Estrogen treatment blocks 8-OH-DPAT- and apomorphine-induced disruptions of prepulse inhibition: Involvement of dopamine D₁ or D₂, serotonin 5-HT₁₆, 5-HT₂A or 5-HT₇ receptors* 

Andrea Gogos, Perrin Kwek, Carolina Chavez and Maarten van den Buuse

Behavioural Neuroscience Laboratory, Mental Health Research Institute of Victoria, Parkville, VIC, Australia (AG, PK, CC, MvdB)
Centre for Neuroscience, University of Melbourne, VIC, Australia (AG)
Department of Pharmacology, University of Melbourne, VIC, Australia (CC, MvdB)
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Corresponding author:

A/Prof Maarten van den Buuse

Behavioural Neuroscience Laboratory, Mental Health Research Institute

155 Oak Street, Parkville (Melbourne), Victoria 3052, Australia

Tel: +61 3 9389 2967, Fax: +61 3 9387 5061, E-mail: m.vandenbuuse@mhri.edu.au

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Abbreviations:

8-OH-DPAT, 8-hydroxy-dipropylaminotetralin

ANOVA, analysis of variance

E20, 5 mm silastic implant containing 20% estradiol

E100, 5 mm silastic implant containing 100% estradiol

OVX, ovariectomized

PP, prepulse

PPI, prepulse inhibition

SEM, standard error of the mean

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Prepulse inhibition (PPI) is a measure of sensorimotor gating and an endophenotype of schizophrenia. We have previously shown in rats that estrogen treatment prevents disruption of PPI by the 5-HT1A/5-HT7 receptor agonist, 8-hydroxy-dipropylaminotetralin (8-OH-DPAT). The aim of the present study was to examine the role of dopamine D1 and D2, 5-HT1A, 5-HT2A and 5-HT7 receptors in these effects. Part 1 of this study investigated the ability of estrogen treatment to reverse PPI disruption induced by 8-OH-DPAT or the dopamine D1/D2 receptor agonist, apomorphine. Part 2 of this study compared these effects to the ability of various antagonists in reversing the action of 8-OH-DPAT and apomorphine on PPI. Female Sprague-Dawley rats were ovariectomized (OVX) and, where appropriate, received silastic implants containing either a low- (E20) or high-dose of estrogen (E100). Two weeks later, PPI was assessed using automated startle boxes. The disruption of PPI by either treatment with 8-OH-DPAT (0.5 mg/kg) or apomorphine (0.3 mg/kg) was similarly prevented by E100 treatment. 8-OH-DPAT-induced PPI disruption was reversed by pretreatment with the 5-HT1A receptor antagonist, WAY 100,635 (1 mg/kg), and the typical antipsychotic and dopamine D2 receptor antagonist, haloperidol (0.25 mg/kg), but not the dopamine D1 receptor antagonist, SCH 23390 (0.1 mg/kg), 5-HT2A/2C receptor antagonist, ketanserin (2 mg/kg) or the 5-HT7 receptor antagonist, SB-269970 (10 mg/kg). Apomorphine-induced disruptions of PPI were reversed by haloperidol and SCH 23390 only. Estrogen may prevent disruptions of PPI induced by both 8-OH-DPAT and apomorphine by an action on dopamine D2 receptors downstream of 5-HT1A receptors.
INTRODUCTION

Epidemiological, clinical and animal studies have led to the hypothesis that the ‘female’ sex steroid hormone, estrogen, plays a protective role in schizophrenia (Seeman and Lang, 1990; Häfner et al., 1993). Men and women with schizophrenia differ in terms of age-at-onset, symptom severity and functional outcome (Häfner et al., 1993). In women, schizophrenia symptoms worsen during the low estrogen stage of the menstrual cycle (Riecher-Rossler et al., 1994). However, the mechanism of action of a protective effect of estrogen is unclear.

Prepulse inhibition (PPI) is a measure of sensorimotor gating which functions as a filter of irrelevant information, preventing information overload and allowing for coherent thought. Patients with schizophrenia have deficient PPI and treatment with atypical antipsychotic medication may reverse this deficit (Braff et al., 2001). The PPI disruption in schizophrenia has been identified as one of the most consistently observed endophenotypes of the illness (Braff et al., 2007). Interestingly, as with schizophrenia, gender differences in PPI have been described, where men show greater PPI than women (Swerdlow et al., 1993). Further, women have greater PPI during the early follicular (low estrogen) phase of the menstrual cycle (Swerdlow et al., 1997; Jovanovic et al., 2004).

PPI can be reliably assessed in humans and experimental animals in a technically similar way (Geyer and Markou, 1995; Gogos and Van den Buuse, 2004; Gogos et al., 2006). Several studies using a variety of experimental conditions in animals have implicated dopamine and serotonin in the regulation of PPI (Geyer et al., 2001). For example, treatment with the dopamine D1/D2 receptor agonist, apomorphine, or the 5-HT1A/5-HT7 receptor agonist, 8-hydroxy-di-propylaminotetralin (8-OH-DPAT), caused a disruption of PPI in male rats.
The 8-OH-DPAT-induced disruption of PPI has been found to be prevented by the 5-HT$_{1A}$ receptor antagonist, WAY 100,135 (Sipes and Geyer, 1995). We previously showed that the effect of 8-OH-DPAT on PPI in female rats could be prevented by estrogen treatment (Gogos and Van den Buuse, 2004). Similarly, we showed that in humans, disruption of PPI by treatment with the 5-HT$_{1A}$ receptor partial agonist, buspirone, could be prevented by treatment with estrogen (Gogos et al., 2006). Thus, our results support the notion that estrogen exerts a functional protection against mechanisms that disrupt PPI, in this case 5-HT$_{1A}$ receptor activation. However, the mechanisms underlying this estrogen effect remain unclear.

It is well established that alterations in dopamine release in the brain play a major role in the symptoms of schizophrenia. Most antipsychotic drugs are dopamine D$_2$ receptor antagonists and the therapeutic effects of antipsychotic drugs are correlated with the level of dopamine receptor blockade in the brain (Harrison, 1999). In addition to dopamine, several studies have suggested a role for serotonin receptors in schizophrenia, particularly the 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor subtypes (Dean, 2003). For example, post-mortem research has shown a significant increase in 5-HT$_{1A}$ receptor and decrease in 5-HT$_{2A}$ receptor binding density in the frontal cortex from patients with schizophrenia (Bantick et al., 2001; Dean, 2003). There is considerable evidence for an interaction of dopaminergic and serotonergic receptor mechanisms in schizophrenia, which may be particularly important in the efficacy of atypical antipsychotic treatment (Alex and Pehek, 2007). 5-HT$_{1A}$ receptor agonists selectively influence dopamine release in particular brain regions (Bantick et al., 2001). In vivo animal studies report that activation of 5-HT$_{1A}$ receptors preferentially increases cortical dopamine release, while not affecting release in the striatum (Bantick et al., 2001; Alex and Pehek,
In terms of schizophrenia, this may improve symptoms while not causing extrapyramidal side-effects, respectively (Bantick et al., 2001).

Previous studies have shown that estrogen dose-dependently modulates dopamine-related behaviors (Van Hartesveldt and Joyce, 1986) and can alter serotonin levels, firing rate, receptor levels, metabolism, and transporter binding and function (Bethea et al., 1998). Thus, the proposed functional protection exerted by estrogen in schizophrenia may be mediated by an action on either dopaminergic or serotonergic activity or both. The aim of the present study was therefore to further investigate the effect of estrogen on PPI. In part one of this study, the action of estrogen on the disruption of PPI induced by 8-OH-DPAT was compared to its effect on the disruption of PPI induced by apomorphine. In the second part of the study, we compared the action of estrogen to that of dopamine D₁ and D₂, 5-HT₁₅, 5-HT₂₅ and 5-HT₇ receptor antagonists.
METHODS

Animals
We used 160 female Sprague-Dawley rats in this study (Monash Animal Services, Monash University, VIC, Australia). The rats were 12 weeks of age at the time of surgery. They were housed in groups of 2-3 in standard rat cages, with free access to standard pellet food and water. The rats were maintained on a 12-h light-dark cycle (lights on at 0630), at a constant temperature of 22 ± 2°C. All surgical techniques, treatments and experimental protocols were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1990) set out by the National Health and Medical Research Council of Australia.

Surgery
For ovariectomy surgery, rats were anaesthetized using an isofluorane/oxygen gas mixture, and placed upon a heat pad in the prone position. The medial region of the lower back was shaved and a 2-3 cm midline incision was made through the skin which was then separated from the underlying muscle in a lateral direction either side of the incision. A 5 mm incision was made through the abdominal wall and the ovaries bilaterally located and removed. The abdominal incision was sutured with silk thread and the skin layer closed using surgical staples. Intact rats were sham-operated, i.e. these rats underwent the same procedure as for ovariectomy, except for removal of the ovaries.

During the ovariectomy procedure, rats were subcutaneously implanted with Silastic implants at the nape of the neck as previously described (Gogos and Van den Buuse, 2004). Briefly, these implants (Dow Corning, I.D. 1.98 mm, O.D. 3.18 mm; Futuremedics Australia, VIC,
Australia) were either empty or filled with crystalline steroid hormones. Estrogen implants were 5 mm long, and filled with either 100% estradiol (17β-estradiol; Sigma Chemical Company, MO, USA), or a 20% estradiol-cholesterol mixture (cholesterol: 5-Cholesten-3β-OL; Steraloids Inc., RI, USA).

After surgery, rats received a subcutaneous injection of 5 mg/kg of the non-steroidal, anti-inflammatory analgesic, carprofen (Zenecarp®; Heriot AgVet, VIC, Australia). Behavioral experiments commenced two weeks after surgery. Three days after completion of experiments, the rats were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (Nembutal®, 60 mg/ml; Merial Australia, QLD, Australia) and killed by decapitation. The uterus was removed, weighed and inspected for any abnormalities. Trunk blood was collected for determining 17β-estradiol levels using a radioimmunoassay kit (Diagnostics Systems laboratories, Beckman Coulter Company, Texas, USA).

Prepulse inhibition (PPI)

PPI of the acoustic startle response was measured with eight automated startle chambers (SR-Lab; San Diego Instruments, San Diego, CA, USA) as previously described (Gogos and Van den Buuse, 2003). Briefly, rats were placed individually into a transparent Plexiglas cylinder in a sound-attenuating cabinet. The PPI session comprised 80 trials presented with variable intervals (8-27 s), including 32 pulse-alone trials (4 blocks of 8 115 dB trials) and 40 prepulse-pulse trials. Prepulse-pulse trials consisted of a prepulse (PP) of an intensity of 2, 4, 8, 12 or 16 dB above the 70 dB background (8 of each), followed 100 ms later by the startle pulse. Startle data was measured using all 4 blocks of pulse-alone trials. The % PPI was calculated as [(pulse-alone trials startle response amplitude - prepulse-pulse trials startle response amplitude)/pulse-alone trials startle response amplitude] * 100.
amplitude) / (pulse-alone trials startle amplitude)] x 100%. The middle 16 pulse-alone trials were used to calculate % PPI.

**Experimental protocol**

All drugs were dissolved or diluted in saline to doses that were selected on the basis of the literature and preliminary experiments. All drugs were administered in a volume of 1 ml/kg. In a randomised, cross-over protocol, all rats in each experiment received all treatments, with 3-4 days allowed between each experiment.

Pre-treatments were administered subcutaneously in the flank 30 mins before the next injection. These included the 5-HT\textsubscript{1A} receptor antagonist, WAY 100,635 (N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyrindinylcyclohexanecarboxamide maleate salt, Sigma-Aldrich Chemical Company, MO, USA), the 5-HT\textsubscript{2A/2C} receptor antagonist, ketanserin (3-(2-[4-(4-Fluorobenzoyl)-1-piperidinyl]ethyl)-2,4(1H,3H)-quinazolinedione (+)-tartrate salt, Tocris, Bristol, UK), the 5-HT\textsubscript{7} receptor antagonist, SB-269970 ((R)-3-[2-[2-(4-Methylpiperidin-1-yl)ethyl]pyrrolidine-1-sulfonyl]phenol hydrochloride, Sigma), the typical antipsychotic and dopamine D\textsubscript{2} receptor antagonist, haloperidol (Serenace, 5 mg/ml ampule, 4-[4-(p-Chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone, Sigma) or the dopamine D\textsubscript{1} receptor antagonist, SCH 23390 (R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride, Research Biochemicals Inc, MA, USA). The treatments, 8-OH-DPAT ((±)-8-Hydroxy-2-(dipropylamino)tetralin, Tocris) and apomorphine (R-(−)-Apomorphine hydrochloride hemihydrate, Sigma) were administered subcutaneously in the flank 10 mins prior to the rat being placed in the PPI chamber.
In experiment 1, rats were randomly chosen to become sham-operated controls receiving an empty implant (n=13), ovariectomized (OVX) rats receiving an empty implant (n=14), OVX rats implanted with the 20% estradiol mixture (E20, n=13), or OVX rats receiving the 100% estradiol implant (E100, n=14). Rats in this experiment were tested for PPI after administration of saline and 0.5 mg/kg 8-OH-DPAT. In experiment 2, rats were randomly chosen to become sham-operated controls receiving an empty implant (n=13), OVX rats receiving an empty implant (n=15), E20-treated OVX rats (n=13), or E100-treated OVX rats (n=12). Rats in this experiment were tested for PPI after administration of saline and 0.3 mg/kg apomorphine.

In experiments 3-7, all rats were OVX rats receiving an empty implant in order to eliminate the confounding effects of sex hormones on PPI. In experiment 3, rats (n=12) were tested for PPI after pre-treatment with saline or 1 mg/kg WAY 100,635 and treatment with either saline, 0.3 mg/kg apomorphine or 0.5 mg/kg 8-OH-DPAT. In experiment 4, rats (n=12) were tested for PPI after pre-treatment with saline or 2 mg/kg ketanserin and treatment with either saline, 0.3 mg/kg apomorphine or 0.5 mg/kg 8-OH-DPAT. In experiment 5, rats (n=8) were tested for PPI after pre-treatment with saline or 10 mg/kg SB-269970 and treatment with saline or 0.5 mg/kg 8-OH-DPAT. In experiment 6, rats (n=9) were tested for PPI after pre-treatment with saline or 0.25 mg/kg haloperidol and treatment with either saline, 0.3 mg/kg apomorphine or 0.5 mg/kg 8-OH-DPAT. In experiment 7, rats (n=12) were tested for PPI after pre-treatment with saline or 0.1 mg/kg SCH 23390 and treatment with either saline, 0.3 mg/kg apomorphine or 0.5 mg/kg 8-OH-DPAT.
Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM) and analyzed using the statistical software package SYSTAT 9.0 (SPSS Inc., Chicago, IL, USA). Average % PPI was used for graphical presentation only and reflects the average of the five prepulse intensities. Body weight, uterus weight and radioimmunoassay results (i.e. circulating estradiol levels) were analyzed with one-way analysis of variance (ANOVA) for group. Body and uterus weights were statistically analyzed for the rats of experiments 1 and 2; estradiol levels were measured in a sample of rats from experiments 1 and 2. PPI and startle data were analyzed with a two-way ANOVA with repeated measures where appropriate. For experiments 1 and 2, a 4 group (intact, OVX, E20, E100) x 2 treatment (saline or 8-OH-DPAT/apomorphine) x 5 prepulse intensity (PP2 – PP16) ANOVA was used. For startle amplitude, a 4 group x 2 treatment x 4 block ANOVA was used. For experiments 3-7, a 2 pre-treatment (saline or antagonist) x 2 treatment (saline or 8-OH-DPAT/apomorphine) x 5 prepulse intensity (PP2 – PP16) ANOVA was used. For startle amplitude, a 2 pre-treatment x 2 treatment x 4 block ANOVA was used. Main effects of prepulse intensity or startle block were always observed and will not be reported here in detail unless there were relevant interactions with other factors. Significant main effects and interactions were further explored with pair-wise ANOVAs. Differences were considered to be significant at \( p < 0.05 \).
RESULTS

Body weight, uterus weight and circulating estradiol levels

Table 1 shows the body and uterus weights of all the rats in the seven experiments. Overall, there was no significant difference in body weight at the time of surgery. In experiment 1 and 2, there were significant group differences in body weight gain (experiment 1, \( F(3,50)=64.7, p<0.001 \); experiment 2, \( F(3,49)=75.5, p<0.001 \)). Compared to sham-operated controls, weight gain was significantly enhanced in untreated OVX rats and significantly reduced in E20- and E100-treated OVX rats (Table 1). Uterus weight was also significantly different between the groups (uterus weight as a % of BW: experiment 1, \( F(3,50)=20.0, p<0.001 \); experiment 2, \( F(3,49)=22.1, p<0.001 \)). Compared with sham-operated rats, untreated OVX rats showed reduced uterus weight, while E20- and E100-treated OVX rats had larger uteri. Further, the untreated OVX rats of experiments 3-7 had comparable body weight gain and uterus weight compared to those in experiments 1 and 2 (Table 1).

Based on the radioimmunoassay results, the levels of circulating 17\(\beta\)-estradiol were: sham-operated rats 7.2 ± 1.1 pg/ml (n=13), untreated OVX rats 3.4 ± 0.4 pg/ml (n=12), E20-treated OVX rats 16.2 ± 1.6 pg/ml (n=15), E100-treated OVX rats 19.3 ± 2.6 pg/ml (n=14). ANOVA indicated that there was a significant difference between the groups (\( F(3,50)=18.3, p<0.01 \)); Bonferroni post-hoc tests showed that both E20- and E100-treated OVX rats had significantly higher levels of circulating estradiol compared to sham-operated and untreated OVX rats (all \( p<0.01 \)).
Experiment 1 – Estrogen treatment and 8-OH-DPAT

When comparing PPI in the four hormone-treated groups, 8-OH-DPAT caused a significant disruption of PPI ($F_{(1,50)}=61.3$, $p<0.001$, Figure 1a). However, the effect of 8-OH-DPAT was different between the four groups (8-OH-DPAT x group interaction $F_{(3,50)}=4.9$, $p<0.01$). Pair-wise ANOVA showed no differences in the effect of 8-OH-DPAT between sham-operated and untreated OVX rats, or between untreated OVX and E20-treated OVX rats. In contrast, when comparing untreated OVX with E100-treated OVX rats, there was a significant reduction in the effect of 8-OH-DPAT when rats received E100 treatment (8-OH-DPAT x group interaction $F_{(1,26)}=10.9$, $p<0.01$). Specifically, treatment with 0.5 mg/kg of 8-OH-DPAT induced a significant 22% reduction of average PPI in untreated OVX rats ($F_{(1,13)}=25.3$, $p<0.001$), but only a non-significant 4% reduction in E100-treated OVX rats (Figure 1a).

Treatment with 8-OH-DPAT caused a slight increase in startle amplitude ($F_{(1,50)}=4.1$, $p<0.05$), which was not different between the groups (Figure 1c).

Experiment 2 – Estrogen treatment and apomorphine

When comparing PPI in the four hormone-treated groups, there was a significant main effect of apomorphine ($F_{(1,49)}=87.3$, $p<0.001$) and an apomorphine x prepulse intensity interaction ($F_{(4,196)}=5.3$, $p<0.001$), indicating that apomorphine treatment disrupted PPI and that this effect was dependent on the prepulse intensity (Figure 1b). Inspection of the data suggested that apomorphine had its greatest effect at the middle prepulse intensities (data not shown). The disruption of PPI caused by treatment with apomorphine was different between the four groups (apomorphine x group interaction $F_{(3,49)}=4.8$, $p<0.01$). Similar to 8-OH-DPAT, there
was no difference in the effect of apomorphine on PPI between sham-operated and untreated OVX rats. However, when comparing untreated OVX rats with E20- or E100-treated OVX rats, there was a significant apomorphine x group interaction ($F_{(1,26)}=4.3$, $p<0.05$ and $F_{(1,25)}=15.4$, $p<0.01$, respectively), reflecting the reduction of the effect of apomorphine with estrogen treatment, in particular with E100 treatment. Treatment with 0.3 mg/kg of apomorphine induced a 32% reduction of average PPI in untreated OVX rats ($F_{(1,14)}=66.9$, $p<0.001$), 21% reduction in E20-treated OVX rats ($F_{(1,12)}=40.3$, $p<0.001$), but only a non-significant 9% reduction in E100-treated OVX rats (Figure 1b).

Apomorphine treatment caused a significant increase in startle amplitude ($F_{(1,49)}=7.7$, $p<0.01$), which was not different between the groups (Figure 1d).

**Experiment 3 – WAY 100,635 vs. 8-OH-DPAT and apomorphine**

Although the main effect of 8-OH-DPAT treatment did not reach significance, there was a significant pre-treatment x 8-OH-DPAT interaction ($F_{(1,11)}=12.6$, $p<0.01$), reflecting the lack of an 8-OH-DPAT-induced PPI disruption after WAY 100,635 pre-treatment (Figure 2a). Indeed, post-hoc analysis showed that 8-OH-DPAT significantly disrupted PPI after saline pre-treatment ($F_{(1,11)}=17.5$, $p<0.01$) but not after WAY 100,635 pre-treatment (Figure 2a). There was also a significant main effect of WAY 100,635 pre-treatment ($F_{(1,11)}=6.7$, $p<0.05$), however, this was not significant when comparing saline/saline and WAY 100,635/saline.

As expected, apomorphine treatment significantly disrupted PPI ($F_{(1,11)}=22.3$, $p<0.01$). There was no main effect of WAY 100,635 pre-treatment or a significant pre-treatment x apomorphine interaction, indicating that the apomorphine-induced PPI disruption was similar
regardless of WAY 100,635 pre-treatment (Figure 2b). Indeed, PPI was significantly disrupted by apomorphine treatment both after saline pre-treatment ($F_{(1,11)}=10.8, p<0.01$) and after WAY 100,635 pre-treatment ($F_{(1,11)}=16.8, p<0.01$). In addition, there was no difference in PPI after apomorphine either in the saline or WAY 100,635 pre-treatment condition.

Analysis of the effect of 8-OH-DPAT on startle amplitude revealed a trend for a pre-treatment x treatment interaction ($F_{(1,11)}=4.3, p=0.06$), but no main effects. This reflected the tendency for startle amplitude to be reduced by 8-OH-DPAT treatment alone, but not after pre-treatment with WAY 100,635 (Figure 2c). Apomorphine treatment tended to cause an increase in startle ($F_{(1,11)}=4.4, p=0.06$, Figure 2d). There was also a significant main effect of WAY 100,635 pre-treatment ($F_{(1,11)}=8.0, p<0.05$) and a pre-treatment x treatment interaction ($F_{(1,11)}=6.1, p<0.05$), indicating that the apomorphine-induced increase in startle amplitude was not seen after WAY 100,635 pre-treatment. Startle was significantly enhanced by apomorphine treatment after saline pre-treatment ($F_{(1,11)}=5.4, p<0.05$) but not after WAY 100,635 pre-treatment (Figure 2d).

Experiment 4 – Ketanserin vs. 8-OH-DPAT and apomorphine

Also in this cohort, 8-OH-DPAT treatment significantly disrupted PPI ($F_{(1,11)}=16.3, p<0.01$). There was no significant main effect or interactions of ketanserin pre-treatment (Figure 3a), suggesting that the 8-OH-DPAT-induced PPI disruption occurred regardless of the presence of ketanserin. Pair-wise comparison confirmed significant PPI disruption by 8-OH-DPAT after saline pre-treatment ($F_{(1,11)}=7.4, p<0.05$) and ketanserin pre-treatment ($F_{(1,11)}=8.4, p<0.05$). In addition, there was no difference in PPI after 8-OH-DPAT either in the saline or ketanserin pre-treatment condition (Figure 3a).
Similarly, apomorphine treatment significantly disrupted PPI \( (F_{(1,11)}=9.1, p<0.05) \) and this was not affected by ketanserin pre-treatment (Figure 3b). PPI was significantly disrupted by apomorphine treatment after saline pre-treatment \( (F_{(1,11)}=13.8, p<0.01) \) although it failed to reach significance after ketanserin pre-treatment \( (F_{(1,11)}=2.5, p=0.14) \). However, there was no difference in PPI after apomorphine between the saline or ketanserin pre-treatment condition.

Analysis of the effect of 8-OH-DPAT on startle amplitude revealed no main effects of ketanserin pre-treatment or 8-OH-DPAT treatment and no interactions (Figure 3c). Apomorphine treatment caused an increase in startle amplitudes \( (F_{(1,10)}=17.7, p<0.01, \text{Figure 3d}) \) but there was no effect of ketanserin pre-treatment or interaction, indicating that the apomorphine-induced increase in startle amplitude was not affected by ketanserin pre-treatment. Startle was significantly enhanced by apomorphine treatment after saline pre-treatment \( (F_{(1,10)}=5.4, p<0.05) \) as well as after ketanserin pre-treatment \( (F_{(1,11)}=13.5, p<0.01, \text{Figure 3d}) \).

**Experiment 5 – SB-269970 vs. 8-OH-DPAT**

8-OH-DPAT treatment significantly disrupted PPI \( (F_{(1,7)}=36.7, p<0.01) \). There was no significant main effect or interactions of SB-269970 pre-treatment (Figure 4a), suggesting that the 8-OH-DPAT-induced PPI disruption occurs regardless of the presence of SB-269970. Pair-wise comparison confirmed significant PPI disruption by 8-OH-DPAT after saline pre-treatment \( (F_{(1,7)}=20.1, p<0.01) \) and SB-269970 pre-treatment \( (F_{(1,7)}=30.3, p<0.01) \). In addition, there was no difference in PPI after 8-OH-DPAT either in the saline or SB-269970 pre-treatment condition (Figure 4a).
Pre-treatment with SB-269970 slightly reduced startle ($F_{(1,7)} = 5.9, p < 0.05$) but there were no significant effects of 8-OH-DPAT or any interactions (Figure 4b).

**Experiment 6 – Haloperidol vs. 8-OH-DPAT and apomorphine**

As expected, 8-OH-DPAT treatment significantly disrupted PPI ($F_{(1,8)} = 16.9, p < 0.01$, Figure 5a). There was a significant main effect of haloperidol pre-treatment ($F_{(1,8)} = 8.6, p < 0.05$), although comparison of the saline/saline and haloperidol/saline conditions was not significantly different. Importantly, the significant pre-treatment x 8-OH-DPAT interaction ($F_{(1,8)} = 5.6, p < 0.05$) reflected that haloperidol pre-treatment blocked the 8-OH-DPAT-induced PPI disruption (Figure 5a). Post-hoc testing confirmed that 8-OH-DPAT disrupted PPI after saline pre-treatment ($F_{(1,8)} = 26.4, p < 0.001$) but not after haloperidol pre-treatment.

Apomorphine treatment significantly disrupted PPI ($F_{(1,8)} = 19.5, p < 0.01$, Figure 5b). In addition, there was a significant effect of haloperidol pre-treatment ($F_{(1,8)} = 12.6, p < 0.01$) and pre-treatment x apomorphine interaction ($F_{(1,8)} = 10.9, p < 0.05$) indicating that haloperidol pre-treatment blocked the apomorphine-induced PPI disruption (Figure 5b). Pair-wise comparison showed that apomorphine disrupted PPI after saline pre-treatment ($F_{(1,8)} = 26.9, p < 0.01$) but not haloperidol pre-treatment.

In the 8-OH-DPAT experiment, haloperidol pre-treatment slightly reduced startle amplitude ($F_{(1,8)} = 26.0, p < 0.01$, Figure 5c) but there was no significant effect of 8-OH-DPAT or interactions. Also in the apomorphine experiment, haloperidol pre-treatment slightly reduced startle ($F_{(1,8)} = 5.9, p < 0.05$, Figure 5d). Apomorphine treatment increased startle ($F_{(1,8)} = 8.2, p < 0.05$), but there was no pre-treatment x treatment interaction (Figure 5d).
Experiment 7 – SCH 23390 vs. 8-OH-DPAT and apomorphine

8-OH-DPAT treatment significantly disrupted PPI ($F_{(1,11)}=13.5$, $p<0.01$, Figure 6a) and pretreatment with SCH 23390 slightly, but significantly increased PPI ($F_{(1,11)}=20.6$, $p<0.01$). Importantly, there was no significant pre-treatment x 8-OH-DPAT interaction suggesting that SCH 23390 pre-treatment did not affect 8-OH-DPAT-induced PPI disruption (Figure 6a). Post-hoc testing confirmed that 8-OH-DPAT disrupted PPI after saline pre-treatment ($F_{(1,11)}=5.5$, $p<0.05$) and after SCH 23390 pre-treatment ($F_{(1,11)}=17.7$, $p<0.01$).

Apomorphine treatment significantly disrupted PPI ($F_{(1,11)}=13.8$, $p<0.01$, Figure 6b). In addition, there was a significant effect of SCH 23390 pre-treatment ($F_{(1,1)}=40.4$, $p<0.001$) and pre-treatment x apomorphine interaction ($F_{(1,11)}=8.6$, $p<0.05$) indicating that SCH 23390 pre-treatment blocked the apomorphine-induced PPI disruption (Figure 6b). Pair-wise comparison showed that apomorphine disrupted PPI after saline pre-treatment ($F_{(1,11)}=14.6$, $p<0.01$) but not SCH 23390 pre-treatment.

In the 8-OH-DPAT experiment, there were no main effects of SCH 23390 pre-treatment or 8-OH-DPAT treatment. However, a significant pre-treatment x 8-OH-DPAT interaction ($F_{(1,11)}=8.8$, $p<0.05$) reflected a trend for an 8-OH-DPAT-induced decrease in startle after saline pre-treatment as opposed to a slight, but significant ($F_{(1,10)}=6.3$, $p<0.05$) 8-OH-DPAT-induced increase in startle after SCH 23390 pre-treatment (Figure 6c). In the apomorphine experiment, SCH 23390 pre-treatment slightly reduced startle ($F_{(1,10)}=13.8$, $p<0.01$, Figure 6d). Although there was no main effect of apomorphine treatment, a significant pre-treatment x apomorphine interaction ($F_{(1,10)}=7.2$, $p<0.05$) reflected a trend for an apomorphine-induced increase in startle after saline pre-treatment as opposed to a slight, but significant ($F_{(1,10)}=6.3$, $p<0.05$) apomorphine-induced decrease in startle after SCH 23390 pre-treatment (Figure 6c).
DISCUSSION

In the present study we aimed to examine the effects and underlying mechanisms of estrogen action on PPI. We showed that estrogen treatment not only reversed disruption of PPI by 8-OH-DPAT treatment but also by apomorphine treatment. Subsequent experiments confirmed that the action of apomorphine on PPI was mediated by dopamine D₁ and D₂ receptors, while the effects of 8-OH-DPAT were mediated by 5-HT₁A receptors. Interestingly, 8-OH-DPAT-induced PPI disruption was also reversed by the dopamine D₂ receptor antagonist, haloperidol, but not the dopamine D₁ receptor antagonist, SCH 23390, 5-HT₂A/2C receptor antagonist, ketanserin or the 5-HT₇ receptor antagonist, SB-269970. Thus, estrogen may prevent disruptions of PPI induced by both 8-OH-DPAT and apomorphine by an action on dopamine D₂ receptors ‘downstream’ of 5-HT₁A receptors.

The present study used OVX rats which had received estrogen implants. Successful ovariectomy of the rats was ascertained by a significant decrease in uterus weight, an increase in body weight gain and a low level of circulating estradiol (Gogos and Van den Buuse, 2004). Estrogen treatment (E20- and E100-treated OVX rats) produced physiological changes compared to both intact and OVX rats, including an increase in uterus weight and decrease in body weight gain, and had high levels of circulating estradiol. The present study used a chronic treatment regimen of hormone replacement as it is advantageous over acute treatment in mimicking the chronic in vivo situation, although a disadvantage is that silastic implants produce stable, continuous hormone release, as opposed to the cyclical levels that occur during the estrous cycle. The estradiol-filled implants used in this study produced levels of circulating estradiol (E20=16 pg/ml, E100=19 pg/ml) that are similar to the mean level of estradiol present throughout the estrous cycle (16 pg/ml) (Albert et al., 1991).
Although E20- and E100-implants produced comparable effects on body and uterus weight, only the E100 implants showed a pharmacological effect and were able to prevent drug-induced disruptions of PPI.

The present results confirm our earlier study on the protective effect of estrogen treatment against 8-OH-DPAT-induced disruptions of PPI in rats (Gogos and Van den Buuse, 2004). We previously showed a similar effect of estrogen treatment in healthy women, where estrogen treatment prevented a disruption of PPI induced by the 5-HT\textsubscript{1A} receptor partial agonist, buspirone (Gogos et al., 2006). Although 8-OH-DPAT is commonly used as the prototypical 5-HT\textsubscript{1A} receptor agonist, it also has some agonist activity at 5-HT\textsubscript{7} receptors (Barnes and Sharp, 1999). This is the first study to show that the specific 5-HT\textsubscript{1A} receptor antagonist, WAY 100,635, blocks 8-OH-DPAT-induced PPI deficits in rats, but the selective 5-HT\textsubscript{7} receptor antagonist, SB-269970, had no effect. Our results suggest that the action of 8-OH-DPAT on PPI is mediated by 5-HT\textsubscript{1A} rather than 5-HT\textsubscript{7} receptors. On its own, this result could then suggest that the action of estrogen on 8-OH-DPAT-induced disruption of PPI is mediated by an effect on 5-HT\textsubscript{1A} receptor function. On the other hand, the present results showed estrogen also inhibited the effect of apomorphine treatment to disrupt PPI in female rats. This supports the notion that estrogen exerts a functional protection against PPI disruption by two neurotransmitter systems, specifically 5-HT\textsubscript{1A} and dopamine D\textsubscript{2} receptor activation. Others have also shown that estrogen can modulate both dopaminergic or serotonergic activity in the brain. For example, estrogen treatment reduces behaviors induced by 8-OH-DPAT, such as the lordosis reflex (Jackson and Etgen, 2001) and hypothermia (Matsuda et al., 1991). In addition, estrogen treatment reduces apomorphine-induced stereotypies in the rat (Van Hartesveldt and Joyce, 1986). Estrogen receptors are located in regions of high 5-HT\textsubscript{1A} receptor density, such as the pre-synaptic autoreceptors of the dorsal...
raphe nucleus as well as post-synaptic regions such as the hippocampus (Azmitia and Whitaker-Azmitia, 1995; Shughrue et al., 1997). Estrogen receptors are also located in regions of high dopamine D2 receptor density, such as the ventral tegmental area (Shughrue et al., 1997).

Apomorphine is a mixed D1/D2 receptor agonist but has greater affinity for the D2 receptor (Kebabian and Calne, 1979). Dopamine D2 receptor antagonists, such as spiperone and raclopride (Swerdlow et al., 1991), and D1 receptor antagonists, such as SCH 23390 (Wan et al., 1996), attenuated apomorphine-induced disruptions of PPI. In previous studies apomorphine effects on PPI involved both D1 and D2 receptors, however, although it has also been suggested that dopaminergic regulation of PPI is predominantly mediated by dopamine D2 receptors (Wan et al., 1996). In our experiments, 8-OH-DPAT-induced disruption of PPI was blocked by pre-treatment with haloperidol, but not SCH 23390, suggesting that D2 receptors play an important ‘downstream’ role in the effects of 5-HT1A receptors on PPI. 5-HT1A and dopamine D2 receptors closely interact in terms of PPI (Van den Buuse and Gogos, 2007). Serotonin can normally inhibit dopamine cell firing and removal of this inhibition by activation of 5-HT1A autoreceptors may therefore increase dopamine cell firing (Alex and Pehek, 2007). Further, studies show that there are direct synaptic contacts between serotonin terminals and dopamine cells in the midbrain (Alex and Pehek, 2007). The 5-HT1A receptor has been found to be located on dopaminergic neurons within the ventral tegmental area (Doherty and Pickel, 2001). This model of primary 5-HT1A receptor activation followed by secondary dopamine D2 receptor activation also helps to explain the similar effect of estrogen on both 8-OH-DPAT- and apomorphine-induced disruption of PPI. Thus, these results suggest that the ‘protective’ effect of estrogen occurs via an action on dopamine D2 receptor mechanisms, blocking both the effect of apomorphine and the ‘upstream’ effect of 8-
OH-DPAT. An interaction of estrogen with D1 receptors is unlikely, as pre-treatment with SCH 23390 did not inhibit the action of 8-OH-DPAT on PPI. Previously, acute or chronic oestrogen treatment in OVX rats increased 5-HT2A receptor binding in the frontal cortex, nucleus accumbens and dorsal raphe nucleus (Sumner and Fink, 1995; Cyr et al., 2000). However, in our experiments pre-treatment with ketanserin did not affect the disruption of PPI either by apomorphine or 8-OH-DPAT, making 5-HT2A receptors less likely as a target for the action of estrogen treatment, at least under these experimental conditions. It is also unlikely that 8-OH-DPAT directly acts on dopamine D2 receptors, as it is not known to have significant affinity for any of the dopamine receptors (Protais et al., 1998). Further, haloperidol has very little affinity for 5-HT1A receptors and a high affinity for dopamine D2 receptors (Newman-Tancredi et al., 2005).

Treatment with 8-OH-DPAT caused a slight increase in startle amplitude, but only in some of the experiments. Previous studies have similarly found either an increase in startle amplitude after 8-OH-DPAT treatment (Gogos and Van den Buuse, 2004), no effect or a partial effect (Sipes and Geyer, 1995; Van den Buuse and Gogos, 2007), suggesting that the role of 5-HT1A receptors on startle amplitude is modest and unlikely to have influenced the present PPI results. Apomorphine treatment significantly increased startle amplitude in several of the experiments of the present study. This is in contrast with previous work where apomorphine treatment had no effect on startle amplitude (Koch, 1998; Van den Buuse, 2003). In the present study, the majority of animals were OVX rats and it may be that ovariectomy and thus the loss of endogenous sex steroid hormones, results in an apomorphine-induced increase in startle amplitude.
In conclusion, these data suggest that 8-OH-DPAT activates 5-HT$_{1A}$ receptors, and not 5-HT$_7$ receptors, which subsequently activate downstream dopamine D$_2$ receptors, resulting in a disruption of PPI. Haloperidol, which blocks the effects of apomorphine treatment on PPI, may act directly on these downstream dopamine receptors to block the action of 5-HT$_{1A}$ receptor activation. WAY 100,635 appears to act directly on 5-HT$_{1A}$ receptors to block the effects of 8-OH-DPAT treatment on PPI. The ‘protective’ effect of estrogen against 8-OH-DPAT- and apomorphine-induced PPI disruptions may be mediated by modulating dopamine D$_2$ receptor function. This may support an antipsychotic-like action of estrogen, at least on PPI. PPI is a valid animal model of sensorimotor gating which is disrupted in schizophrenia (Geyer and Markou, 1995) and some other psychiatric illnesses (Gogos et al., 2009). The findings of the present study suggest an interaction between 5-HT$_{1A}$ and dopamine D$_2$ receptors in mediating PPI and may be of importance in schizophrenia. For example, the newer atypical antipsychotic, aripiprazole, is clinically effective and has partial agonist activity at 5-HT$_{1A}$ and D$_2$ receptors and acts as a dopamine-serotonin system stabiliser (Burris et al., 2002). The present findings suggest a mechanism of action for a protective role of estrogen in PPI and therefore schizophrenia, that is, estrogen acts on the dopamine D$_2$ receptor. Furthermore, the present results may help to explain the beneficial effects of estrogen treatment on symptoms of the illness (Kulkarni et al., 2001).
REFERENCES


receptors: affinity, efficacy and potential implications for treatment of schizophrenia.


FOOTNOTES

* Funding for this study was provided by the National Health and Medical Research Council of Australia in the form of a Project Grant (ID 509234), a Peter Doherty Fellowship (ID 435690 to A.G.) and a senior research fellowship (ID 435500 to MvdB); the J. & P. Clemenger Trust; and the Operational Infrastructure Support (OIS) from the Victorian State Government.

Reprint requests to:
Maarten van den Buuse
Mental Health Research Institute
155 Oak St, Parkville, VIC, 3052, Australia
Email: m.vandenbuuse@mhri.edu.au
LEGENDS FOR FIGURES

Figure 1: Average % PPI (panels (a) and (b)) and startle amplitude (arbitrary units; panels (c) and (d)) of female sham-operated controls (Sham), untreated ovariectomized rats (OVX), 20% estradiol (E20)- or 100% estradiol (E100)-treated OVX rats (n=12-15 per group). All rats were administered saline and 0.5 mg/kg 8-OH-DPAT (left panels) or saline and 0.3 mg/kg apomorphine (right panels). *p<0.05 compared to saline treatment.

Figure 2: Average % PPI (panels (a) and (b)) and startle amplitude (arbitrary units; panels (c) and (d)) of untreated ovariectomized rats (n=12) that were pre-treated with saline or 1 mg/kg WAY 100,635, followed 30 mins later by saline, 0.5 mg/kg 8-OH-DPAT (left panels) or 0.3 mg/kg apomorphine (right panels). *p<0.05 compared to saline treatment.

Figure 3: Average % PPI (panels (a) and (b)) and startle amplitude (arbitrary units; panels (c) and (d)) of untreated ovariectomized rats (n=12) that were pre-treated with saline or 2 mg/kg ketanserin, followed 30 mins later by saline, 0.5 mg/kg 8-OH-DPAT (left panels) or 0.3 mg/kg apomorphine (right panels). *p<0.05 compared to saline treatment.

Figure 4: Average % PPI (panel (a)) and startle amplitude (arbitrary units; panel (b)) of untreated ovariectomized rats (n=8) that were pre-treated with saline or 10 mg/kg SB-269970, followed 30 mins later by saline or 0.5 mg/kg 8-OH-DPAT. *p<0.05 compared to saline treatment.

Figure 5: Average % PPI (panels (a) and (b)) and startle amplitude (arbitrary units; panels (c) and (d)) of untreated ovariectomized rats (n=9) that were pre-treated with saline or
0.25 mg/kg haloperidol, followed 30 mins later by saline, 0.5 mg/kg 8-OH-DPAT (left panels) or 0.3 mg/kg apomorphine (right panels). *p<0.05 compared to saline treatment.

**Figure 6:** Average % PPI (panels (a) and (b)) and startle amplitude (arbitrary units; panels (c) and (d)) of untreated ovariectomized rats (n=12) that were pre-treated with saline or 0.1 mg/kg SCH 23390, followed 30 mins later by saline, 0.5 mg/kg 8-OH-DPAT (left panels) or 0.3 mg/kg apomorphine (right panels). *p<0.05 compared to saline treatment.
TABLES

Table 1: Body weight (BW) and uterus weight (UW) of female rats.

<table>
<thead>
<tr>
<th>Experiment 1 – 8-OH-DPAT</th>
<th>Surgery BW</th>
<th>Weight gain</th>
<th>Uterus weight</th>
<th>% UW/BW</th>
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<tbody>
<tr>
<td>Sham-operated</td>
<td>252 ± 8</td>
<td>32 ± 6</td>
<td>0.47 ± 0.03</td>
<td>0.17 ± 0.01</td>
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<tr>
<td>OVX</td>
<td>244 ± 5</td>
<td>96 ± 5*</td>
<td>0.14 ± 0.01*</td>
<td>0.04 ± 0.00*</td>
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<tr>
<td>E20</td>
<td>270 ± 12</td>
<td>10 ± 5*†</td>
<td>1.03 ± 0.12*†</td>
<td>0.39 ± 0.05*†</td>
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<tr>
<td>E100</td>
<td>257 ± 7</td>
<td>10 ± 4*†</td>
<td>0.82 ± 0.08*†</td>
<td>0.32 ± 0.05*†</td>
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<th>Experiment 2 – Apomorphine</th>
<th>Surgery BW</th>
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<th>Uterus weight</th>
<th>% UW/BW</th>
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<tr>
<td>Sham-operated</td>
<td>258 ± 10</td>
<td>26 ± 4</td>
<td>0.46 ± 0.02</td>
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<td>OVX</td>
<td>250 ± 7</td>
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<td>E20</td>
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<td>7 ± 4*†</td>
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<th>Experiment 3 - WAY 100,635</th>
<th>Surgery BW</th>
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<td></td>
<td>234 ± 6</td>
<td>83 ± 5</td>
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<th>Experiment 4 - Ketanserin</th>
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<th>% UW/BW</th>
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<td>241 ± 5</td>
<td>64 ± 6</td>
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<th>Experiment 5 – SB-269970</th>
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<td></td>
<td>243 ± 6</td>
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<th>Experiment 6 - Haloperidol</th>
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<th>Uterus weight</th>
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<td>226 ± 4</td>
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<th>Experiment 7 – SCH 23390</th>
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<th>Weight gain</th>
<th>Uterus weight</th>
<th>% UW/BW</th>
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<td>241 ± 6</td>
<td>76 ± 3</td>
<td>0.12 ± 0.00</td>
<td>0.04 ± 0.00</td>
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In experiments 1 and 2, rats were either sham-operated controls, untreated ovariectomized (OVX) rats, or 20% estradiol (E20) or 100% estradiol (E100) treated OVX rats. Experiments 3-7 included only OVX rats. All weights are in grams and are expressed as mean ± SEM for n=8-15 per group. Weight gain is the difference between body weight on the day of surgery and final body weight. In experiment 1 and 2: *p<0.05 compared with sham-operated rats; †p<0.05 compared with untreated OVX rats.
Fig 4

(a) SB-269970 & 8-OH-DPAT

Average % PPI

Saline

8-OH-DPAT

* *

(b) Saline

8-OH-DPAT

Startle amplitude

Saline

SB-269970

Pre-treatment
Fig 5

(a) Haloperidol & 8-OH-DPAT

(b) Haloperidol & Apomorphine

(c) Haloperidol & 8-OH-DPAT

(d) Haloperidol & Apomorphine
Fig 6

(a) SCH 23390 & 8-OH-DPAT

- Saline
- 8-OH-DPAT

(b) SCH 23390 & Apomorphine

- Saline
- Apomorphine

(c) SCH 23390 & 8-OH-DPAT

- Saline
- 8-OH-DPAT

(d) SCH 23390 & Apomorphine

- Saline
- Apomorphine

Startle amplitude