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8-OH-DPAT-induced effects on prepulse inhibition: Pre- vs. post-synaptic 5-HT$_{1A}$ receptor activation

Andrea Gogos[a,*], Snezana Kusljic[a,b], Maarten van den Buuse[a]

[a]*Behavioural Neuroscience Laboratory, Mental Health Research Institute of Victoria, Parkville, VIC 3052, Australia*
[b]*Department of Pharmacology, The University of Melbourne, Melbourne, VIC 3010, Australia*

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Abstract

Prepulse inhibition (PPI) is a measure of sensorimotor gating that is deficient in schizophrenia. In rats, administration of the serotonin-1A (5-HT$_{1A}$) receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), causes a disruption of PPI. It is unclear whether this effect is due to the activation of pre- or post-synaptic 5-HT$_{1A}$ receptors, however pre-synaptic receptors located in the dorsal raphe nucleus (DRN) may play a role. Our previous research showed that castrated rats have a reduced sensitivity to 8-OH-DPAT-induced disruptions of PPI. Therefore, in Experiment 1, male Sprague–Dawley rats were sham-operated or castrated and micro-injected with 8-OH-DPAT directly into the DRN. In Experiment 2, male rats were sham-operated or received a selective serotonergic, 5,7-dihydroxytryptamine lesion of the DRN. 8-OH-DPAT was injected subcutaneously in these rats. In both sham-operated and castrated rats, a micro-injection of 8-OH-DPAT into the DRN did not disrupt PPI. Instead, in castrated rats, 8-OH-DPAT treatment tended to increase PPI. A DRN lesion caused a significant reduction in 5-HT content in the frontal cortex (70% reduction), striatum (69%) and ventral hippocampus (76%). In both sham-operated and DRN-lesioned rats, systemic 8-OH-DPAT significantly disrupted PPI. Taken together, these data suggest that the disruption of PPI observed in rats with systemic 8-OH-DPAT treatment is predominantly due to an activation of post-synaptic, rather than pre-synaptic, 5-HT$_{1A}$ receptors.

Keywords: 5-HT$_{1A}$ receptors; 5,7-DHT lesions; Serotonin; Prepulse inhibition; Schizophrenia; Dorsal raphe nucleus

1. Introduction

Prepulse inhibition (PPI) is an operational measure of sensorimotor gating that is deficient in schizophrenia. Sensorimotor gating is a normal protective mechanism in the CNS that functions to 'gate' or filter irrelevant sensory, motor or cognitive information, allowing for coherent thought (Kodsi and Swerdlow, 1994). The finding that PPI is disrupted in schizophrenia patients has been replicated in many studies (for review see Braff et al., 2001). PPI of the startle response is similarly shown in humans as well as in experimental animals (Braff et al., 2001; Geyer et al., 2001; Paylor and Crawley, 1997). The startle response can be defined as a fast response to sudden, intense stimuli that functions to protect the organism from injury (Koch, 1999). PPI of the acoustic startle response involves the brief presentation of a high intensity sound stimulus that results in a normal startle reflex response. When this stimulus is preceded by a weak, non-startling stimulus (a prepulse), the subsequent startle response is reduced (Koch and Schnitzler, 1997; Kumari et al., 2000). Over the years, many studies have used a variety of experimental conditions in animals in order to study brain mechanisms involved in PPI. These studies include pharmacological manipulation of different transmitter systems, brain lesions and developmental manipulations of the CNS, and have implicated several neurotransmitters (e.g. dopamine, serotonin (5-HT), glutamate) and developmental mechanisms (e.g. isolation rearing, prenatal stress) in the regulation of PPI (Geyer et al., 2001; Koch, 1999).

It has been well established that administration of the prototypical 5-HT$_{1A}$ receptor agonist, 8-hydroxy-2-(di-*n*-

* Corresponding author. Tel.: +61 3 9388 1633; fax: +61 3 9387 5061.
E-mail address: agogos@mhri.edu.au (A. Gogos).

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propylamino)tetralin (8-OH-DPAT), results in a disruption of PPI in rats (Czyrak et al., 2003; Fletcher et al., 2001; Gogos and Van den Buuse, 2003; Gogos and Van den Buuse, 2004; Kinney et al., 1999; Rigdon and Weatherspoon, 1992; Sipes and Geyer, 1995; Sipos et al., 2000). Several studies have confirmed that 8-OH-DPAT disrupts PPI via activation of 5-HT_{1A} receptors (Czyrak et al., 2003; Rigdon and Weatherspoon, 1992; Sipes and Geyer, 1995). However, it is unclear where in the brain this occurs. Only two studies have examined this issue and suggested an important role of the raphe nuclei (Fletcher et al., 2001; Sipes and Geyer, 1995).

Generally, a reduction in 5-HT levels causes a disruption of PPI. Systemic administration of *p*-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor, results in depletion of 5-HT levels in the entire brain. In rats, PCPA treatment causes a disruption of PPI (Fletcher et al., 2001; Prinssen et al., 2002). More specifically, a 5,7-dihydroxytryptamine (5,7-DHT) lesion of 5-HT neurons in both the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) (Fletcher et al., 2001), or MRN alone (Kusljic et al., 2003), also causes a disruption of PPI.

8-OH-DPAT treatment potently reduces 5-HT synthesis and release in the rat brain (Hjorth et al., 1987; Hjorth and Magnusson, 1988). The question then arises whether the 8-OH-DPAT-induced disruption of PPI in rats is due to a reduction of 5-HT levels in the brain, similar to serotonergic raphe lesions. Sipes and Geyer (1995) micro-injected 8-OH-DPAT directly into the DRN or MRN of male rats and found that this caused a disruption of PPI. The authors suggested that the disruption of PPI observed with systemic administration of 8-OH-DPAT is the result of 8-OH-DPAT activating pre-synaptic 5-HT_{1A} receptors located in the raphe nuclei (Sipes and Geyer, 1995). However, in the study by Sipes and Geyer (1995) the disruption of PPI induced by systemic 8-OH-DPAT administration was greater than that caused by intra-raphe administration. Interestingly, Fletcher et al. (2001) showed that systemic administration of 8-OH-DPAT to raphe-lesioned rats further enhanced the disruption of PPI that was caused by the lesion alone (Fletcher et al., 2001). Together, these studies suggest that 8-OH-DPAT also activates post-synaptic 5-HT_{1A} receptors in causing a disruption of PPI. The question therefore arises, which receptor population is responsible for the disruption of PPI induced by 8-OH-DPAT treatment, pre- or post-synaptic 5-HT_{1A} receptors, or a combination. Furthermore, it is unclear if the activation of pre-synaptic 5-HT_{1A} receptors by 8-OH-DPAT treatment elicits the same response as does the activation of post-synaptic receptors.

This study aimed to further examine the role of pre-synaptic 5-HT_{1A} receptors located in the raphe nuclei. The first part of this study involved the administration of 8-OH-DPAT directly into the DRN. We have previously shown that castrated (CAST) rats have a reduced sensitivity to the disruption of PPI caused by 8-OH-DPAT (Gogos and Van den Buuse, 2003). In addition, we found that CAST rats showed a 'biphasic' PPI response to 8-OH-DPAT treatment, where a low dose of 8-OH-DPAT (0.02 mg/kg) tended to increase PPI and a higher dose (0.5 mg/kg) decreased PPI (Gogos and Van den Buuse, 2003). The PPI response that predominates may depend on the circulating level of sex steroid hormones, the dose of 8-OH-DPAT administered, or on the population of 5-HT_{1A} receptors activated. Therefore, in the present study we compared the PPI responses from sham-operated and CAST rats that received a micro-injection of 8-OH-DPAT in the DRN. The DRN was selected, rather than the MRN, as the DRN contains a higher density of 5-HT_{1A} receptors (Jacobs and Azmitia, 1992; Palacios et al., 1987), it is a more compact nucleus (Paxinos and Watson, 1998) and the ventral hippocampus and nucleus accumbens, both of which are strongly implicated in PPI regulation (Koch, 1999), receive dense serotonergic input from the DRN (Azmitia and Whitaker-Azmitia, 1995). The second part of this study further investigated the role of the DRN in PPI by selectively lesioning this nucleus. Thus, rats were sham-operated or DRN-lesioned and then received subcutaneous injections of 8-OH-DPAT before being tested in PPI. In the lesioned rats, 8-OH-DPAT will predominantly target the post-synaptic 5-HT_{1A} receptors.

2. Methods

2.1. Animals

This study used 39 male Sprague–Dawley rats, which were obtained from the Department of Pathology and Anatomy Animal Services, University of Melbourne (Parkville, VIC, Australia). Animals were housed at the Mental Health Research Institute in groups of two to three in standard rat cages and had free access to standard pellet food and tap water. The animals were maintained on a 12 h light–dark cycle (lights on at 0630) at an average temperature of 22 °C. All surgical techniques, treatments and experimental protocols were carried out during the light phase and were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council of Australia, 1990).

2.2. Surgery

2.2.1. Castration

This surgery was done as published previously (Gogos and Van den Buuse, 2003). Briefly, rats underwent surgery at 10 weeks of age and weighed an average of 445 g. Male rats were randomly selected to become CAST rats or sham-operated rats. Rats were anesthetized using a gas mixture of oxygen and isoflurane (I.S.O.™, Inhalation anesthetic, 1 ml/ml; Veterinary Companies of Australia, Artarmon, NSW, Australia) and were placed on a heat pad. A small midline incision was made through the skin of the scrotum and through the muscle layer. The blood vessels were ligated using silk suture, after which the testicle was removed. The

procedure was repeated on the other side, before the muscle layer and skin was suture-closed.

2.2.2. Cannula implantation

This surgery was done as published previously (Cornish et al., 1997). Briefly, 10 days after castration or sham surgery, rats were anesthetized with an intraperitoneal (i.p.) injection of sodium pentobarbitone (Nembutal®, 60 mg/kg; Merial Australia, Rhone Merieux, QLD, Australia) and mounted in a Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). A skin incision exposed the skull and a hole was drilled at the appropriate coordinates obtained from the atlas of Paxinos and Watson (1998) and preliminary experiments. The incisor bar was set at −3.3 mm and the angle of the stereotaxic arm was set at 25°. Using the bregma as zero, coordinates for the DRN were anterior/posterior (AP) −8.5 mm, left/right (LR) +2.9 mm and dorsal/ventral (DV) −5.8 mm (Paxinos and Watson, 1998). A stainless steel guide cannula (Plastics Products Company, Roanoke, VA, USA) was inserted through a burr hole and implanted into the DRN. The guide cannula was fixed to the skull with dental cement (Dentimex, Zeist, Netherlands) and 3 anchoring screws (Fine Science Tools, Foster City, CA, USA). Finally, a dummy cannula (Plastic Products Company) was inserted into the guide cannula.

2.2.3. DRN lesion

This surgery was done as published previously (Kusljic et al., 2003). Briefly, all rats underwent surgery at about 7 weeks of age and weighed between 250 and 300 g. Rats were pre-treated with 20 mg/kg of the noradrenergic re-uptake inhibitor, desipramine, 30 min prior to lesions, to prevent destruction of noradrenergic neurons by 5,7-DHT (Jonsson, 1980; Towle et al., 1984). Desipramine HCl (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in distilled water and administered as an i.p. injection. Similar to the cannula implantation surgery described above, rats were anesthetized with sodium pentobarbitone and mounted into a stereotaxic apparatus with the incisor bar set at −3.3 mm and the angle of the stereotaxic arm set at 25°. Rats were micro-injected with 1 μl of the selective serotonergic neurotoxin 5,7-DHT (5 μg/μl; Sigma Chemical Co.), or the vehicle solution for sham-operated rats, directly into the DRN. 5,7-DHT was dissolved in sterile saline with 0.1% ascorbic acid to prevent oxidation. The solution was micro-injected into the DRN by hand, using a 10 μl syringe held in a micrometer. Using the bregma as zero, stereotaxic coordinates were AP −8 mm, LR +2.9 mm, DV −6.8 mm (Kusljic et al., 2003; Paxinos and Watson, 1998).

2.2.4. Post-operative procedures

After each of the above-mentioned surgeries, antiseptic cream (Betadine®, povidone–iodine 10%; Faulding Consumer, Salisbury, SA, Australia) was applied over the sutures. Rats were also given a subcutaneous (s.c.) injection of the non-steroidal, anti-inflammatory analgesic, carprofen (Zenecarp®, 50 mg/ml; Heriot AgVet, Rowville, VIC, Australia). Carprofen was diluted in saline to give a concentration of 5 mg/ml. After surgery, rats were routinely examined and allowed approximately 10 days to recover from castration, 7 days to recover from cannula implantation and 14 days to recover from a raphe lesion, before experimentation.

2.3. PPI of the acoustic startle response

PPI of the acoustic startle response was measured as previously described (Gogos and Van den Buuse, 2003), using automated startle chambers (SR-Lab; San Diego Instruments, San Diego, CA, USA). Briefly, rats were placed individually into transparent acrylic cylinder that was closed on each end. A piezoelectric transducer mounted underneath the cylinder detected whole body startle responses. Background noise and acoustic pulses were presented through a speaker and responses measured with the SR-Lab software (San Diego Instruments).

There were 100 trials in a single PPI session, including forty 115 dB pulse-alone trials, fifty prepulse trials and ten no-stimulus trials. Prepulse trials consisted of a prepulse of an intensity of 2, 4, 8, 12 or 16 dB above the 70 dB background (ten each), followed by a startle pulse of 115 dB, 100 ms later. Trials were presented with variable inter-trial intervals (10–37 s), to prevent learning and anticipatory responses. Startle amplitude was assessed using the four blocks of ten 115 dB pulses. The percentage (%) PPI was calculated as the difference in amplitude between the startle response to the pulse-alone trials and the prepulse-startle pulse trials, divided by the pulse-alone trial × 100% (Gogos and Van den Buuse, 2003, 2004).

2.4. Experimental protocol

2.4.1. Experiment 1 — Central DRN micro-injections

This experiment included 7 sham-operated rats and 10 CAST rats. One week after the cannula implantation surgery, rats were randomly injected with saline, 0.3, 1.0 or 3.0 μg/0.5 μl of 8-OH-DPAT (Tocris, Ellisville, MO, USA). All rats received all doses with a randomized sequence of treatments, with 3–4 days allowed between experiments. These doses were selected on the basis of the literature and preliminary experiments (Fletcher, 1991; Martin et al., 1990; Sipes and Geyer, 1995). 8-OH-DPAT was dissolved in sterile saline immediately prior to use. 8-OH-DPAT or saline were administered by hand via the guide cannula in a volume of 0.5 μl, infused over 30 s. The cannula was left in place for a further minute to prevent backflow of the solution, after which the dummy cannula was replaced. Five minutes later the rat was placed in the PPI chamber.

2.4.2. Experiment 2 — DRN lesions

This experiment included 12 sham-operated rats and 10 DRN-lesioned rats. Two weeks after surgery, rats were randomly injected with saline or 0.5 mg/kg of 8-OH-DPAT.

All rats received these two treatments with a randomized sequence. The dose of 8-OH-DPAT used was selected on the basis that it causes a significant and reproducible disruption of PPI (Gogos and Van den Buuse, 2003; Gogos and Van den Buuse, 2004). 8-OH-DPAT was administered with an injection volume of 1 ml/kg body weight and was administered s.c. in the nape of neck, 10 min prior to the rat being placed in the PPI chamber.

2.5. Evaluation of surgery

2.5.1. Castration and cannula implantation

Three days after completion of experiments, the rats were killed by decapitation and their seminal vesicles removed, weighed and morphologically observed in order to assess the accuracy of the castration procedure. The brain was removed and frozen. Twenty micrometer sections of the region of the DRN were cut on a cryostat and stained with cresyl violet (ProSciTech, Thuringowa, QLD, Australia) to verify the accuracy of the cannula placement. Only rats that had correct cannula placement were included in the analysis of behavior.

2.5.2. DRN lesions

Three days after completion of experiments, the rats were killed by decapitation. Individual brain regions (cerebellum, hypothalamus, frontal cortex, striatum, dorsal and ventral hippocampus) were dissected by hand and frozen. High-pressure liquid chromatography (HPLC) was used to determine the 5-HT content in these tissues, as previously described (Kusljic and Van den Buuse, 2004). Only DRN-lesioned rats with marked 5-HT depletion in the brain were included in the analysis of behavior.

2.6. Statistical analysis

PPI and startle data are expressed as mean ± standard error of the mean (S.E.M.). Data were evaluated with analysis of variance (ANOVA) with repeated measures, where appropriate, using the statistical software package SYSTAT 9.0 (SPSS Inc., Chicago, IL, USA). When significant differences were found in the main analysis, further pair-wise ANOVAs were completed for relevant comparisons. For both experiments, the between-subjects variable was group and the within-subjects variables were dose or treatment and prepulse intensity. Differences were considered to be significant at $p<0.05$.

3. Results

3.1. Experiment 1 — Central DRN micro-injections

3.1.1. Body and seminal vesicle weight

Body weight at the time that PPI experiments began was not significantly different between the groups. In addition, there was no significant difference in body weight gain between the groups. As expected, CAST rats had significantly smaller seminal vesicle weight compared with sham-operated rats, both for absolute ($F_{(1,13)}=255.5$, $p<0.001$) and per body weight values ($F_{(1,13)}=416.1$, $p<0.001$; Table 1).

3.1.2. Startle amplitude

Comparison of startle amplitude in sham-operated and CAST rats showed that there was no significant main effect of group or dose, or a dose × group interaction (Fig. 1). This indicated that micro-injection of 8-OH-DPAT into the DRN did not significantly affect startle amplitude. Furthermore, while both groups showed significant habituation of the startle response ($F_{(3,45)}=17.2$, $p<0.001$; data not shown), there was no effect of 8-OH-DPAT treatment on habituation.

3.1.3. Prepulse inhibition

With all data combined, there was a highly significant main effect of prepulse intensity ($F_{(4,60)}=142.2$, $p<0.001$), reflecting the expected progressive reduction of startle responses with increasing prepulse intensity (Fig. 1). However, there was no significant main effect of dose, nor a dose × group interaction, indicating that 8-OH-DPAT micro-injection into the DRN did not affect PPI in either group. Furthermore, there was no significant main effect of group or any interactions of dose or group with prepulse intensity. However, inspection of the average PPI graph (Fig. 1, right panel) shows a clear trend for an increase in PPI in CAST rats with 8-OH-DPAT treatment and no effect on PPI in sham-operated rats. In an analysis comparing PPI of sham-operated and CAST rats in response to saline treatment and the highest dose of 8-OH-DPAT (3 μg/0.5 μl), there was a significant dose × group interaction ($F_{(1,15)}=7.3$, $p=0.016$), reflecting a differential effect of 8-OH-DPAT treatment on PPI in CAST rats vs. sham-operated rats. Further analysis comparing the responses to saline and 3 μg/0.5 μl 8-OH-DPAT in CAST rats, revealed a significant main effect of dose ($F_{(1,9)}=6.2$, $p=0.034$), reflecting an increase in PPI with this dose of 8-OH-DPAT. In contrast, there was no significant change in PPI in sham-operated rats when comparing saline and 3 μg/0.5 μl 8-OH-DPAT treatment.

3.2. Experiment 2 — DRN lesions

3.2.1. 5-HT content

In DRN-lesioned rats, 5-HT content was differentially, but significantly reduced in each of the six brain regions

Table 1
Body and seminal vesicle weight of sham-operated and castrated rats

	BW at PPI (g)	Final BW (g)	Weight gain (g)	SV weight (mg)	SV weight/ BW
Sham	480±17	496±26	31±21	1322±80	2.67±0.12
CAST	447±18	479±25	53±17	127±6*	0.28±0.02*

Weight gain (g, grams) is the difference between body weight (BW) on the day of castration (CAST) surgery and final BW on the day of sacrifice. Seminal vesicle (SV) weight was measured at sacrifice (mg, milligrams). Weights are expressed as mean±S.E.M. (*$p<0.05$).

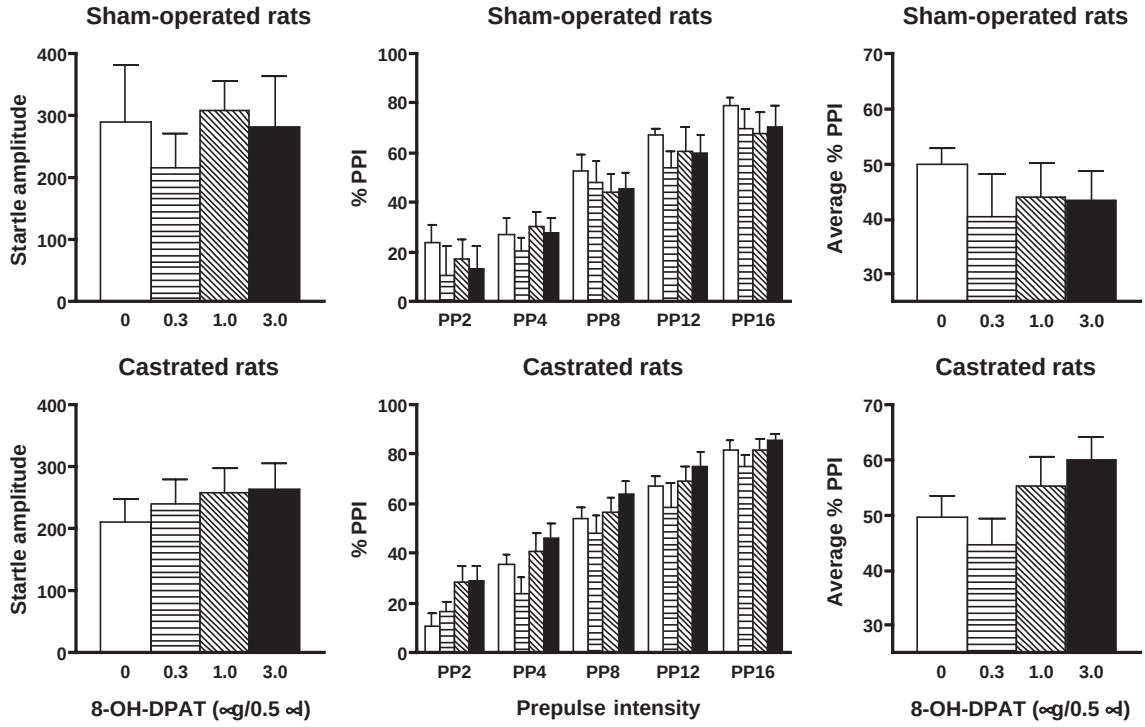

Fig. 1. Startle responses (in arbitrary units; left panel), percentage (%) PPI (middle panel) and average % PPI (right panel) of sham-operated rats (top, $n=7$) and castrated rats (bottom, $n=10$). Both groups were micro-injected with saline (0; open bars), or 0.03 μg/0.5 μl (striped bars), 1.0 μg/0.5 μl (hatched bars) and 3.0 μg/0.5 μl 8-OH-DPAT (black bars) into the DRN. Data are expressed as mean±S.E.M.

analyzed, compared to sham-operated rats. There was a highly significant main effect of group (all $p<0.001$; Table 2) when analyzing 5-HT content in the frontal cortex ($F_{(1,20)}=426.1$; 70.4% reduction in DRN-lesioned rats), striatum ($F_{(1,20)}=540.3$; 68.8% reduction), dorsal hippocampus ($F_{(1,20)}=139.8$; 33.4% reduction), ventral hippocampus ($F_{(1,20)}=395.5$; 76.2% reduction), cerebellum ($F_{(1,20)}=123.5$; 36.8% reduction) and hypothalamus ($F_{(1,20)}=673.8$; 63.1% reduction). This depletion pattern is consistent with the previously published projection pattern of the DRN.

3.2.2. Startle amplitude

When comparing the startle responses of both rat groups, there was a significant main effect of dose ($F_{(1,20)}=15.0$, $p=0.001$), indicating that 0.5 mg/kg of 8-OH-DPAT significantly increased startle amplitude (Fig. 2). However, there was no significant main effect of group or a dose × group interaction, reflecting a similar increase in startle amplitude in the groups. Furthermore, while both groups showed significant habituation of the startle response ($F_{(3,60)}=16.7$, $p<0.001$; data not shown), there was no effect of 8-OH-DPAT treatment on habituation.

Table 2
5-HT content (expressed as ng/mg tissue) in various brain regions of sham-operated and DRN-lesioned rats

	Sham-operated ($n=12$)	DRN-lesioned ($n=10$)
Frontal cortex	0.75±0.02	0.22±0.02*
Striatum	0.86±0.02	0.27±0.01*
Hypothalamus	2.18±0.03	0.80±0.04*
Dorsal hippocampus	1.23±0.02	0.82±0.03*
Ventral hippocampus	1.36±0.03	0.32±0.04*
Cerebellum	0.63±0.02	0.40±0.01*

* $p<0.05$.

3.2.3. Prepulse inhibition

As expected, there was a highly significant main effect of prepulse intensity ($F_{(4,80)}=156.6$, $p<0.001$; Fig. 2). In addition, there was a significant main effect of dose ($F_{(1,20)}=18.3$, $p<0.001$), where treatment with 8-OH-DPAT significantly disrupted PPI compared to saline treatment. There was also a significant dose × prepulse intensity interaction ($F_{(4,80)}=3.9$, $p=0.006$), reflecting a greater PPI disruption by 8-OH-DPAT treatment at the lower prepulse intensities (Fig. 2). However, there was no significant main effect of group or interaction of dose × group or dose × group × prepulse intensity, suggesting that the disruption caused by 8-OH-DPAT treatment was similar in the sham-operated and DRN-lesioned rat groups.

4. Discussion

This study explored whether pre- or post-synaptic 5-HT_{1A} receptors are involved in 8-OH-DPAT-mediated disruption of PPI. We found that there was no significant effect on PPI when injecting 8-OH-DPAT directly into the

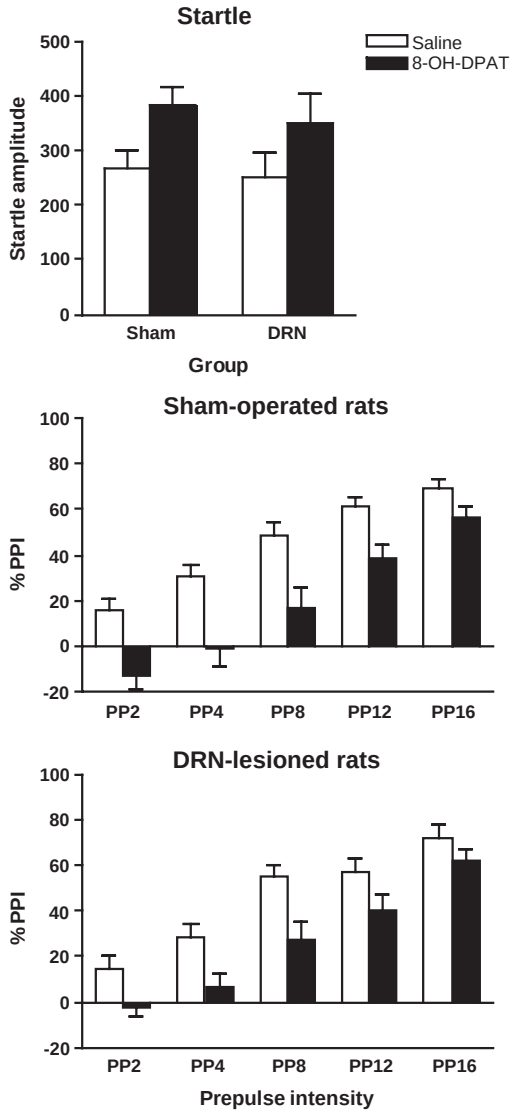

Fig. 2. Startle responses (in arbitrary units; top panel) of sham-operated rats (Sham, $n=12$) and DRN-lesioned rats (DRN, $n=10$). Percentage (%) PPI of sham-operated rats (middle panel, $n=12$) and DRN-lesioned rats (bottom panel, $n=10$). Both groups were subcutaneously administered saline (open bars) and 0.5 mg/kg 8-OH-DPAT (black bars). Data are expressed as mean ± S.E.M.

DRN of sham-operated or CAST male rats. Furthermore, brain serotonin depletion by lesion of the DRN did not significantly alter the PPI disruption induced by 8-OH-DPAT. This study suggests that the 8-OH-DPAT-induced disruption of PPI and increase in startle amplitude seen with systemic administration are mediated by activation of post-synaptic 5-HT_{1A} receptors, rather than pre-synaptic receptors, at least in the DRN.

4.1. Central injections

Castration almost completely eliminates circulating testosterone levels in the rat (Dijcks et al., 1994; Nnane et al., 1999). In the present study, successful castration was ascertained by a decrease in seminal vesicle weight (Gogos and Van den Buuse, 2003; McNeilly et al., 1983). Neither sham-operated nor CAST rats showed an increase in startle amplitude in response to 8-OH-DPAT micro-injection into the DRN. It is well established that subcutaneous administration of 8-OH-DPAT increases startle amplitude (Fletcher et al., 2001; Gogos and Van den Buuse, 2003; Nanry and Tilson, 1989). The present results therefore suggest that this increase is due to an activation of 5-HT_{1A} receptors in a brain region other than the DRN. Sipes and Geyer (1995) also found no effect on startle amplitude with infusion of 8-OH-DPAT into the DRN or MRN (Sipes and Geyer, 1995).

In terms of PPI, 8-OH-DPAT injected into the DRN did not significantly affect PPI of sham-operated or CAST rats. However, close inspection of the data showed that 8-OH-DPAT micro-injection into the DRN caused a dose-dependent increase of PPI in CAST rats, which reached significance at the highest dose of 8-OH-DPAT tested. A trend for an increase in PPI was previously observed in CAST rats treated systemically with a low dose (0.02 mg/kg) of 8-OH-DPAT (Gogos and Van den Buuse, 2003; preliminary experiments). The results of this experiment are in contrast to those of Sipes and Geyer (1995) who found a significant disruption of PPI with infusion of 8-OH-DPAT into either the DRN or MRN of intact, male rats (Sipes and Geyer, 1995). This disruption occurred only with the highest dose of 8-OH-DPAT tested, 5 μg/0.5 μl, while there was no significant effect of 0.5 or 1 μg/0.5 μl. The cause of this discrepancy is unclear, but may be due to slight differences in doses of 8-OH-DPAT used, differences between Sprague–Dawley rat sub-strains, or differences in the PPI protocol used. The effects of 8-OH-DPAT micro-injected into the DRN of CAST rats have not been examined before.

4.2. DRN lesions

The serotonergic neurotoxin, 5,7-DHT, is taken up by the serotonergic neuron via the 5-HT transporter and causes a loss of serotonergic terminals and cell bodies (Jonsson, 1980; Kusljic et al., 2003; Towle et al., 1984). Successful DRN 5,7-DHT lesions were ascertained by markedly reduced 5-HT concentrations in several brain regions, particularly in the ventral hippocampus, frontal cortex and striatum, compared to sham-operated rats. This depletion pattern is consistent with anatomical studies, showing that these regions are innervated predominantly by projections from the DRN, rather than the MRN (Adell and Myers, 1995; Azmitia and Whitaker-Azmitia, 1995; McQuade and Sharp, 1997; Mokler et al., 1998).

All the rats in this experiment showed a similar increase in startle amplitude with 8-OH-DPAT treatment. The lack of effect of a DRN lesion on 8-OH-DPAT-induced increases in startle amplitude, suggests that the activation of pre-synaptic 5-HT_{1A} receptors by 8-OH-DPAT treatment, does not influence the regulation of acoustic startle. This is in line with the results of Experiment 1, further supporting the

notion that the DRN is not involved in the increase in startle amplitude observed with systemic 8-OH-DPAT administration.

Sham-operated and DRN-lesioned rats showed a similar disruption of PPI with 8-OH-DPAT treatment. Fletcher et al. (2001) found that systemic 8-OH-DPAT treatment in DRN/MRN-lesioned rats disrupted PPI to a greater extent than in sham-operated rats (Fletcher et al., 2001). This discrepancy may be explained by a combined raphe lesion (Fletcher et al., 2001), compared to a selective DRN lesion (present study). A combined lesion destroys most of the pre-synaptic 5-HT_{1A} receptors, whereas a selective DRN lesion only destroys the pre-synaptic 5-HT_{1A} receptors that are located in the DRN and its associated projection areas. Fletcher et al. (2001) also found a greater reduction in 5-HT content than in the present study (90% reduction vs. 69% in the striatum) and used a lower dose of 8-OH-DPAT (0.1 mg/kg). It is possible that this large 5-HT depletion led to an up-regulation of post-synaptic 5-HT_{1A} receptors, rendering the lower dose of 8-OH-DPAT more effective. In the present study with slightly lower 5-HT depletion, this up-regulation may not have become apparent. In any case, the 8-OH-DPAT-induced disruption of PPI was not reduced after a raphe lesion in either the present study or in that of Fletcher et al. (2001).

4.3. Mechanism of action

The 5-HT_{1A} receptors are widely distributed in the brain. The pre-synaptic receptors located in the raphe nuclei are autoreceptors that inhibit 5-HT release in the forebrain (Barnes and Sharp, 1999). Overall, the results of the present study suggest that the increase in startle amplitude and disruption of PPI, that occur in response to systemic 8-OH-DPAT treatment, are due to the activation of post-synaptic 5-HT_{1A} receptors, rather than pre-synaptic, or at least 5-HT_{1A} receptors located in a brain region other than the DRN. It is possible that differential activation of pre- vs. post-synaptic 5-HT_{1A} receptors may explain the different PPI responses that occur, such as an increase in PPI in male CAST rats and a decrease in PPI in intact, sham-operated rats with systemic 8-OH-DPAT treatment. For example, a low dose of 8-OH-DPAT administered systemically might increase PPI by preferentially activating 5-HT_{1A} autoreceptors in the raphe nuclei, as supported by the present findings with direct micro-injection of 8-OH-DPAT into the DRN of CAST rats. The observed disruption of PPI with higher doses of 8-OH-DPAT may be due to the additional activation of the post-synaptic 5-HT_{1A} receptors, as supported by the finding of DRN-lesioned rats still showing a disruption of PPI in response to systemic 8-OH-DPAT treatment. However, the brain region where the post-synaptic receptors are activated is less clear.

We propose that 8-OH-DPAT-induced increases in PPI may occur only by activation of pre-synaptic 5-HT_{1A} receptors in the DRN, that subsequently inhibit 5-HT and dopamine release in the nucleus accumbens. 8-OH-DPAT directly administered to the DRN decreases 5-HT cell firing in this area (Blier et al., 1998) and decreases 5-HT and dopamine extracellular levels in the nucleus accumbens (Yoshimoto and McBride, 1992). Reduction of dopaminergic activity in the nucleus accumbens, by inhibition of dopamine release or treatment with dopamine receptor antagonists, causes an increase in PPI (Swerdlow and Geyer, 1993). On the other hand, a disruption of PPI may be predominantly mediated by 8-OH-DPAT directly activating post-synaptic 5-HT_{1A} receptors, perhaps in the hippocampus or ventral tegmental area. This activation would increase dopamine release in the nucleus accumbens, subsequently disrupting PPI. Treatment with dopamine receptor agonists and releasing agents causes a disruption of PPI (Van den Buuse, 2003; Varty and Higgins, 1995). While it is tempting to speculate about differential 8-OH-DPAT mechanisms involving pre- vs. post-synaptic 5-HT_{1A} receptors, at this stage this is purely hypothetical and further studies are needed to clarify these mechanisms.

Alternatively, some studies have suggested that there may be different sub-types of the 5-HT_{1A} receptor. In the rat, the mRNA for the 5-HT_{1A} receptor appears to be present as three different mRNAs (De Vry, 1995). Further, the 5-HT_{1A} receptors can be coupled to a variety of second messengers (De Vry, 1995). For example, one study found that in the hippocampus, 8-OH-DPAT inhibits forskolin-stimulated adenylate cyclase activity, but in the DRN 8-OH-DPAT inhibits phosphoinositide hydrolysis (Johnson et al., 1997). Some researchers have suggested that the 5-HT_{1A} receptor exists in two forms: as a high- and low-affinity 5-HT_{1A} receptor (Abe et al., 1999; Mongeau et al., 1992). In the present study, low doses of 8-OH-DPAT or direct administration to the DRN may preferentially activate the high-affinity receptor, while systemic 8-OH-DPAT may act at the low- and high-affinity receptor sites, resulting in different PPI responses. Castration may favour the 5-HT_{1A} receptor-mediated mechanism that causes an increase in PPI.

Why is an increase in PPI observed only in CAST rats? It is possible that sham-operated rats show two simultaneous effects of 8-OH-DPAT injection into the DRN: an increase and a decrease in PPI. These effects may cancel each other out in sham-operated rats, but in CAST rats, the increase in PPI predominates. The exact mechanism of action underlying such an effect is unknown. If the observed increase in PPI in CAST rats was due to an activation of pre-synaptic 5-HT_{1A} receptors, then it would be expected that a serotonergic depletion by a lesion of the raphe nuclei in CAST rats would prevent the increase in PPI. The result observed in CAST rats resembles the increase in PPI that occurs in mice in response to 8-OH-DPAT treatment (Dulawa et al., 2000). Until now, it was assumed that species differences accounted for the opposite effects of 8-OH-DPAT treatment seen in mice and rats. The present study, however, suggests that there may be some similarity between mice and

castrated rats in the function of their 5-HT_{1A} receptors in PPI. Further studies in mice are required to determine why this species shows an increase in PPI in response to systemic 8-OH-DPAT treatment.

In conclusion, the results of the present study suggest that the 8-OH-DPAT-induced increase in startle amplitude and disruption of PPI is not due to an activation of pre-synaptic 5-HT_{1A} receptors located in the DRN. Instead, it is suggested that these effects may be the result of an activation of post-synaptic 5-HT_{1A} receptors, or at least 5-HT_{1A} receptors located in a region other than the DRN. Furthermore, these results suggest that pre-synaptic receptors may be distinct from the post-synaptic 5-HT_{1A} receptors in their response to 8-OH-DPAT treatment in PPI. In fact, under certain circumstances, an opposite effect in PPI can be observed by activating pre- vs. post-synaptic receptors, where in CAST rats only, an increase in PPI may occur in response to activation of 5-HT_{1A} autoreceptors located in the DRN. Therefore, it is important to recognize that 5-HT_{1A} receptors are not only pre-synaptic, inhibitory receptors, but are also active as post-synaptic 5-HT_{1A} receptors.

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