, produces the atypical antipsychotic effect (Kapur & Seeman, 2001). These data also matched clinical brain-imaging findings showing that haloperidol remained constantly bound to D₂ receptors in humans undergoing two positron emission tomography scans 24 h apart, whereas the occupation of D₂ receptors by clozapine has mostly disappeared after 24 h (Seeman, 2002).

Table 2 Average prepulse inhibition of rats with 5,7-DHT induced lesions of the DRN or MRN when compared with sham-operated controls

Treatment	Sham (n = 8)	DRN-lesioned (n = 10)	MRN-lesioned (n = 10)
Saline	56.3 ± 3.5	45.5 ± 4.9	38.3 ± 4.4
Haloperidol 0.05 mg kg ⁻¹	54.3 ± 3.7	40.2 ± 5.8	36.4 ± 3.5
Haloperidol 0.25 mg kg ⁻¹	56.5 ± 4.9	42.4 ± 7.2	47.1 ± 3.3*
Clozapine 1 mg kg ⁻¹	55.8 ± 4.5	45.3 ± 4.6	44.8 ± 6.0*
Clozapine 5 mg kg ⁻¹	53.3 ± 4.0	55.9 ± 4.8	50.3 ± 3.8*

The animals were treated with saline, haloperidol or clozapine.

Data values represent mean ± s.e.m. tissue. Average prepulse inhibition was calculated as the mean % inhibition seen at PP2, PP4, PP8, PP12 and PP16 (see Methods). Differences between the groups were analysed by combined and pairwise ANOVA (see text for details).

**P* < 0.05 Group × Prepulse interaction when compared to saline treatment in the same group.

The nucleus accumbens is one of the brain sites where excessive dopamine activity disrupts prepulse inhibition (Swerdlow *et al.*, 1990; Koch, 1999). We suggest that sensorimotor gating deficits observed by MRN lesions in the present study occurred as a result of dopamine overactivity in the nucleus accumbens brought about by 5-HT depletion in limbic structures such as the dorsal hippocampus (Kusljic & van den Buuse, 2004). The dopamine receptors critical for modulation of prepulse inhibition appear to be located within both the dorsal striatum and the nucleus accumbens (Swerdlow *et al.*, 1986; 1990). Both of these regions contain dense populations of D₂ receptors (Garau *et al.*, 1978). Following intraraphe 5,7-DHT injections, significant increases in 5-HT_{1B/1D} binding were detected in the nucleus accumbens, whereas similar increases in 5-HT_{2A/2C} binding were restricted to the medial striatum (Compan *et al.*, 1998). The ability of clozapine to occupy D₂ receptors at the same time as 5-HT₂ receptors, and then rapidly dissociate from D₂ receptors to allow physiological dopamine neurotransmission, may be responsible for increasing prepulse inhibition in MRN-lesioned rats. In addition, occupancy of dopamine D₂ receptors by a relatively high dose of haloperidol is responsible

for restoring prepulse inhibition deficits in rats with MRN lesions. Neuroimaging data showed that optimal dopamine D₂ receptor occupancy is sufficient to produce the atypical antipsychotic effect (Kapur & Seeman, 2001). If dopamine D₂ receptor occupancy is excessive, the atypical profile is lost even in the presence of high 5-HT₂ occupancy (Kapur & Seeman, 2001). Therefore, our results suggest that appropriate modulation of the dopamine D₂ receptor alone is sufficient to restore sensorimotor gating deficits produced by selective lesions of the brain 5-HT system. In conclusion, these findings highlight the regulatory and behavioural consequences of 5-HT depletion and importance of the dopamine D₂ receptor modulation in regulation of sensorimotor gating.

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