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RESEARCH PAPER

# The effect of 'two hit' neonatal and young-adult stress on dopaminergic modulation of prepulse inhibition and dopamine receptor density

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**Background and purpose:** A combination of early neurodevelopmental insult(s) and young-adult stress exposure may be involved in the development of schizophrenia. We studied prepulse inhibition (PPI) regulation in rats after an early stress, maternal deprivation, combined with a later stress, simulated by chronic corticosterone treatment, and also determined whether changes in brain dopamine receptor density were involved.
**Experimental approach:** Rats were subjected to either 24 h maternal deprivation on postnatal day 9, corticosterone treatment from 8 to 10 weeks of age, or both. At 12 weeks of age, the rats were injected with 0.1, 0.3 or 1.0 mg·kg$^{-1}$ of apomorphine or 0.5 or 2.5 mg·kg$^{-1}$ of amphetamine and PPI was determined using automated startle boxes. Dopamine $D_1$ and $D_2$ receptor levels were assessed in the nucleus accumbens and caudate nucleus using receptor autoradiography.
**Key results:** Young-adult treatment with corticosterone resulted in attenuated disruption of PPI by apomorphine and amphetamine. In some rats, maternal deprivation resulted in reduced baseline PPI which added to the effect of corticosterone treatment. There was no down-regulation of dopamine $D_1$ or $D_2$ receptors.
**Conclusions and implications:** These results confirm and extend our finding of an inhibitory interaction of developmental stress on dopaminergic regulation of PPI. No corresponding changes in dopamine receptor density were observed in brain regions with a major involvement in PPI regulation, suggesting long-lasting desensitization of dopamine receptor signalling or indirect changes in PPI regulation.
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**Keywords:** dopamine; prepulse inhibition; rat; schizophrenia; stress

**Abbreviations:** ACTH, adrenocorticotropic hormone; APO-UNSUS, apomorphine unsusceptible; APO-SUS, apomorphine-susceptible; BDNF, brain-derived neurotrophic factor; CC, cingulate cortex; CN, caudate nucleus; Chol, cholesterol; CORT, corticosterone; CREB, cyclic-AMP response element binding protein; ETE, estimated tissue equivalent; MD, maternal deprivation; NAc, nucleus accumbens core; Nas, nucleus accumbens shell; ND, non-deprived; NSB, non-specific binding; PPI, prepulse inhibition; TB, total binding

## Introduction

With its early onset of symptoms in the late teens or early twenties, schizophrenia is commonly referred as a developmental disorder. The developmental hypothesis of schizophrenia suggests that disturbances which occurred early in development, such as malnutrition, viral infection, obstetric complications or genetic deficits, predispose to the illness (Bayer *et al.*, 1999; Murray and Fearon, 1999; Cannon *et al.*, 2003). More recently, it has been suggested that these early neurodevelopmental insults are not sufficient to cause schizophrenia and, instead, a combination of early neurodevelopmental insult(s) and young-adult stress exposure may be involved in the illness (Pantelis *et al.*, 2003).

Several animal models have been proposed to better understand the developmental aspects of schizophrenia (van den Buuse *et al.*, 2003). However, most of the animal models include a single developmental insult, for example neonatal hippocampal or prefrontal cortex lesions, neonatal hypoxia or isolation rearing (Geyer *et al.*, 1993; Lipska *et al.*, 1995; Brake *et al.*, 2000; Rehn *et al.*, 2004). Therefore, we recently established an animal model, comprising of a combination of an early stress, induced by neonatal maternal deprivation (MD), and a later stress, simulated by young-adult corticosterone (CORT) treatment (Garner *et al.*, 2007; Choy and van den Buuse, 2008; Choy *et al.*, 2008) These animals showed selective impairments of learning and memory and reduced brain-derived neurotrophic factor (BDNF) gene expression,

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changes which were not observed in animals after either of the stress episodes (Choy *et al.*, 2008).

In this model, we also assessed dopaminergic regulation of prepulse inhibition (PPI), a measure of sensorimotor gating, which is disrupted in patients with schizophrenia (Braff and Geyer 1990; Braff *et al.*, 1995). Dopaminergic pathways in regions such as striatum (caudate putamen) and nucleus accumbens play an important role in PPI regulation via activation of both dopamine $D_1$ and $D_2$ receptors (Swerdlow *et al.*, 1986; 1991; Wan and Swerdlow, 1993; Wan *et al.*, 1994). Stress and developmental insults may result in long-term changes in dopamine receptor responsiveness via alterations in the activity of the hypothalamic-pituitary-adrenal axis (Walker and Diforio, 1997). In animals treated with neonatal MD and young-adult CORT, treatment with either the dopamine receptor agonist, apomorphine, or the dopamine releaser, amphetamine, failed to cause the disruption of PPI seen in controls (Choy and van den Buuse, 2008). Because we previously only used a single dose of apomorphine and amphetamine, the aim of the present study was to replicate and extend our findings by testing multiple doses of both drugs in animals treated with the two developmental insults. Similar dose-response studies have also been done on other neurodevelopmental models (Lipska *et al.*, 1995; Swerdlow *et al.*, 2000). In addition, the density of dopamine $D_1$ and $D_2$ receptors in the nucleus accumbens and caudate putamen was assessed, in order to investigate whether the functional dopaminergic desensitization was correlated with dopamine receptor down-regulation, as observed in other studies (Al-Amin *et al.*, 2000; Tenn *et al.*, 2003).

## Methods

*Animals*

All animal experiments and procedures were approved by the University of Melbourne Animal Experimentation Committee.

Wistar outbred rats (Animal Resources Centre, Canning Vale, WA, Australia) were housed in plastic cages (28 cm $h \times 30$ cm $w \times 43$ cm $l$) at 21°C on a 12 h light/dark cycle (light on 6:00). Rats received *ad libitum* access to food and water. The rats' cages were cleaned twice weekly excluding the pregnant mothers during the last week of gestation, or mothers and pups prior to weaning. Litters found at 9:00 were classified as postnatal day 1 (pnd 1) and those found at 17:00 as pnd 0. All litters were sexed and culled to a maximum of 8 within 24 h after birth, with healthy male pups as the primary choice. Pups were weaned at pnd 21.

Body weights were obtained at the time of weaning (3 weeks of age), when Chol or CORT pellets were implanted (young adult, 8 weeks of age), 3 days after implantation (week 8 + 3 days), 2 weeks after implantation at the time of removal of the pellets (10 weeks of age) and 1–3 days after the last behavioural test (adult, 14 weeks of age).

*MD and CORT pellet implants*

Maternal deprivation and CORT pellet implants were carried out as previously described (Choy and van den Buuse, 2008). In brief, the litters were separated from their mothers for 24 h on pnd 9, and the same animals were implanted with a 100 mg CORT pellet from week 8 to week 10. As in our previous studies, no mortality was found among pups. Behavioural testing commenced at week 12, i.e. a minimum of 2 weeks after removal of the CORT pellets. Together with the controls of MD (non-deprived, ND) and CORT (cholesterol pellet, Chol), there were four experimental groups after the MD/ND and Chol/CORT treatments in each cohort: ND Chol (controls), ND CORT (CORT treated), MD Chol (maternally deprived) and MD CORT (maternally deprived and CORT treated). Two cohorts of animals comprising 39–40 pups per cohort from 14 to 16 litters were used in this study, with 1–2 pups from each litter allocated to different pre-treatment (ND or MD), treatment (Chol or CORT), and either apomorphine ($n = 10$ each group) or amphetamine ($n = 9$–10 each group) PPI behavioural groups.

*PPI of acoustic startle*

Prepulse inhibition experiments were performed using automated startle boxes (SR-LAB, San Diego Instruments, San Diego, CA, USA) with Plexiglas cylinders (9 cm diameter). A piezoelectric accelerometer unit attached underneath the platform of the Plexiglas cylinders detected the startle responses of the animals.

The session started with a 5 min habituation period and included three startle blocks, with the first and the last consisting of ten 115 dB startle pulse-alone trials. The second block was part of the main PPI protocol and consisted of another two blocks of ten 115 dB startle pulse-alone trials. There were 50 prepulse trials, during which a startle pulse was preceded by a prepulse of 2, 4, 8, 12 or 16 dB above the 70 dB background (10 of each), and 10 'no stimulus' trials. Each startle pulse was of 40 ms duration, while the prepulse was 20 ms and there was 100 ms between the prepulse and the pulse. Percentage PPI was calculated with the formula $100 \times$ [(pulse-alone trials – prepulse trials)/pulse-alone trials].

Saline (baseline), three different doses (0.1, 0.3 and 1.0 $mg \cdot kg^{-1}$) of apomorphine (Sigma) or two different doses (0.5 and 2.5 $mg \cdot kg^{-1}$) of amphetamine (Sigma) were subcutaneously (s.c.) injected 10 min before the session start. Using a pseudo-randomized, within-animal protocol, all rats were treated with all doses of either apomorphine or amphetamine, with 3–4 days of washout between individual sessions. PPI testing thus lasted for about 2 weeks. From these animals, eight per group were randomly chosen for dopamine $D_1$ and $D_2$ receptor autoradiography.

*Dopamine $D_1$ and $D_2$ receptor autoradiography*

Animals were culled by decapitation 3 days after the last behavioural tests and brains were rapidly dissected out and snap frozen on powdered dry ice. Fourteen µm coronal sections from the anterior part of the brain including the nucleus accumbens and caudate nucleus were cut on a cryostat and mounted on gel-coated slides where three sections were used for total binding (TB) as a triplicate and another three for non-specific binding (NSB).

The sections were pre-incubated in buffer containing 50 $mmol \cdot L^{-1}$ Tris-HCl, 120 $mmol \cdot L^{-1}$ NaCl, 5 $mmol \cdot L^{-1}$ KCl,

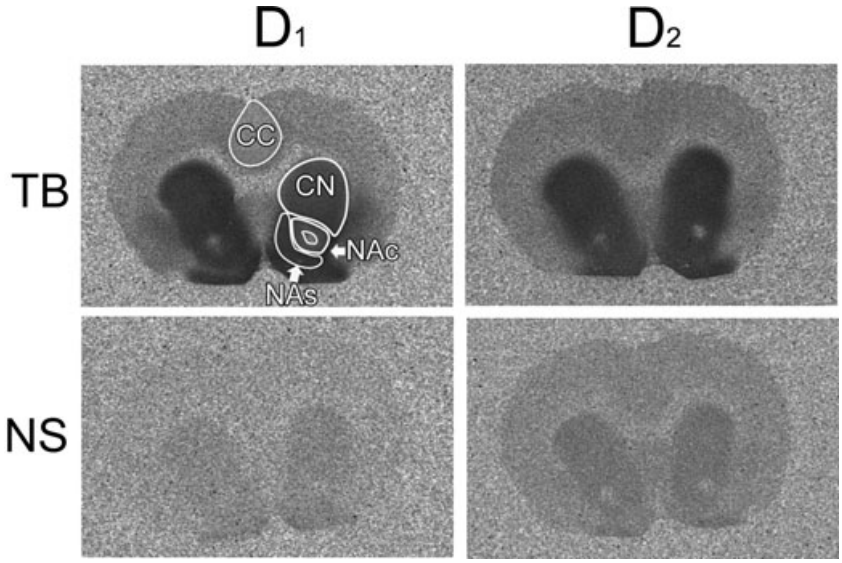


**Figure 1** Representative example of dopamine $D_1$ receptor (left column) and $D_2$ receptor (right column) autoradiography of the rat forebrain. Top panel shows the total binding (TB) of [$^3$H]SCH23390 (left, $D_1$) or [$^3$H]YM091512 (right, $D_2$) and the bottom panel shows the corresponding non-specific binding (NS). CC, cingulate cortex; CN, caudate nucleus; NAc, nucleus accumbens core; NAs, nucleus accumbens shell.

2 mmol·L$^{-1}$ $CaCl_2$ and 1 mmol·L$^{-1}$ $MgCl_2$ (pH 7.4) for 30 min. For dopamine $D_1$ receptor binding we used 30 min incubation with 1 nmol·L$^{-1}$ of [$^3$H]SCH23390 (Perkin Elmer) for TB and additional 0.5 μmol·L$^{-1}$ cis-flupenthixol (Sigma) for NSB (Figure 1, left panel). This concentration of ligand was similar to that in other studies (Filloux *et al.*, 1987; McCabe *et al.*, 1987) and is approximately twice the $K_D$ of dopamine $D_1$ receptors in these conditions (Billard *et al.*, 1984). For dopamine $D_2$ receptor binding, we used 1 nmol·L$^{-1}$ of [$^3$H]YM09151-2 (nemonapride, Perkin Elmer), 0.1 μmol·L$^{-1}$ of pindolol (Sigma) and 0.5 μmol·L$^{-1}$ of 1,3-di-o-tolyguanidine (Sigma) for TB and additional 10 μmol·L$^{-1}$ of (-)-sulpiride (Sigma) for NSB (Figure 1, right panel). The reported $K_D$ value for dopamine $D_2$ receptors is between 0.1 and 0.6 nmol·L$^{-1}$ (Cox and Waszczak, 1991; Yokoyama *et al.*, 1994; Unis *et al.*, 1998) and our ligand concentration, which has also been used by others (Landwehrmeyer *et al.*, 1993), therefore approximated to between 1.7× and 10× the $K_D$.

Both radiolabelled slides and [$^3$H] microscales were apposed to a BAS-TR2025 phosphoimaging plate (Fuji Imaging Plates, Berthold, Australia) for 6 days at room temperature. On the scanned image, relevant brain areas were identified and outlined on the computer screen using the dopamine $D_1$ and $D_2$ receptor density pattern (see Figure 1) and a brain atlas (Paxinos and Watson, 1986). Data from the left and right sides of the brain were averaged as we did not observe any significant left to right differences for either $D_1$ or $D_2$ receptor density (data not shown). The photo-stimulated luminescence of the analysed image was converted to dpm·mg$^{-1}$ tissue equivalents using a standard curve obtained by linear regression of the values derived from the tritium scales exposed to the same phosphor-imaging plate (AIS image analysis software). Then dpm·mg$^{-1}$ values were converted to fmol·mg$^{-1}$ taking into account the ligand's specific activity (in Ci mmol$^{-1}$), a decay factor (a constant) and a dpm to Ci conversion constant ($2.22 \times 10^{12}$ dpm·Ci$^{-1}$). The density of $D_1$ and $D_2$ receptors is thus presented as fmol per milligram (fmol·mg$^{-1}$) estimated tissue equivalent by subtracting the NSB density value from the TB value (Figure 1) in the caudate putamen, nucleus accumbens core, nucleus accumbens shell and cingulate cortex.

Receptor nomenclature conforms to British Journal of Pharmacology's Guide to Receptors and Channels (Alexander *et al.*, 2008).

*Data analysis*

All data were analysed with ANOVA with repeated measures when necessary, using the statistical software package Systat version 9.0 (SPSS Inc., Chicago, IL, USA). Where $P < 0.05$, differences were considered to be statistically significant. In this $2 \times 2$ design, the between-group factors were the pretreatment (two levels: ND or MD) and the treatment (two levels: Chol or CORT implants). Within-group factors were drug dose (apomorphine: saline, 0.1, 0.3 and 1.0 mg·kg$^{-1}$ and amphetamine: saline, 0.5 and 2.5 mg·kg$^{-1}$) and prepulse intensity (72, 74, 78, 82 and 86 dB). In the first experiment on apomorphine effects, a possible influence of MD-induced changes in baseline PPI was excluded by selecting a subgroup ($n = 5$) of animals from each treatment group on the basis of baseline PPI and repeating the ANOVA. Dopamine $D_1$ and $D_2$ autoradiography data of individual brain regions were analysed with two-way ANOVA.

## Results

*Effect of MD and CORT on body weight*

Body weight was significantly lower in maternally deprived animals at weaning at 3 weeks of age [main effect $F(1,77) = 24.3$, $P < 0.001$]. This overall effect of MD lasted to at least 3 days after the pellet administration [week 8:

**Table 1** Body weights of rats subjected to either maternal deprivation (MD) or young-adult corticosterone (CORT) treatment or both

| *Age* | *ND* | | *MD* | |
|---|---|---|---|---|
| Week 3 | 57.6 ± 1.0 | | 49.5 ± 1.3* | |
| Week 8 | 321.3 ± 4.1 | | 304.2 ± 5.5* | |
| | *ND Chol* | *ND CORT* | *MD Chol* | *MD CORT* |
| Week 8 + 3 days | 332.3 ± 5.6 | 314.1 ± 6.9* | 314.0 ± 8.2 | 300.0 ± 8.1* |
| Change from week 8 ($g \cdot day^{-1}$) | 2.60 ± 0.48 | −1.33 ± 0.62* | 3.05 ± 0.24 | −1.20 ± 0.47*,** |
| Week 10 | 392.8 ± 8.6 | 373.4 ± 9.7 | 373.7 ± 10.5 | 357.8 ± 11.6* |
| Change from week 8 ($g \cdot day^{-1}$) | 4.88 ± 0.25 | 3.95 ± 0.34* | 4.92 ± 0.27 | 3.87 ± 0.36*,** |
| Week 14 | 492.5 ± 8.7 | 465.5 ± 7.9* | 468.2 ± 10.2 | 460.8 ± 12.1* |

Controls were non-deprived (ND) or received a cholesterol (Chol) control pellet. Data are presented as mean ± standard error of the mean (SEM) and were obtained from all animals used in the PPI tests (both apomorphine and amphetamine groups). The number of animals in these combined groups was ND Chol = 20, ND CORT = 20, MD Chol = 19 and MD CORT = 20. *$P < 0.05$ for difference with control (ND Chol), **$P < 0.05$ for difference between Chol- and CORT-treated maternally deprived animals (i.e. MD Chol vs. MD CORT).

$F(1,77) = 6.2$, $P = 0.015$, week 8 + 3 days: $F(1,75) = 5.0$, $P = 0.029$] and diminished thereafter (e.g. week 10, $P > 0.05$). A significant CORT effect on body weight was observed 3 days after pellet implantation [$F(1,75) = 4.9$, $P = 0.030$] and, similarly, the CORT effect diminished at week 10 ($P > 0.05$). There were no significant effects of MD or CORT on body weight after week 10 (Table 1). Growth rate in gram per day ($g \cdot day^{-1}$) showed a significant reduction from week 8 to week 10 [$F(1,75) = 19.2$, $P = 0.002$] caused by CORT treatment; however, there were no other differences or interactions among the four groups in other time periods (Table 1).

*Experiment 1: effect of apomorphine on PPI and startle*

After saline treatment, there was no significant main effect of CORT treatment on PPI in this cohort and a trend for a main effect of MD [$F(1,36) = 3.5$, $P = 0.068$] (Figure 2). The effect of MD was significant at PP4 [$F(1,36) = 5.3$, $P = 0.027$] and PP8 [$F(1,36) = 5.5$, $P = 0.025$]. This confirms our previous observations of a small reduction of baseline PPI after MD (Garner *et al.*, 2007; Choy and van den Buuse, 2008), which in this cohort is dependent on the prepulse intensity. In contrast to PPI, there were no effects of MD or CORT treatment on baseline startle (Table 2).

Apomorphine treatment caused a dose-dependent reduction of PPI (Figure 2). Both 0.3 and 1.0 $mg \cdot kg^{-1}$ of apomorphine disrupted PPI in ND Chol and ND CORT animals, whereas only the highest dose induced a significant effect in the MD Chol group and no significant effect of apomorphine was observed in the MD CORT group (Figure 2). There was a significant overall dose-dependent effect of apomorphine treatment [$F(3,108) = 22.0$, $P < 0.001$] and a significant pretreatment (MD) by treatment (CORT) interaction [$F(1,36) = 5.0$, $P = 0.032$] (Figure 2).

To exclude any influence of changes in baseline PPI (see above and Figure 2), further analysis was done on data from a balanced selection of animals ($n = 5$) from each group (Table 3). In these selected animals, after saline treatment there were no differences in average baseline PPI (Table 3) nor was there an interaction with prepulse level (data not shown). Other than the expected main effect of apomorphine treatment on PPI in these selected rats [$F(3,48) = 14.0$, $P < 0.001$], there were no other main effects but there was an interaction of CORT treatment and apomorphine dose which was near significance [$F(3,48) = 2.8$, $P = 0.051$]. This was more clear if only the 0.3 $mg \cdot kg^{-1}$ dose was compared with saline treatment [apomorphine dose × CORT interaction $F(1,16) = 4.7$, $P = 0.045$]. *Post hoc* analysis revealed that this dose caused significant disruption of PPI in both Chol-treated groups [effect of apomorphine $F(1,8) = 32.7$, $P < 0.001$, no additional effect of MD] but not in CORT-treated groups. This result suggests that CORT treatment, but not MD, affects the action of apomorphine on PPI if the rat groups are balanced for baseline.

For startle amplitude, analysis of data from all rats in this cohort (Table 2) revealed a main effect of apomorphine treatment [$F(3,108) = 22.7$, $P < 0.001$] but no statistical interactions of MD and CORT treatment, as seen for PPI (Table 2).

*Experiment 2: effect of amphetamine on PPI and startle*

Similar to the apomorphine cohort, there was no significant main effect of either MD or CORT treatment on PPI or startle in saline-treated rats in this cohort (Figure 3, Table 2). In addition to the expected main effect of prepulse level, there was no interaction between prepulse level and any other factor in this cohort.

In contrast to the effect in the apomorphine cohort (above), analysis of the effect of amphetamine on PPI revealed no statistical interaction of MD by CORT treatment (Figure 3). Instead, in addition to the expected main effect of amphetamine dose [$F(2,70) = 28.0$, $P < 0.001$], there was an amphetamine dose by CORT treatment interaction [$F(2,70) = 4.2$, $P = 0.019$] and a prepulse by amphetamine dose by CORT interaction [$F(8,280) = 2.5$, $P = 0.011$]. The dose by CORT interaction was due to significant PPI disruption caused by 0.5 $mg \cdot kg^{-1}$ amphetamine in Chol-treated animals [ND Chol: $F(1,9) = 8.0$, $P = 0.020$ and MD Chol: $F(1,8) = 10.5$, $P = 0.012$] being absent in CORT-treated animals. In contrast, significant amphetamine-induced PPI disruption was observed at the dose of 2.5 $mg \cdot kg^{-1}$ in all groups: ND Chol [$F(1,9) = 12.4$, $P = 0.007$], ND CORT [$F(1,9) = 13.7$, $P = 0.005$] and MD Chol [$F(1,8) = 18.3$, $P = 0.003$], although only at borderline significance in the MD CORT group [$F(1,9) = 5.3$, $P = 0.047$]. With

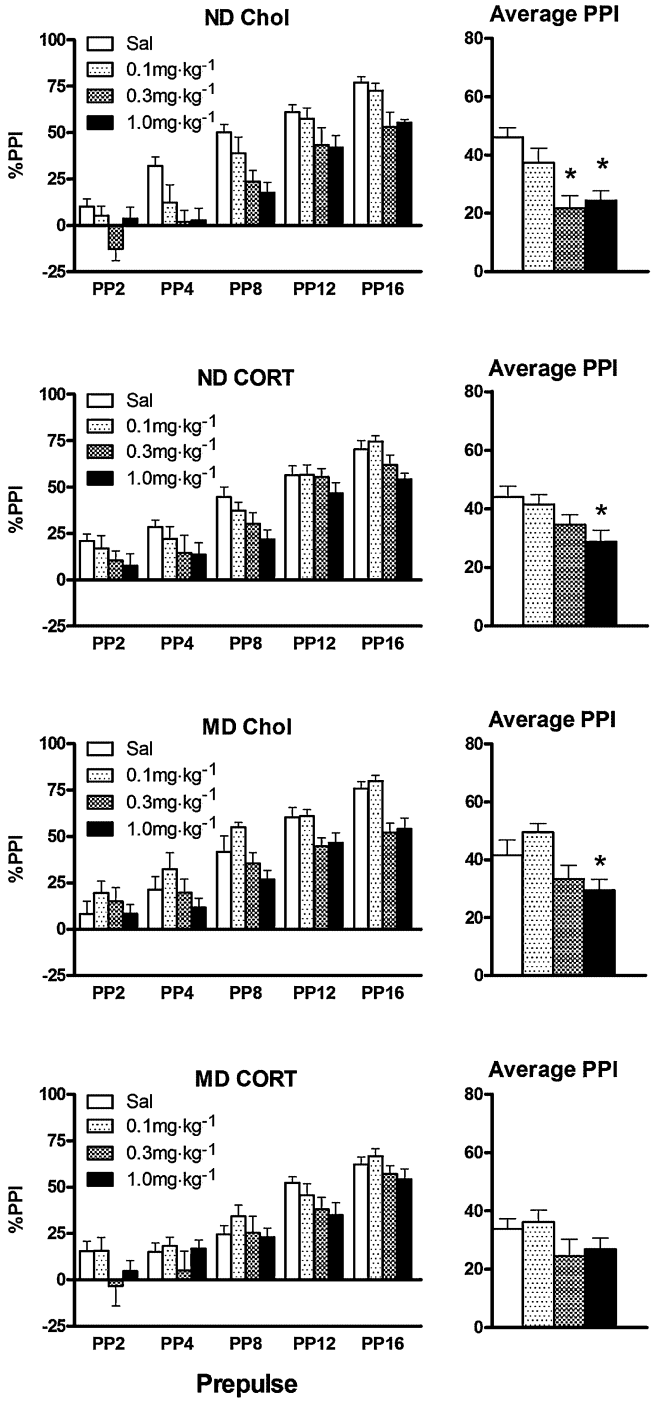


**Figure 2** The effect of different doses of apomorphine (0.1, 0.3 and 1.0 mg·kg$^{-1}$) on prepulse inhibition of startle (PPI) in rats after maternal deprivation (MD) and chronic corticosterone (CORT) treatment. MD consisted of a 24 h separation of the pups from their mothers on postnatal day 9, whereas controls were non-deprived (ND). CORT treatment consisted of subcutaneous implantation of a 100 mg CORT pellet between 8 and 10 weeks of age, whereas controls received a cholesterol (Chol) implant. Behavioural experiments were done between 12 and 14 weeks of age. Left panels show PPI expressed per prepulse intensity (PP) level, whereas right panels show PPI data expressed as average across all prepulse intensities. Data are mean ± standard error of the mean (SEM) for 10 rats per group. *$P < 0.05$ for difference with saline (Sal) treatment. For details of statistical analysis, see text.

respect to the prepulse by amphetamine dose by CORT interaction, further analysis of individual prepulse intensities revealed that at PP2, in addition to a main effect of amphetamine dose [$F(2,70) = 6.1$, $P = 0.004$], there was also a dose × CORT interaction [$F(2,70) = 5.6$, $P = 0.005$] reflecting that amphetamine reduced PPI at this prepulse intensity only in the Chol-treated groups, but not after CORT treatment (Figure 3, left panel). There were no interactions between amphetamine dose and MD or CORT at any of the other prepulse intensities. Statistical analysis of startle data revealed neither main effects nor interactions (Table 2).

*Dopamine $D_1$ and $D_2$ receptor autoradiography*

Dopamine $D_1$ and $D_2$ receptor binding was highest in the caudate nucleus, followed by slightly lower levels in the nucleus accumbens core and shell. Dopamine $D_1$ binding was low in the cingulate cortex, whereas $D_2$ receptor binding was close to the level of NSB in this region (Table 4).

For $D_1$ receptor levels, there were no main effects or interactions observed in the caudate nucleus. Instead, a significant, although weak, effect of MD was observed in the nucleus accumbens core [$F(1,28) = 4.5$, $P = 0.042$] and shell [$F(1,28) = 4.4$, $P = 0.044$]. Further analysis revealed no differences between the ND Chol and other groups in any of the brain regions (Table 4). For $D_2$ receptor levels, there was a MD by CORT interaction observed in the caudate nucleus [$F(1,28) = 6.1$, $P = 0.020$] and nucleus accumbens core [$F(1,28) = 5.1$, $P = 0.032$]. This statistical interaction reflected the tendency for a CORT-induced decrease of $D_2$ receptor density in ND animals compared with a tendency for a CORT-induced increase of $D_2$ receptor density in maternally deprived animals in these regions (Table 4).

## Discussion

The present study confirmed and extended our previous observations that MD and CORT treatment induced differential changes in the PPI responses to dopaminergic stimulation by apomorphine and amphetamine (Choy and van den Buuse, 2008). Apomorphine is a direct dopamine receptor agonist, with limited selectivity for dopamine $D_2$ over $D_1$ receptors. As a dopamine releaser, amphetamine indirectly activates dopamine receptors, with no selectivity between them. As expected, the control animals showed dose-dependent disruption of PPI in response to treatment with either of the dopaminergic psychotomimetics. The main finding of our study was that in animals previously treated as young adults with CORT, both the apomorphine- and amphetamine-induced disruption of PPI was attenuated. It also appeared that MD may influence the results through changes in baseline PPI. This could explain some of our previous findings where the greatest inhibition of PPI disruption was observed in rats which had been previously treated with a combination of MD and young-adult CORT (Choy and van den Buuse, 2008). However, if the rat groups were balanced for baseline PPI, as we did in the current study in the apomorphine cohort, or if baseline PPI was not significantly different, as in the amphetamine cohort, the additional effect of

**Table 2** Average startle responses of rats after acute treatment of either saline or different doses of apomorphine or amphetamine

| | *ND Chol* | *ND CORT* | *MD Chol* | *MD CORT* |
|---|---|---|---|---|
| Apomorphine cohort | | | | |
| Saline | 603 ± 81 | 725 ± 103 | 786 ± 88 | 707 ± 111 |
| 0.1 mg·kg$^{-1}$ | 380 ± 54* | 403 ± 46* | 543 ± 53* | 589 ± 95 |
| 0.3 mg·kg$^{-1}$ | 586 ± 69 | 566 ± 80 | 597 ± 71* | 687 ± 168 |
| 1.0 mg·kg$^{-1}$ | 856 ± 129 | 1013 ± 163 | 914 ± 115 | 870 ± 161 |
| Amphetamine cohort | | | | |
| Saline | 936 ± 123 | 837 ± 163 | 967 ± 96 | 875 ± 79 |
| 0.5 mg·kg$^{-1}$ | 818 ± 106 | 756 ± 68 | 827 ± 126 | 932 ± 89 |
| 2.5 mg·kg$^{-1}$ | 805 ± 125 | 755 ± 67 | 906 ± 122 | 1003 ± 128 |

The rats were subjected to either maternal deprivation (MD) or young-adult corticosterone (CORT) treatment or both. Controls were non-deprived (ND) or received a cholesterol (Chol) control pellet. Data are presented as mean ± standard error of the mean (SEM). The number of animals in the apomorphine cohort was $n = 10$ in each group. The number of animals in the amphetamine cohort was $n = 10$ each, except MD Chol where $n = 9$. Differences between the groups were analysed with ANOVA. *$P < 0.05$ for difference with saline within group.

**Table 3** Effect of apomorphine (0.1–1.0 mg·kg$^{-1}$) on prepulse inhibition (PPI) of rat subgroups selected from the first cohort of animals and balanced for baseline

| | *ND Chol* | *ND CORT* | *MD Chol* | *MD CORT* |
|---|---|---|---|---|
| Saline | 40.8 ± 2.1 | 39.7 ± 4.6 | 40.7 ± 5.4 | 39.9 ± 3.8 |
| 0.1 mg·kg$^{-1}$ | 27.2 ± 5.8 | 36.1 ± 5.6 | 47.5 ± 4.6 | 37.8 ± 5.7 |
| 0.3 mg·kg$^{-1}$ | 14.2 ± 6.1* | 30.6 ± 2.2 | 22.8 ± 5.6* | 40.5 ± 7.1 |
| 1.0 mg·kg$^{-1}$ | 18.0 ± 4.5* | 27.4 ± 4.2 | 24.1 ± 2.3* | 35.8 ± 3.1 |

The rats were subjected to either maternal deprivation (MD) or young-adult corticosterone (CORT) treatment or both. Controls were non-deprived (ND) or received a cholesterol (Chol) control pellet. Data are presented as mean ± standard error of the mean (SEM) of $n = 5$ per group. Differences between the groups were analysed with ANOVA (see text for details). *$P < 0.05$ for difference with saline in the same group (paired *t*-test).

MD on drug-induced PPI disruption disappeared. Importantly, the altered responses to dopaminergic drugs were not associated with CORT-induced dopamine $D_1$ or $D_2$ receptor down-regulation in forebrain areas with a major involvement in PPI regulation.

The effect of CORT treatment on the action of apomorphine and amphetamine in the absence of major changes in receptor density suggests long-lasting effects on dopamine receptor signalling or dopamine release involved in PPI. The additional effect of MD through changes in baseline PPI could also involve effects on dopaminergic activity; however, further experiments are needed to confirm this. In any case the effect of MD on baseline PPI in the current experiments (in the first cohort) and our previous studies (Garner *et al.*, 2007; Choy and van den Buuse, 2008) was far smaller than that seen in previous studies using the same protocol (Ellenbroek *et al.*, 1998). The reason for these differences is unclear, but could be related to the use of different Wistar substrains or details of the MD and/or PPI procedure. Interestingly, a previous study focusing on baseline PPI, found that the disruption caused by either MD or post-weaning isolation was absent in animals which had undergone both stress episodes (Ellenbroek and Cools, 2002). This confirms that two stress episodes along the neurodevelopmental trajectory can interact and lead to an apparently reduced disruption of PPI.

The observation that there were only minor changes of dopamine receptor levels in the nucleus accumbens and caudate nucleus is in line with some previous studies, although overall the literature on the effects of MD or CORT treatment on dopamine receptor densities has been inconclusive. For example, MD increased dopamine $D_1$ receptor levels in one study (Brake *et al.*, 2004), although other studies showed no change (Henry *et al.*, 1995; Ploj *et al.*, 2003). Inconclusive results were also reported for dopamine receptors after chronic stress, as simulated in the present study with chronic CORT treatment. For example, unavoidable stresses increased $D_1$ receptor binding density and decreased $D_2$ binding in the nucleus accumbens (Scheggi *et al.*, 2002). In contrast, another study showed that chronic restraint stress caused a decrease of $D_1$ and no change of $D_2$ receptor density in the nucleus accumbens (Giardino *et al.*, 1998), whereas in another study, chronic immobilization stress was reported to cause increased $D_2$ receptor density (Lucas *et al.*, 2007). Prenatal stress or stress during pregnancy caused a significant increase of $D_2$ receptor expression at an adult age (Henry *et al.*, 1995; Berger *et al.*, 2002) although the neonatal MD in our experiment did not. Instead of increasing $D_2$ receptor density, in our study the maternally deprived animals showed slightly higher $D_1$ receptor expression. However, in these animals there were no major changes of the amphetamine and apomorphine effect on PPI, suggesting that the minor $D_1$ receptor up-regulation has little functional consequence. In this context it is of interest to note that the role of dopamine $D_1$ receptors in PPI regulation may be largely focused in the prelimbic frontal cortex (Peng *et al.*, 1990; de Jong and van den Buuse, 2006), where no difference in densities was observed in the present experimental groups.

Because our receptor binding studies did not reveal receptor down-regulation which could explain the apparent CORT-induced desensitization to the effect of apomorphine

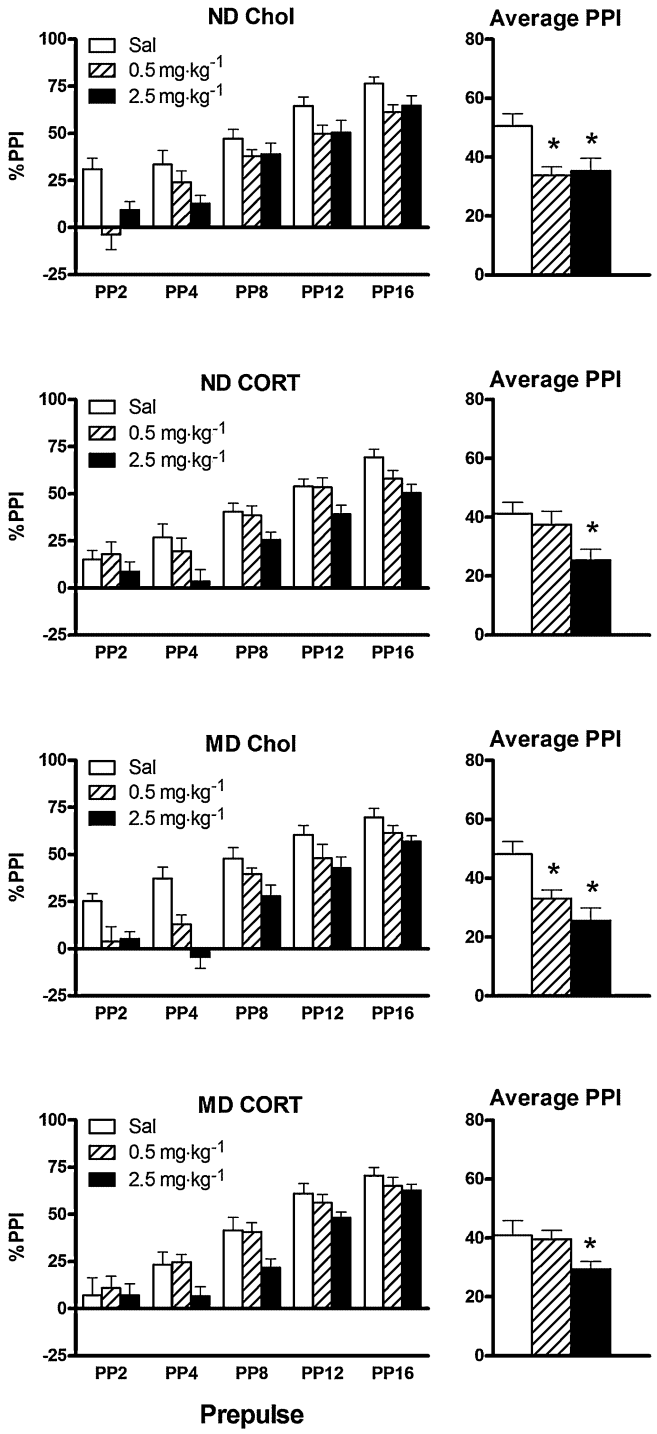


**Figure 3** The effect of different doses of amphetamine (0.5 and 2.5 mg·kg$^{-1}$) on prepulse inhibition of startle (PPI) in rats after maternal deprivation (MD) and chronic corticosterone (CORT) treatment. MD consisted of a 24 h separation of the pups from their mothers on postnatal day 9, whereas controls were non-deprived (ND). CORT treatment consisted of subcutaneous implantation of a 100 mg CORT pellet between 8 and 10 weeks of age, whereas controls received a cholesterol (Chol) implant. Behavioural experiments were done between 12 and 14 weeks of age. Left panels show PPI expressed per prepulse intensity (PP) level, whereas right panels show PPI data expressed as average across all prepulse intensities. Data are mean ± standard error of the mean (SEM) for 9–10 rats per group. *$P < 0.05$ for difference with saline (Sal) treatment. For details of statistical analysis, see text.

and amphetamine on PPI, mechanisms 'behind' the receptor, that is receptor signalling, are likely to be involved. Other studies have also shown a lack of receptor density or mRNA changes despite alterations in drug responsiveness caused by developmental disruptions (Al-Amin *et al.*, 2001). The identity of the dopamine receptor signalling mechanisms remains to be elucidated. Previously, the effects of chronic treatment with the dopamine $D_2/D_3$ receptor agonist, quinpirole, were studied (Culm *et al.*, 2004). These authors showed that the disruption of PPI by acute quinpirole treatment was absent after a 4-week daily treatment with the same drug. This relative insensitivity to quinpirole was associated with increased phosphorylation of cyclic AMP response element binding protein (CREB) which is a second messenger component downstream of dopamine receptors (Culm *et al.*, 2004). This finding could indicate a possible molecular mechanism relevant for our CORT pretreatment effect, whereby enhanced dopaminergic tone, by chronic quinpirole treatment in the Culm *et al.* (2004) study or intrinsic in our animals, induces reduced sensitivity to acute dopaminergic stimulation [quinpirole in Culm *et al.* (2004) or apomorphine/amphetamine in our studies] by enhanced CREB phosphorylation. Another possibility to explain the present results is BDNF, as we have recently found a decrease of BDNF expression in animals that were subjected to both MD and young-adult CORT treatment (Choy *et al.*, 2008). BDNF expression is regulated at the level of CREB phosphorylation (Miyata *et al.*, 2001), and the decrease of BDNF expression could be a reflection of changes in the levels of CREB phosphorylation, which additionally contributes to attenuation of dopaminergic responsiveness in PPI regulation. However, at this point, the association between functional dopaminergic desensitization, CREB phosphorylation and BDNF expression remains speculative and further experiments are needed to explore these links.

Another animal model with relevance to the present data is the apomorphine-unsusceptible (APO-UNSUS) Wistar rat developed by Cools *et al.* (1990). These animals and their controls, the apomorphine susceptible (APO-SUS) rats, were selectively bred by these researchers according to their responsiveness to a 1.5 mg·kg$^{-1}$ apomorphine dose assessed by their gnawing scores (Cools *et al.*, 1990). Similar to the present results, these animals displayed a lower $D_2$ receptor-associated PPI response (Rots *et al.*, 1996; van der Elst *et al.*, 2006). These authors furthermore observed lower corticotrophin-releasing hormone mRNA levels in the paraventricular nucleus, lower baseline plasma adrenocorticotropic hormone (ACTH) levels, and lower and shorter plasma ACTH and CORT secretion responses (Rots *et al.*, 1995; de Kloet *et al.*, 1996). The APO-UNSUS also exhibited a smaller supply of dopamine, which was determined by measuring the levels of factors limiting dopamine synthesis, such as tyrosine hydroxylase (van der Elst *et al.*, 2005). Because APO-UNSUS rats appear similar to our CORT-treated animals in terms of low apomorphine responsiveness, it is possible that our animals also exhibit these changes in components of the hypothalamus-pituitary-adrenal axis. On the other hand, APO-UNSUS exhibited lower dopamine $D_1$ and $D_2$ receptor expression (Rots *et al.*, 1996; van der Elst *et al.*, 2006), which is not in line with our findings

**Table 4** Density of dopamine $D_1$ and $D_2$ receptors in rats subjected to either maternal deprivation (MD) or young-adult corticosterone (CORT) treatment or both

| | *ND Chol* | *ND CORT* | *MD Chol* | *MD CORT* |
|---|---|---|---|---|
| $D_1$ receptor | | | | |
| Accumbens core | 60.1 ± 5.7 | 59.5 ± 2.5 | 72.3 ± 5.3 | 65.5 ± 2.5 |
| Accumbens shell | 65.1 ± 6.4 | 65.1 ± 2.7 | 80.6 ± 6.3 | 70.7 ± 3.3 |
| Caudate | 67.1 ± 3.8 | 69.4 ± 3.1 | 74.0 ± 3.1 | 73.5 ± 1.5 |
| Cingulate cortex | 13.3 ± 0.6 | 14.0 ± 1.2 | 13.8 ± 0.5 | 14.9 ± 0.7 |
| $D_2$ receptor | | | | |
| Accumbens core | 59.0 ± 4.0 | 51.0 ± 2.3 | 57.1 ± 2.0 | 64.0 ± 4.2 |
| Accumbens shell | 57.5 ± 3.7 | 50.7 ± 2.9 | 57.9 ± 1.9 | 64.8 ± 5.3 |
| Caudate | 82.0 ± 4.2 | 69.3 ± 4.1 | 76.1 ± 5.0 | 86.5 ± 5.4 |
| Cingulate cortex | 4.5 ± 1.1 | 4.7 ± 2.6 | 2.9 ± 0.5 | 2.4 ± 0.8 |

Data are mean ± SEM of eight rats per group and expressed as fmol·mg$^{-1}$ estimated tissue equivalent (ETE). Controls were non-deprived (ND) or received a cholesterol (Chol) control pellet. [$^3$H]SCH23390 ($D_1$ receptor) or [$^3$H]YM091512 ($D_2$ receptor) binding density was assessed by autoradiography.

of a lack of receptor down-regulation. Furthermore, while the apomorphine responsiveness in APO-UNSUS and APO-SUS rats was linked to changes of mineralocorticoid and glucocorticoid receptor binding and expression in the hippocampus (de Kloet *et al*., 1996; de Bruin *et al*., 2001), we have conducted preliminary *in situ* hybridization measurements of glucocorticoid receptor expression and found it to be not significantly altered in rats after either MD, CORT treatment, or both (unpublished results).

In conclusion, early developmental insults have been implicated in the development of schizophrenia and it has been proposed that two or more developmental insults are required. Our experimental model, combining early stress by MD with later, young-adult stress in the form of CORT treatment, has unmasked significant effects of these neurodevelopmental interventions on dopaminergic regulation of PPI. Particularly young-adult CORT treatment resulted in reduced effects of apomorphine and amphetamine on PPI. In some animals, MD induces a reduction of baseline PPI which added to the effect of CORT. These findings could help to find behavioural and neurochemical mechanisms involved in the effect of stress on schizophrenia development, and also be a potential model for therapeutic approaches.

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## Conflicts of interest

None.

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