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RESEARCH PAPER

The importance of baseline in identifying 8-OH-DPAT-induced effects on prepulse inhibition in rats

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Background and purpose: Prepulse inhibition (PPI) of the acoustic startle response is a model of sensorimotor gating which is disrupted in schizophrenia and other mental illnesses. We and others have shown that treatment with the 5-hydroxytryptamine-1A (5-HT_{1A}) receptor agonist, 8-OH-DPAT, disrupts PPI in rats. In the present study, we highlight the importance of baseline levels on the effect of 8-OH-DPAT on PPI.
Experimental approach: Adult male and female Sprague-Dawley rats were gonadectomised. These rats were treated with saline, 0.02 and 0.5 mg kg^{-1} of 8-OH-DPAT using a random-sequence, repeated-measures protocol. The rats were allocated into high and low baseline groups depending on their baseline PPI observed after saline treatment.
Key results: Treatment with 0.5 mg kg^{-1} of 8-OH-DPAT significantly disrupted PPI in both male and female rats. In male rats only, 0.02 mg kg^{-1} 8-OH-DPAT caused a small, but significant, increase in PPI. When these male rats were allocated to either a high or low baseline PPI group, 0.5 mg kg^{-1} 8-OH-DPAT disrupted PPI in the high baseline group only. In contrast, treatment with 0.02 mg kg^{-1} 8-OH-DPAT increased PPI only in the low baseline PPI group. There were no changes in the effect of 8-OH-DPAT administration in female rats when they were divided into high and low baseline PPI groups.
Conclusions and implications: The level of baseline PPI is an important variable that can influence the direction of drug effects induced by 8-OH-DPAT. The explanation for this phenomenon could be differential activation of pre- and postsynaptic 5-HT_{1A} receptors.
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Keywords: 5-HT_{1A} receptors; 8-OH-DPAT; prepulse inhibition; schizophrenia; rats; castrated; ovariectomized; startle; baseline

Abbreviations: DRN, dorsal raphe nucleus; GTPγS, [^{35}S]guanosine-5′-*O*-(3-thio)triphosphate; 5-HT_{1A}, 5-hydroxytryptamine-1A; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; OVX, ovariectomized; PP, prepulse; PPI, prepulse inhibition

Introduction

Prepulse inhibition (PPI) is a measure of sensorimotor gating which is deficient in schizophrenia (Braff *et al*., 2001). Sensorimotor gating is a crucial component of sensory information processing and is a normal protective mechanism in the central nervous system that functions to 'gate' or filter irrelevant sensory, motor or cognitive information, therefore allowing for coherent thought (Kodsi and Swerdlow, 1994). 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 (PP)), the subsequent startle response is reduced (Koch, 1999; Braff *et al*., 2001). Over the years, many studies have used a variety of experimental conditions in animals in order to study the brain mechanisms involved in PPI. These studies have implicated several neurotransmitters (e.g. dopamine, 5-hydroxytryptamine (serotonin; 5-HT), glutamate) in the regulation of PPI (Koch, 1999; Geyer *et al*., 2001; Van den Buuse *et al*., 2003).

It has been well established that administration of the prototypical 5-hydroxytryptamine-1A (5-HT_{1A}) receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), results in a disruption of PPI in rats (Rigdon and Weatherspoon, 1992; Sipes and Geyer, 1995; Kinney *et al*., 1999; Sipos *et al*., 2000; Fletcher *et al*., 2001; Czyrak *et al*., 2003; Gogos and Van den Buuse, 2003, 2004; Gogos *et al*., 2005). Several studies have confirmed that 8-OH-DPAT disrupts PPI via activation of 5-HT_{1A} receptors (Sipes and Geyer, 1995; Czyrak *et al*., 2003). However, it is unclear where in the brain this occurs. Only few studies have examined this issue and suggested an important role of presynaptic 5-HT_{1A} receptors located in the raphe nuclei (Sipes and Geyer, 1995) and also of postsynaptic 5-HT_{1A} receptors (Fletcher *et al*., 2001; Gogos *et al*., 2005).

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We have previously shown that castrated male rats have a reduced sensitivity to the disruption of PPI caused by 8-OH-DPAT administration (Gogos and Van den Buuse, 2003). In addition, we found that castrated rats showed a 'biphasic' PPI response to 8-OH-DPAT treatment, where a low dose of 8-OH-DPAT (0.02 mg kg^{-1}) tended to increase PPI and a higher dose (0.5 mg kg^{-1}) decreased PPI (Gogos and Van den Buuse, 2003). We suggested that the PPI response that predominates may depend on the dose of 8-OH-DPAT administered and the population of 5-HT$_{1A}$ receptors activated. Therefore, in a subsequent study, we compared the PPI responses from intact and castrated male rats that received a micro-injection of 8-OH-DPAT into the dorsal raphe nucleus (DRN) (Gogos *et al.*, 2005). We found that there was a trend for an increase in PPI when injecting 8-OH-DPAT directly into the DRN of male castrated rats (Gogos *et al.*, 2005). We concluded that a differential activation of pre- vs postsynaptic 5-HT$_{1A}$ receptors may explain the different PPI responses that may occur. Thus, an increase in PPI could occur in male castrated rats when presynaptic 5-HT$_{1A}$ receptors are activated by local injection into the DRN or by low systemic doses of 8-OH-DPAT and a decrease in PPI could occur by higher systemic 8-OH-DPAT doses. The net effect of 8-OH-DPAT on PPI could therefore be a mixture of increased PPI mediated by activation of presynaptic 5-HT$_{1A}$ receptors and decreased PPI mediated by activation of postsynaptic 5-HT$_{1A}$ receptors, perhaps in the hippocampus or ventral tegmental area (Gogos *et al.*, 2005).

While investigating the mechanisms involved in 8-OH-DPAT-induced disruption of PPI, we observed that only some rats appeared to show low-dose 8-OH-DPAT-induced PPI enhancement, whereas only some rats showed clear high-dose 8-OH-DPAT-induced PPI disruption. Closer inspection of our previous data suggested that the level of baseline PPI in the rats appeared to be an important factor determining whether the increase/decrease in PPI was observed or reached significance. Therefore, in the present study, we re-analysed PPI responses to low- and high-dose 8-OH-DPAT in relation to baseline PPI. We only used gonadectomized male and female rats to exclude any effect of sex steroid hormones (Gogos and Van den Buuse, 2003, 2004).

Methods

Animals

This study used 30 male and 25 female Sprague–Dawley rats, which were obtained from the Department of Pathology and Anatomy Animal Services, University of Melbourne (VIC, Australia). Some of these rats were included in another study (Gogos and Van den Buuse, 2003, 2004). Animals were housed at the Mental Health Research Institute in groups of 2–3 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 hours) 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).

Gonadectomy

Castration of male rats and ovariectomy of female rats was carried out as described previously (Gogos and Van den Buuse, 2003, 2004). Briefly, rats underwent surgery at 12 weeks of age when they weighed an average of 492 g (range: 368–652 g) for male rats and 279 g (range: 224–314 g) for female rats. Rats were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (Nembutal, 60 mg ml^{-1}; Merial Australia, QLD, Australia). Castration involved a small midline incision through the skin of the scrotum and then 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. Ovariectomy involved a midline incision through the skin and the muscle layer of the abdominal area. The ovaries were located and a piece of silk suture was used to ligate the fallopian tubes, after which the ovaries were carefully removed. The muscle layer and skin were suture-closed.

After surgery, antiseptic cream (Betadine, povidone-iodine 10%, Faulding Consumer, SA, Australia) was applied over the sutures. Rats were also given a subcutaneous (s.c.) injection of 5 mg kg^{-1} of the non-steroidal, anti-inflammatory analgesic, carprofen (Zenecarp, 50 mg ml^{-1}, Heriot AgVet, VIC, Australia). All drug solutions were injected with an injection volume of 1 ml kg^{-1}. Behavioural experiments commenced 2 weeks after surgery when normal body weight gain had returned. Three days after completion of experiments, the rats were killed by decapitation and the seminal vesicles and uteri removed and weighed to confirm successful gonadectomy.

PPI of the acoustic startle response

PPI was measured as described previously (Gogos and Van den Buuse, 2003, 2004), using automated startle chambers (SR-Lab; San Diego Instruments, San Diego, CA, USA). Briefly, rats were placed individually into a transparent acrylic cylinder and a piezoelectric transducer mounted underneath the cylinder detected the whole-body startle responses. Sounds were presented through a speaker and responses measured with the SR-Lab software (San Diego Instruments). The PPI session included 40 115 dB pulse-alone trials, 50 PP trials and 10 no-stimulus trials. PP trials consisted of a PP of an intensity 2, 4, 8, 12 or 16 dB above the 70 dB background, followed by a startle pulse of 115 dB, 100 ms later. The % PPI was calculated as the difference in amplitude between the startle response to the pulse-alone trials and the PP-startle pulse trials, divided by the pulse-alone trial $\times$ 100%. Startle amplitude was assessed using the four blocks of 10 115 dB pulses. To show that the percentage calculations did not influence the conclusions (Swerdlow *et al.*, 2000), data from male rats were also analysed as absolute startle values.

Experimental protocol

Two weeks after gonadectomy surgery, rats were randomly injected with saline, 0.02 or 0.5 mg kg^{-1} of 8-OH-DPAT ($\pm$8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide; Tocris

Bioscience, Avonmouth, UK). These doses were selected on the basis of previous experiments (Gogos and Van den Buuse, 2003, 2004). 8-OH-DPAT was dissolved in sterile saline (0.9% sodium chloride, Baxter Healthcare, NSW, Australia) and administered s.c. in the flank, 10 min before the rat was placed in the PPI chamber. Using a randomized, crossover protocol, all rats received all treatments, with 3–4 days allowed between each PPI experiment.

The group baseline average % PPI (i.e. after saline treatment) was used to determine which rats had a high or low baseline PPI. To select rats for the low and high baseline groups, 2 × standard error of the mean (s.e.m.) was added and subtracted from the average % PPI. The average % PPI for the 30 male rats was 49.3 ± 2.5%; therefore, low baseline male rats were those that had an average PPI < 44.3% ($n = 10$; new group mean 34.9 ± 3.5%), high baseline male rats were those that had an average PPI > 54.3% ($n = 10$; new group mean 62.6 ± 1.8%). The average % PPI for the 25 female ovariectomized (OVX) rats was 57.6 ± 2.4%; therefore, low baseline female rats were those with an average PPI < 52.8% ($n = 10$; new group mean 46.0 ± 1.6%), high baseline female rats were those with an average PPI > 62.4% ($n = 9$; new group mean 70.9 ± 1.9%). Consequent to these criteria, some rats had an average % PPI that fell within 49.3 ± 5% for male rats ($n = 10$) and 57.6 ± 5% for female rats ($n = 6$). These rats were not included in the dataset as they were not within the range classified as 'low' or 'high' baseline.

Data analysis

All data are expressed as mean ± s.e.m. Data were analysed using two-way analysis of variance with repeated measures, where appropriate, using the statistical software package SYSTAT 9.0 (SPSS Inc., Chicago, IL, USA).

In order to ascertain that the baseline effects were not influenced by expressing the data as percentage PPI, male rat data were also analysed as absolute startle values, in line with previous studies (Swerdlow *et al*., 2000, 2004; Van den Buuse, 2003). Because of the lack of effect of baseline on responding to 8-OH-DPAT in female rats, further analysis of absolute startle values was not done in this group.

The repeated measures variables were *dose* (saline and two doses of 8-OH-DPAT) and *PP intensity* (PP2–PP16 for %PPI, P115 and PP2–PP16 for absolute values). Differences between means were considered to be significant when $P < 0.05$.

Results

Male rats: startle amplitude

Analysis of startle amplitude data of all male rats showed a significant main effect of dose ($F_{(2,58)} = 7.7$, $P = 0.001$), indicating a significant increase in startle amplitude (Figure 1). Further analysis comparing saline and 8-OH-DPAT treatment showed that startle amplitude was significantly increased after treatment with 0.02 mg kg^{-1} ($F_{(1,29)} = 11.6$, $P = 0.002$) and 0.5 mg kg^{-1} ($F_{(1,29)} = 16.8$, $P < 0.001$) of 8-OH-DPAT. There was no significant difference between the effect of 0.02 and 0.5 mg kg^{-1} of 8-OH-DPAT.

Analysis of the effect of 8-OH-DPAT in male rats with low baseline PPI showed a modest, significant increase in startle amplitude (main effect of dose: $F_{(2,18)} = 3.8$, $P = 0.042$), reflecting the trend for an increase in startle amplitude after 0.02 mg kg^{-1} ($F_{(1,9)} = 4.4$, $P = 0.065$) and 0.5 mg kg^{-1} 8-OH-DPAT ($F_{(1,9)} = 5.1$, $P = 0.051$), compared to saline treatment (Figure 1). Analysis of the effect of 8-OH-DPAT in male rats with high baseline PPI showed a main effect of dose ($F_{(2,18)} = 5.4$, $P = 0.015$), reflecting the increase in startle amplitude caused after injection of 0.02 mg kg^{-1} ($F_{(1,9)} = 7.0$, $P = 0.027$) and 0.5 mg kg^{-1} 8-OH-DPAT ($F_{(1,9)} = 10.9$, $P = 0.009$), compared to saline treatment (Figure 1). There was no significant difference between 0.02 and 0.5 mg kg^{-1} of 8-OH-DPAT. Thus, 8-OH-DPAT treatment increased startle amplitude in male rats, regardless of whether their baseline PPI was low or high.

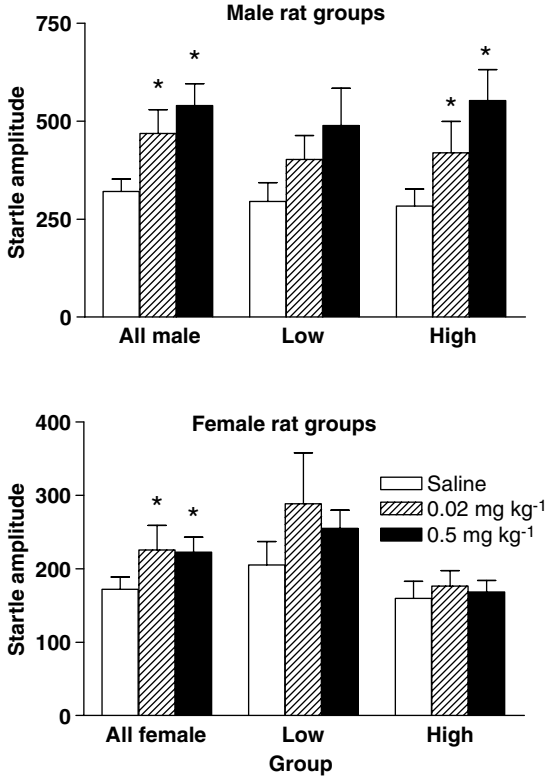

Figure 1 Startle amplitude (arbitrary units) in response to saline, 0.02 or 0.5 mg kg^{-1} 8-OH-DPAT. Top panel shows all male rats combined ($n = 30$) and male rats defined as having a low ($n = 10$) or high ($n = 10$) baseline PPI. Bottom panel shows all female rats combined ($n = 25$) and female rats defined as having a low ($n = 10$) or high ($n = 9$) baseline PPI. *$P < 0.05$, compared to saline treatment.

Male rats: PPI expressed as percentage

Analysis of the data of all male rats combined showed a significant main effect of PP intensity ($F_{(4,116)} = 200.3$, $P < 0.001$), reflecting the expected progressive reduction of startle responses with increasing PP intensity. There was also a significant main effect of dose ($F_{(2,58)} = 27.1$, $P < 0.001$) and a dose × PP intensity interaction ($F_{(8,232)} = 3.6$, $P = 0.001$), reflecting that 8-OH-DPAT treatment altered PPI particularly at the middle PP intensities (Figure 2). Further analysis

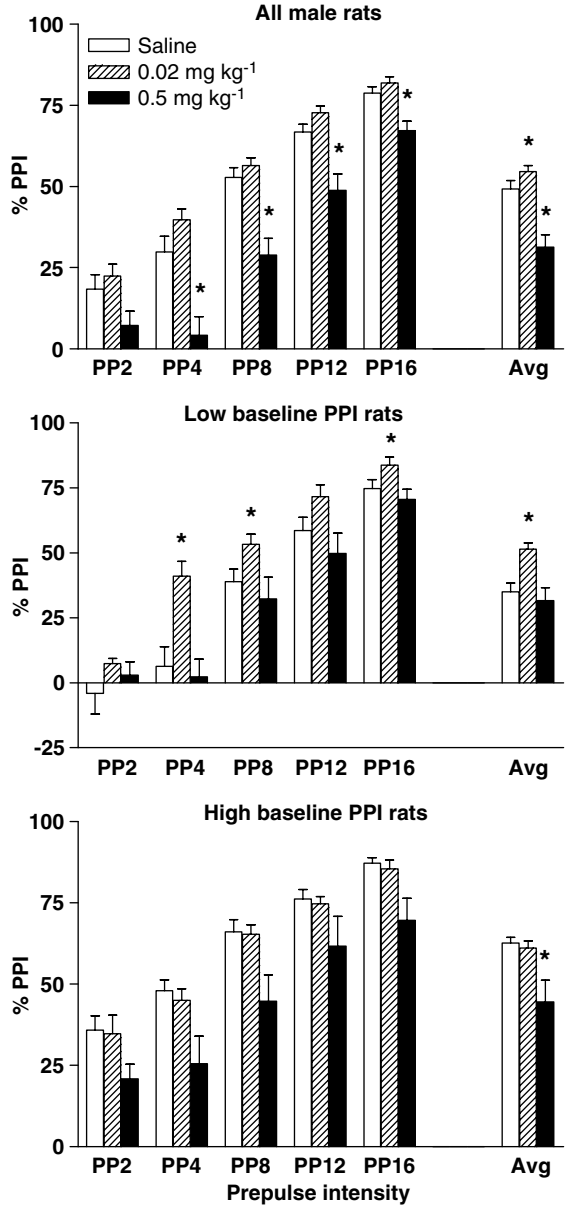

Figure 2 Mean (±s.e.m.) % PPI of all male rats combined ($n=30$; top panel) and male rats defined as having a low ($n=10$; middle panel) or high ($n=10$; bottom panel) baseline PPI. All rats were injected with either saline, 0.02 or 0.5 mg kg^{-1} 8-OH-DPAT. PP2, PP4, PP8, PP12 and PP16 indicate PP intensities of 2, 4, 8, 12 and 16 dB over the 70 dB background. Average (Avg) % PPI is the mean of the five different PP intensities±s.e.m. *$P<0.05$, compared to saline treatment.

revealed that compared to saline treatment, 0.02 mg kg^{-1} 8-OH-DPAT caused a small but significant increase in PPI (main effect of dose: $F_{(1,29)}=4.8$, $P=0.036$; no dose × PP intensity interaction). In contrast, treatment with 0.5 mg kg^{-1} 8-OH-DPAT caused a decrease in PPI (main effect of dose: $F_{(1,29)}=19.2$, $P<0.001$; dose × PP intensity interaction: $F_{(4,116)}=3.0$, $P=0.022$; Figure 2), which was significant at all PP intensities ($P<0.01$) except PP2 ($P=0.054$).

Analysis of the effect of 8-OH-DPAT in male rats with low baseline PPI (Figure 2) showed a main effect of dose ($F_{(2,18)}=9.0$, $P=0.002$) and a dose × PP intensity interaction ($F_{(8,72)}=3.1$, $P=0.005$). Further analysis indicated that the main effect of dose was due to an increase in PPI after treatment with 0.02 mg kg^{-1} 8-OH-DPAT ($F_{(1,9)}=15.6$, $P=0.003$; dose × PP intensity: $F_{(4,36)}=3.8$, $P=0.011$), which was significant at PP4 ($P=0.004$), PP8 ($P=0.005$) and PP16 ($P=0.017$). There was no significant effect of treatment with 0.5 mg kg^{-1} 8-OH-DPAT, compared to saline treatment (Figure 2).

Analysis of the effect of 8-OH-DPAT in male rats with high baseline PPI revealed a main effect of dose ($F_{(2,18)}=8.0$, $P=0.003$; no dose × PP intensity interaction), reflecting the decrease in PPI caused by 0.5 mg kg^{-1} 8-OH-DPAT ($F_{(1,9)}=10.4$, $P=0.010$). There was no significant effect of 0.02 mg kg^{-1} 8-OH-DPAT, compared to saline treatment (Figure 2). Thus, 8-OH-DPAT treatment increased PPI in male rats with low baseline PPI and decreased PPI in male rats with high baseline PPI.

Male rats: PPI expressed as absolute startle amplitude

There was a main effect of PP intensity in each condition (Table 1). Analysis of all male rats combined found that compared to saline treatment, injection of 0.02 mg kg^{-1} 8-OH-DPAT caused a trend for a main effect of dose, reflecting the increase in startle amplitude induced by this treatment. There was also a dose × PP intensity interaction, reflecting the relatively greater impact of PP intensity on startle amplitude after this treatment (Table 1) and consistent with increased %PPI (see above). Treatment with 0.5 mg kg^{-1} 8-OH-DPAT similarly increased startle amplitude (main effect of dose) but *reduced* PPI (dose × PP intensity interaction).

In male rats selected for low baseline PPI, treatment with 0.02 mg kg^{-1} 8-OH-DPAT tended to increase startle amplitude; however, this effect failed to reach significance. Nevertheless, there was a significant dose × PP intensity interaction, again reflecting the greater impact of PP intensity on startle amplitude (Table 1) and consistent with increased %PPI. 8-OH-DPAT (0.5 mg kg^{-1}) increased startle amplitude (main effect of dose) but had no effect on PPI compared to saline treatment (lack of dose × PP intensity interaction).

In male rats selected for high baseline PPI, treatment with 0.02 mg kg^{-1} 8-OH-DPAT again tended to cause an increase in startle (Table 1); however, this did not reach significance. There was no dose × PP intensity interaction, reflecting the similarity of PP effects with 8-OH-DPAT compared to saline treatment. In contrast, 0.5 mg kg^{-1} 8-OH-DPAT increased startle amplitude (main effect of dose) and *reduced* PPI (dose × PP intensity interaction).

The differential effect of 8-OH-DPAT depending on baseline PPI was supported by further comparison of absolute startle amplitude. Thus when analysing all male rats, startle amplitudes were significantly reduced by all PP levels in saline-treated and low-dose-treated rats, but no significant decrease of startle occurred at PP2 and PP4 after injection of 0.5 mg kg^{-1} 8-OH-DPAT (Table 1). Selection for low baseline resulted in PP2 not having an effect after saline treatment; however, the response to injection of 0.02 mg kg^{-1} 8-OH-DPAT was still present in this subgroup, reflecting the

Table 1 Absolute startle responses to either the P115 pulse-alone stimulus or prepulses of 2, 4, 8, 12 or 16 dB over baseline 100 ms before the 115 dB pulse (PP2, PP4, PP8, PP12, PP16)

	P115	*PP2*	*PP4*	*PP8*	*PP12*	*PP16*	*DPAT vs Saline F, P*	*Prepulse intensity F, P*	*Dose × prepulse F, P*
All rats (n = 30)									
Saline	311 ± 28	250 ± 27*	210 ± 20*	148 ± 18*	102 ± 12*	67 ± 10*			
DPAT 0.02	425 ± 60	341 ± 55*	267 ± 47*	197 ± 38*	122 ± 24*	94 ± 27*	3.8, 0.059	74.7, <0.001	4.1, 0.002
DPAT 0.5	558 ± 62	536 ± 72	561 ± 62	404 ± 59*	276 ± 38*	182 ± 27*	26.2, <0.001	63.3, <0.001	6.9, <0.001
Low baseline (n = 10)									
Saline	282 ± 39	295 ± 55	249 ± 32*	169 ± 27*	117 ± 23*	73 ± 16*			
DPAT 0.02	399 ± 73	369 ± 64*	219 ± 32*	191 ± 49*	113 ± 26*	77 ± 30*	NS	33.3, <0.001	3.2, 0.014
DPAT 0.5	499 ± 97	512 ± 125	495 ± 107	391 ± 125*	277 ± 69*	169 ± 56*	5.4, 0.045	27.6, <0.001	NS
High baseline (n = 10)									
Saline	290 ± 40	183 ± 26*	156 ± 28*	105 ± 23*	68 ± 12*	37 ± 7*			
DPAT 0.02	374 ± 67	253 ± 60*	223 ± 56*	131 ± 26*	92 ± 16*	51 ± 10*	NS	31.1, <0.001	NS
DPAT 0.5	594 ± 67	451 ± 70*	407 ± 59*	303 ± 50*	193 ± 40*	156 ± 32*	17.0, 0.003	29.0, <0.001	3.1, 0.018

Abbreviations: ANOVA, analysis of variance; NS, nonsignificant; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; PPI, prepulse inhibition.
Male rats were treated with saline or 8-OH-DPAT (DPAT): 0.02 and 0.5 mg kg^{-1}.
*$P<0.05$ compared with the P115 response. Statistical results in the last three columns refer to F and *P*-values obtained by ANOVA comparison of absolute startle values after treatment with 8-OH-DPAT vs saline, either in all rats combined, rats selected on the basis of a low baseline PPI, or in rats selected on the basis of a high baseline PPI.

enhancement of PPI by this dose. The pattern of responding after treatment with 0.5 mg kg^{-1} 8-OH-DPAT was not altered in the low baseline group, as shown by the persistent lack of effect of PP2 and PP4 (Table 1). In the high baseline group, as expected all PP intensities had significant effects.

Female rats: startle amplitude

Analysis of startle amplitude data of all female rats showed a trend for a main effect of dose ($F_{(2,48)}=2.8$, $P=0.068$). Further analysis comparing saline and 8-OH-DPAT treatment showed that startle amplitude was significantly increased after 0.02 mg kg^{-1} ($F_{(1,24)}=5.3$, $P=0.031$) and 0.5 mg kg^{-1} ($F_{(1,24)}=5.3$, $P=0.031$) of 8-OH-DPAT (Figure 1). There was no significant difference between 0.02 and 0.5 mg kg^{-1} of 8-OH-DPAT. Analysis of startle amplitude data of female rats with low baseline PPI or those with high baseline PPI showed no significant main effect of dose, reflecting the lack of effect of 8-OH-DPAT on startle amplitude in these rats (Figure 1).

Female rats: PPI

Analysis of the data of all female rats showed a significant main effect of PP intensity ($F_{(4,96)}=170.9$, $P<0.001$), reflecting the expected progressive reduction of startle responses with increasing PP intensity. There was also a significant main effect of dose ($F_{(2,48)}=7.6$, $P=0.001$) and a dose × PP intensity interaction ($F_{(8,192)}=3.1$, $P=0.003$; Figure 3). Further analysis indicated that, compared to saline treatment, 0.5 mg kg^{-1} 8-OH-DPAT caused a disruption of PPI (main effect of dose: $F_{(1,24)}=16.2$, $P<0.001$; dose × PP intensity interaction: $F_{(4,96)}=3.9$, $P=0.006$), which was significant at all PP intensities ($P<0.05$) except PP4 ($P=0.058$). In contrast, there was no significant effect of 0.02 mg kg^{-1} 8-OH-DPAT in female rats (Figure 3).

Analysis of the effect of 8-OH-DPAT in female rats with low baseline PPI (Figure 3) showed a main effect of dose ($F_{(2,18)}=5.9$, $P=0.011$), reflecting a significant disruption of PPI after treatment with 0.5 mg kg^{-1} 8-OH-DPAT ($F_{(1,9)}=5.8$, $P=0.040$; no dose × PP intensity interaction). There was no significant effect on PPI after treatment with 0.02 mg kg^{-1} of 8-OH-DPAT (Figure 3) in this group.

Analysis of the effect of 8-OH-DPAT in female rats with high baseline PPI showed a main effect of dose ($F_{(2,16)}=10.3$, $P=0.001$), reflecting a significant decrease in PPI caused by treatment with 0.02 mg kg^{-1} (main effect of dose: $F_{(1,8)}=13.3$, $P=0.007$; dose × PP intensity interaction: $F_{(4,32)}=4.1$, $P=0.008$) and 0.5 mg kg^{-1} 8-OH-DPAT (main effect of dose: $F_{(1,8)}=16.8$, $P=0.003$; dose × PP intensity interaction: $F_{(4,32)}=4.8$, $P=0.004$), compared to saline treatment (Figure 3). The effect of 0.02 mg kg^{-1} 8-OH-DPAT on PPI only reached significance at PP2 ($P=0.016$), whereas treatment with 0.5 mg kg^{-1} 8-OH-DPAT caused a significant disruption at PP2 ($P=0.013$), PP4 ($P=0.026$) and PP8 ($P=0.033$). There was a trend for a difference between 0.02 and 0.5 mg kg^{-1} of 8-OH-DPAT ($F_{(1,8)}=3.7$, $P=0.091$). Thus, 8-OH-DPAT treatment disrupted PPI in all female rats, regardless of high or low baseline PPI. Because of the lack of effect of baseline on responding to 8-OH-DPAT in female rats, further analysis of absolute startle values was not done in this group.

Discussion

In the present study, we have investigated the effect of baseline levels of PPI on 8-OH-DPAT-induced PPI responses. We found that by allocating rats to either low or high baseline PPI groups, we could obtain opposite effects of 8-OH-DPAT on PPI. Thus, male rats selected for low baseline showed an emphasis of low-dose 8-OH-DPAT-induced PPI *enhancement*, whereas male rats selected for high baseline showed an emphasis of high-dose 8-OH-DPAT-induced PPI *disruption*. No influence of baseline PPI was found on the action of 8-OH-DPAT on startle amplitude in male rats, and

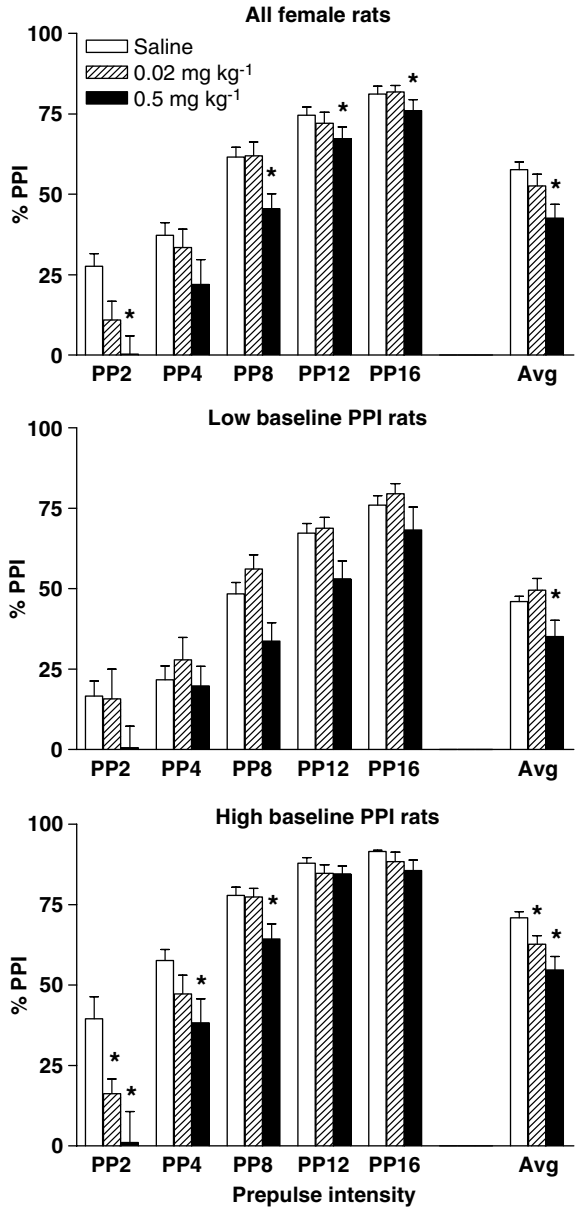

Figure 3 Mean (±s.e.m.) % PPI of all female rats combined ($n=25$; top panel) and female rats defined as having a low ($n=10$; middle panel) or high ($n=9$; bottom panel) baseline PPI. All rats were injected with either saline, 0.02 or 0.5 mg kg^{-1} 8-OH-DPAT. PP2, PP4, PP8, PP12 and PP16 indicate PP intensities of 2, 4, 8, 12 and 16 dB over the 70 dB background. Average (Avg) % PPI is the mean of the five different PP intensities ± s.e.m. *$P<0.05$, compared to saline treatment.

no influence of baseline PPI was found on the action of 8-OH-DPAT on either PPI or startle amplitude in female rats.

Several previous studies have shown disruption of PPI after treatment of male and female rats with a relatively high dose of 8-OH-DPAT (0.5 mg kg^{-1}) (Rigdon and Weatherspoon, 1992; Sipes and Geyer, 1995; Gogos and Van den Buuse, 2003, 2004; Gogos *et al*., 2005). We showed in male castrated rats that a low dose of 8-OH-DPAT (0.02 mg kg^{-1}) tended to cause an increase in PPI (Gogos and Van den Buuse, 2003, 2004). In the present study, these findings are extended as we now show that the increase in PPI can only be observed in male rats that were selected on the basis of having a low baseline PPI, whereas the decrease in PPI can only be shown in rats that were selected on the basis of having a high baseline PPI. Conversely, male rats with high baseline PPI do not show an increase in PPI caused by a low dose of 8-OH-DPAT and rats with low baseline PPI do not show a decrease in PPI normally caused by a relatively high dose of 8-OH-DPAT. These responses were observed regardless of whether we calculated %PPI or absolute startle amplitude.

These findings extend our understanding of the action of 5-HT$_{1A}$ receptor stimulation on PPI. It is unlikely that the differential effect of 8-OH-DPAT seen in male rats at different levels of baseline PPI is simply the result of a floor/ceiling effect. If that were the case, we would have observed the same result in female rats. Furthermore, some drugs, such as phencyclidine, can reduce average PPI to values much lower than that seen in the low baseline group. Even in the high baseline group, lower PP intensities induce only small levels of PPI, which could easily increase to levels seen with higher PP intensities; however, this was not observed. Therefore, the differential effect of 8-OH-DPAT in low vs high baseline rats is more likely to be caused by differential 5-hydroxytryptaminergic activity in the brain.

It is well known that 5-HT$_{1A}$ receptors are present either as presynaptic/autoreceptors on 5-hydroxytryptaminergic projections and cell bodies, respectively, or as postsynaptic receptors in areas such as the hippocampus and ventral tegmental area (Barnes and Sharp, 1999). It has been suggested that in regards to the distinction between pre- and postsynaptic 5-HT$_{1A}$ receptors, that there may in fact be two receptor subtypes (De Vry, 1995). In support of this hypothesis, the signal transduction pathway activated by presynaptic 5-HT$_{1A}$ receptors appears to be different from that activated by postsynaptic 5-HT$_{1A}$ receptors (Clarke *et al*., 1996). In addition, presynaptic 5-HT$_{1A}$ receptors have been shown to be more sensitive to receptor agonists such as 8-OH-DPAT (De Vry, 1995).

While activation of presynaptic 5-HT$_{1A}$ receptors may result in inhibition of 5-hydroxytryptamine release and behavioural inhibition, and direct activation of postsynaptic 5-HT$_{1A}$ receptors leads to behavioural activation (including forepaw treading and flat body posture), a similar opposite effect of 5-HT$_{1A}$ receptor activation depending on differential involvement of pre- and postsynaptic receptors has not been shown previously for PPI. Our results suggest that the presynaptic component of 5-HT$_{1A}$ receptor activation can be unmasked by selecting rats on the basis of a low baseline PPI. This could reflect high resting levels of 5-hydroxytryptaminergic activity in the brain, allowing for a greater inhibitory effect to be seen. An increase in PPI induced by low doses of 8-OH-DPAT was not seen in rats that had been selected for having a high baseline PPI. Possibly this high baseline could reflect low 5-hydroxytryptaminergic activity in the brain, allowing only an increase of 5-hydroxytryptaminergic activation to be evident. However, at this point these explanations are speculative and would need to be confirmed with direct measurement of 5-hydroxytryptamine release in the brain by, for instance, microdialysis.

Surprisingly, selecting female rats on the basis of their baseline PPI did not result in the same unmasking of the

effect of a low dose of 8-OH-DPAT on PPI as seen in male rats. It should be noted, however, that the 'low' baseline in male rats was around 35%, whereas it was 46% in female rats. Similarly, the 'high' baseline was 63% in male rats, whereas it was 71% in female rats. It could be that the 'low' baseline in female rats is simply not low enough and consequently that there is not enough baseline 5-hydroxytryptaminergic activity to allow the presynaptic 5-HT_{1A} receptor-mediated increase in PPI to be unmasked. Alternatively, there may be an inherent sex difference in the way pre- and postsynaptic 5-HT_{1A} receptors are involved in PPI regulation in male and female rats. There are several studies suggesting differential regulation of 5-HT_{1A} receptor function in male vs female rats. For example, 8-OH-DPAT-induced hypothermia and increases in corticosterone, prolactin and adrenocorticotropic hormone levels are more pronounced in female than in male rodents (Carlsson and Eriksson, 1987; Haleem *et al*., 1989; Matsuda *et al*., 1991a, b). In addition, the hypothermic and corticosterone response to 8-OH-DPAT were attenuated by ovariectomy and enhanced by chronic oestradiol treatment in female mice (Matsuda *et al*., 1991a, b). In rats, oestrogen treatment reduces 5-HT_{1A} receptor gene expression in the hippocampus and amygdala (Österlund and Hurd, 1998; Birzniece *et al*., 2001). Oestrogen and testosterone also may be altering 5-HT_{1A} receptor signalling. Acute oestrogen treatment in OVX rats desensitizes the 5-HT_{1A} receptor in the hippocampus, as measured by [^{35}S]guanosine-5′-*O*-(3-thio)triphosphate (GTPγS)-stimulated signalling (Mize and Alper, 2000), presumably through activation of protein kinases A and C (Mize *et al*., 2003). In the present experiments, another sex difference was observed in that male rats showed a significant 8-OH-DPAT-induced increase in startle, which was negligible in female rats.

The result observed in male castrated rats resembles the increase in PPI that occurs in mice in response to 8-OH-DPAT treatment (Dulawa *et al*., 2000; Gogos *et al*., 2006). 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. In mice, similar to castrated rats in the present study, the presynaptic component of 8-OH-DPAT action may predominate, favouring an increase in PPI. However, further studies in mice are required to confirm such a possibility, for example mouse data may be re-analysed after allocating the animals into 'low' baseline and 'high' baseline groups.

In conclusion, the results of the present study suggest that in male castrated rats, an increase or decrease in PPI may predominate depending on the dose of 8-OH-DPAT administered and the level of baseline PPI. The explanation for this phenomenon could be differential activation of pre- and postsynaptic 5-HT_{1A} receptors. We found that by allocating rats into either 'high' or 'low' baseline PPI groups, the subsequent responses to different doses of 8-OH-DPAT appear to be differentially enhanced. It seems that the level of baseline PPI is an important variable that can influence the direction of drug effects, such as those induced by 8-OH-DPAT. This study has wider implications for research on drug effects on PPI. Further studies are needed to investigate if similar baseline effects are important for other neurotransmitter systems such as dopaminergic control of PPI.

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Conflict of interest

The authors state no conflict of interest.

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