Dynamic regulation of dopamine and serotonin responses to salient stimuli during chronic haloperidol treatment

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Abstract

Antipsychotic drugs are the clinical standard for the treatment of schizophrenia. Although these drugs work initially, many compliant patients relapse due to treatment failure. The known biomarkers can not sufficiently explain antipsychotic treatment failure. We, therefore, enquired how the dynamic responses of the neurotransmitters, dopamine and serotonin, change in relation to treatment action and failure. Rats received either short-term (2–6 d) or long-term (12–14 d) treatment with haloperidol, which resembled human D₂ receptor occupancy, using osmotic mini-pumps. Dopamine and serotonin basal levels and responses to novelty, appetitive food, and to an aversive tail pinch were measured in the prefrontal cortex, nucleus accumbens and caudate putamen using in-vivo microdialysis, and the behaviour was recorded. Subsequently, we used in-vivo voltammetry to measure dopamine overflow in the nucleus accumbens. Haloperidol decreased dopamine, but not serotonin baseline levels in a time-dependent way. Salient stimuli induced dopamine and serotonin responses. Short-term haloperidol treatment attenuated the mesolimbic dopamine responses to aversive stimulation, while the responses to appetitive stimulation were largely preserved. After long-term treatment, the initial response adaptations were reversed. Similar changes were also observed at the behavioural level. In-vivo voltammetry showed that nucleus accumbens dopamine adaptations and their reversal were mediated by changes in extracellular dopamine release. Chronic haloperidol treatment, which resembles human D₂ receptor occupancy, modulates dopamine and behavioural responses to aversive and appetitive stimulation depending on the duration of treatment. Specific changes in dopamine response dynamics and their reversal may be a functional substrate of antipsychotic action and failure respectively.

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Introduction

Antipsychotics have been the mainstay of schizophrenia treatment. While these drugs are initially effective for most of the patients, sooner or later these treatments fail leading to relapses and recurrences (Lieberman et al. 2005). Even when medication compliance is assured by depot injection, the rate of relapse at 1 yr is up to 44% in some studies (Schooler, 2003) suggesting that relapse is in large part due to treatment failure (Buckley et al. 2007). Thus the major translational challenge for the field is not only to study antipsychotic efficacy, but also to understand reasons for antipsychotic failure. Early studies on treatment failure measured adaptive changes in the rodent brain after up to 18 months of chronic antipsychotic treatment in a withdrawal sensitivity model (Murugaiah
et al. 1982). However, the consequences of these adaptations have not been followed up. Moreover, the drugs were given in repeated doses with the consequence that the drug did not achieve peak occupancy similar to human levels (Kapur et al. 2003).

A recent and preliminary approach using clinically relevant doses of antipsychotics via infusion pumps showed that treatment efficacy diminished over time at the behavioural level in two animal models (Samaha et al. 2007, 2008). In particular, haloperidol (Hal) initially blocked amphetamine-induced hyperlocomotion, but lost its efficacy after 2 wk of chronic treatment. A conditioned avoidance response was also lost within the second week of treatment. These changes in the antipsychotic’s efficacy were not completely explained by the D₂ receptor up-regulation (Samaha et al. 2007). Individuals with schizophrenia manifest abnormal functional networks (Andreasen et al. 1997) and dynamic models may help to understand both schizophrenia (Stephan et al. 2008) and the action of antipsychotic medications (Carlsson, 2006). Thus, the measurement of protein expression captures only discrete aspects of a more complex phenomenon, while the recording of dynamic responses to environmental stimuli is likely to provide a better biomarker of the switch to the reversal effect. As yet, little information on how the dynamics of the dopaminergic and other transmitter systems change in animals has been provided (Kapur & Mamo, 2003).

Unconditioned stimuli with a positive or negative salience are well known to activate the dopamine (DA), and also the serotonin (5-HT) system. The DA response is essential for immediate behavioural responses as well as for long-term behavioural adaptations (Berridge, 2007), which both are disturbed in schizophrenia patients (Kapur, 2003). Addressing this issue, we systematically mapped the dynamic responses of mesocorticolimbic DA and its relationship with 5-HT and locomotor activity, to a range of naturally relevant salient stimuli using in-vivo microdialysis under chronic treatment of different durations. In order to address the question whether observed effects were mediated by changes in DA overflow, we used in-vivo voltammetry after stimulation of the medial forebrain bundle (MFB).

### Materials and methods

All experiments were approved by local ethics committees and were conducted in compliance with the Animals (Experimental Procedures) Act 1986, UK, and the State Provincial Office of Eastern Finland.

### Subjects

Male Sprague–Dawley rats (Charles River, UK; n = 5–7/group) weighing 200–250 g upon arrival were socially housed in groups of six for at least 7 d, with food and water available ad libitum, under controlled environmental conditions.

### Microdialysis guide cannula implantation

Rats were anaesthetized using 0.2 ml/100 g (i.p.) of a mixture of Ketamine (100 mg/ml), Medetomidine (1 mg/ml) and sterile water (analgesic treatment: 5 mg/kg Rimadyl, s.c.). Three guide cannulae (MAB 6.14., Microbiotech, Sweden) were implanted targeting the medial prefrontal cortex (mPFC; AP + 2.8, ML ± 0.8, DV − 4.4 mm), caudate putamen (CPu; AP + 0.0, ML ± 3.5, DV − 4.0 mm; 10− to the midline), and nucleus accumbens (NAc core); AP + 1.6, ML ± 2.6, DV − 5.8 mm, 10− to the midline (Paxinos & Watson, 1986) (for method see Pum et al. 2007, 2008). After surgery 5–6 d recovery was allowed before implantation of osmotic mini-pumps.

### Mini-pump implantation and drug delivery

According to studies reported in the literature we used a representative time schedule of treatment which yielded neurochemical changes over time in the rat brain. Short-term (2–6 d) and long-term (12–14 d) Hal (0.5 mg/kg,d) treatments were used. Hal or vehicle (Veh) were delivered over a period of either 6 d or 14 d (expt 1) or 2 d or 12 d (expt 2) via an osmotic mini-pump (see Samaha et al. 2007 for the implantation description). We used the shorter time treatment for the voltammetry experiment as no different responses within these days were found.

### Microdialysis and analytical procedures

On the night prior to testing, microdialysis probes with a membrane length of 2 mm (mPFC and NAc) or 3 mm (CPu) and a 15-kDa molecular cut-off (MAB 6, Microbiotech) were inserted into the guide cannulae under isoflurane anaesthesia. Probes were perfused with artificial cerebrospinal fluid (aCSF) with a flow rate of 0.5 μl/min. Animals were returned to their home cage and moved to the experimental room. Animals were given ad libitum access to water but restricted access to food (one pellet of standard food). On the day of experimentation, perfusion flow was increased to 1.5 μl/min and was allowed to
stabilize for 60 min. Samples were taken every 20 min. After a stable baseline was established, each animal was tested in three behavioural protocols: novelty, an appetitive stimulus, and an aversive stimulus. For each stimulus a separate baseline was established and sampling continued for 60 min (three samples).

**Novelty**

animals were placed into a novel open field (40 × 40 × 39 cm). Locomotor activity was monitored automatically by a TruScan (Coulbourn, USA) system. Data from the tip of the tail for 20 min. At the end of the experiment, rats were deeply anaesthetized, transcardially perfused and brains were collected for probe verification. Unconsumed Fonzies were removed thereafter. **Aversive stimulus**

A tail pinch (TP) was applied to each animal with a Mohr clip at 2.5 cm from the tip of the tail for 20 min. At the end of the experiment, rats were deeply anaesthetized, transcardially perfused and brains were collected for probe verification.

The samples were immediately assayed after collection using high-performance liquid chromatography with electrochemical detection. The column was an ET 125/2, Nucleosil 120-5, C-18 reversed phase column (Macherey & Nagel, Germany) perfused with a mobile phase composed of 75 mM NaH₂PO₄, 4 mM KCl, 20 μM EDTA, 1.5 mM SDS, 100 μL/1 diethylamine, 12% methanol and 12% acetonitril adjusted to pH 6.0 using phosphoric acid. The electrochemical detector (Intro, The Netherlands) was set at 500 mV vs. an ISAAC reference electrode (Antec, The Netherlands) at 30 °C. This set-up allows the measurement of 5-HT and DA. The detection limit of the assay was 0.1 pg for 5-HT and DA with a signal-to-noise ratio of 2:1. Neurochemical data were not corrected for recovery (Pum et al. 2007, 2008).

**Voltammetry**

Either 2 d or 12 d after mini-pump implantations, rats (n = 6/group) were anaesthetized with 450 mg/10 ml/kg (i.p.) chloral hydrate and implanted with a pre-calibrated carbon-fibre working electrode in the NAc (shell) (AP +1.6 mm, ML ±2.8 mm, DV −8.3 mm). A bipolar stimulating electrode was implanted in the MFB (AP −2.1 mm, ML 2.0 mm, DV −8.7 mm) (coordinates vs. bregma; Paxinos & Watson, 1986). The final position of the stimulating electrode within the DV coordinate was adjusted for maximal DA release. A small leak-free Ag/AgCl reference electrode (AH 69-0023, Harvard Apparatus, USA) in a saline bridge was placed on the skull. A stainless-steel screw fixed into occipital bone served as an auxiliary electrode. After the measurements, the locations of the working electrode in the striatum were electrolytically marked using a 12-s application of anodic current at 6 V. Stimulated DA release was measured by constant potential amperometry with a single carbon fibre, 30 μm in diameter (WPI, USA) insulated with epoxy glue in a pulled capillary glass. The fibre was cut to a length of 300 μm from the end of the glass seal. A custom-built three-electrode potentiostat was used to hold potential at the working electrode at 0.4 V against an Ag/AgCl reference electrode. Data from the potentiostat were digitized and saved for offline analysis.

**Experimental procedure**

The working electrode was lowered into the NAc. Recording was started 90 min after the implantation of the electrode. Two schedules of stimulation were used one after another. In the first schedule, 2-s stimulations of increasing frequencies (10–50 Hz) were applied to the MFB. In the second schedule, stimulations of increasing lengths (0.1–1.6 s at 50 Hz) were applied to the MFB at increasing intervals (30–300 s), which eliminates interaction between stimulations. For the stimulation we used a battery-operated constant current unit (A365, WPI) run by a PC. Constant bipolar pulses (1-ms duration) of 200–400 μA were delivered to the stimulation electrode. At the end of the experiment, rats were decapitated to collect brains for verification of the positions of the working electrodes (Yavich et al. 2007).

**Data analysis: microdialysis**

Changes in DA and 5-HT baseline levels after 6-d and 14-d Hal treatment were expressed as percentages of the Veh group. DA and 5-HT responses to the behavioural stimuli were analysed as percentage of pre-stimulus baseline (mean ± S.E.M.). Statistical analysis was performed using Fisher’s LSD test (Ramsey, 1993).

**Voltammetry**

DA overflow was expressed as percentage of release induced by minimal stimulation (2 s, 10 Hz and 0.1 s, 50 Hz for the first and second schedule, respectively). Changes in DA overflow after 2-d and 12-d Hal treatment were expressed as percentages of Veh group (mean ± S.E.M.). Statistical analysis was performed using ANOVAs (factors: treatment and different parameters of stimulation of the MFB) and Bonferroni...
Haloperidol treatment

Hal treatment reduced DA levels in all three brain areas (p < 0.01). 6-d Hal treatment decreased 5-HT levels in the CPu (p < 0.001), but not in the CPu or NAc (Fig. 1). The 14-d Hal treatment significantly modulated 5-HT responses to novelty in the mPFC. There was an effect of treatment (F2,32 = 9.25, p < 0.001), but no effect of time or interaction. Novelty had no effect on 5-HT levels in the mPFC in the control group. After 6-d Hal treatment, novelty induced a significant 5-HT increase in the mPFC in the first (p = 0.001) and second (p = 0.017) treatment interval. This effect was abolished after 14-d Hal treatment.

Hal treatment significantly modulated the DA response to novelty in the NAc. There was an effect of treatment (F2,35 = 3.50, p = 0.035), but no effect of time or interaction. Novelty increased DA levels in the NAc in the control group in the first treatment interval (p = 0.014). This effect was abolished after 6-d and 14-d Hal treatment. Novelty had no significant effect on NAc 5-HT levels. Neither Hal treatment changed this. Novelty had no significant effect on CPu DA levels. Neither Hal treatment changed this. The Hal treatment significantly modulated the 5-HT response to novelty in the CPu. There was an effect of treatment (F2,35 = 3.55, p = 0.033), but no effect of time or interaction. However, pre-planned comparisons did not show significant changes from baseline in all three test groups.

**Results**

**Hal effects on DA and 5-HT baseline levels**

DA baseline concentrations in the Veh group were 5.8 ± 2.3, 10.2 ± 3.2 and 6.4 ± 1.4 pg/30 µl in the mPFC, CPu, and NAc, respectively. The 6-d Hal treatment reduced DA levels significantly vs. Veh in the mPFC (p = 0.001), but not in the CPu or NAc (Fig. 1). The 14-d Hal treatment reduced DA levels in all three brain areas (p < 0.01). 5-HT baseline concentrations in the Veh group were 0.9 ± 0.2, 5.0 ± 2.6, and 0.9 ± 0.3 pg/30 µl in the mPFC, CPu, and NAc, respectively. The 6-d Hal treatment decreased 5-HT levels in the CPu (p < 0.05), but had no significant effect on 5-HT levels in the mPFC or NAc. After 14-d Hal treatment 5-HT levels in all brain areas did not differ significantly from Veh.

**Hal effects on DA and 5-HT responses to novelty**

The Hal treatment significantly modulated the DA response to novelty in the mPFC (Fig. 2). There was an effect of treatment (F2,32 = 4.51, p = 0.014), but no effect of time or interaction. Novelty had no effect on DA levels in the mPFC in the control group and after 6-d Hal treatment. However, a significant increase was observed after 14-d Hal treatment in the first (p = 0.044) and third (p = 0.035) treatment interval. The Hal treatment significantly modulated the 5-HT responses to novelty in the mPFC. There was an effect of treatment (F2,32 = 9.25, p < 0.001), but no effect of time or interaction. Novelty had no effect on 5-HT levels in the mPFC in the control group. After 6-d Hal treatment, novelty induced a significant 5-HT increase in the mPFC in the first (p = 0.001) and second (p = 0.017) treatment interval. This effect was abolished after 14-d Hal treatment.

Hal treatment did not modulate the DA response in the mPFC to preferred food (Fig. 3). There was a tendency for a time effect (F3,92 = 2.53, p = 0.063), but no overall effect of treatment or interaction. Food increased mPFC DA levels in the Veh group in the second sample vs. baseline (p = 0.016). After 6-d Hal treatment this effect was attenuated, but fully preserved after 14-d Hal treatment (p = 0.027, second sample vs. baseline). Hal treatment also modulated the 5-HT response in the mPFC to food. There was an effect of treatment (F3,92 = 8.99, p < 0.001), but no effect of time or interaction. Preferred food did not increase 5-HT levels in the mPFC in the Veh group. However, after 6-d Hal treatment there was a significant 5-HT increase after food, which was significant vs. baseline in the second and third samples (p = 0.014, p = 0.009).

After 14-d Hal treatment, food did not induce any 5-HT response in the mPFC. Food-induced eating
behaviour was reduced by 6-d Hal (*p < 0.01) and by 14-d Hal (as a tendency, *p = 0.07; Fig. 4) treatment.

Hal treatment significantly modulated the DA response in the NAc to food. There was a tendency for an overall effect of treatment (*F_{2,95} = 2.54, p = 0.085), but no effect of time or interaction. Food increased DA levels in the NAc in the Veh group in the second sample *vs. baseline (*p = 0.004). This response was attenuated after 6-d Hal treatment and completely abolished after 14-d Hal treatment. The 14-d Hal treatment modulated the 5-HT response in the NAc to food. The food stimulus increased 5-HT levels in the NAc. There was a tendency for an effect of time (*F_{3,95} = 2.43, p = 0.07), but no treatment effect or interaction. Food increased NAc 5-HT levels in the Veh group in the second treatment interval (*p = 0.009 *vs. baseline). This effect was largely unaffected by 6-d Hal (second treatment interval: *p = 0.05 *vs. baseline). Food had no significant effect on 5-HT levels after 14-d Hal treatment. There was no significant effect of the food stimulus on DA or 5-HT levels in the CPu in control or Hal-treated animals.
Hal effects on DA and 5-HT responses to aversive stimulation

Hal treatment significantly modulated the DA response in the mPFC to TP. There was an effect of treatment ($F_{2,92} = 9.89$, $p < 0.001$), but no effect of time or interaction. The TP did not affect DA levels in the mPFC in the Veh group. After 6-d Hal treatment, however, the TP significantly increased DA levels in all three samples ($p = 0.008$, $p = 0.03$, $p = 0.04$ vs. baseline). After 14-d Hal treatment, the TP no longer affected DA levels (Fig. 5). Hal treatment also modulated the 5-HT response in the mPFC to TP. Although there were no significant overall effects in the ANOVA, pre-planned comparisons showed an increase in 5-HT in the Veh group in the second sample ($p = 0.007$). This effect was abolished by 6-d Hal treatment, and only partially recovered after 14-d Hal treatment.
Hal treatment significantly modulated the DA response in the NAc to TP. There was an effect of treatment ($F_{2,95} = 3.76$, $p = 0.027$), time ($F_{1,35} = 5.33$, $p = 0.002$), but no interaction. TP increased DA levels within the NAc with respect to baseline in the Veh group in the first ($p = 0.001$) and second ($p = 0.002$) samples. The TP effect on NAc DA levels was abolished after 6-d Hal, but re-established after 14-d Hal treatment ($p = 0.008$, $p = 0.067$, first and second sample vs. baseline). Hal did not modulate the 5-HT response in the NAc to TP. The TP increased 5-HT levels in the NAc. There was an effect of time ($F_{2,91} = 3.54$, $p = 0.018$), but no treatment effect or interaction. The TP increased NAc 5-HT levels in the Veh group in the first treatment interval ($p = 0.05$). This effect was not changed by Hal treatment after 6 d or 14 d.

Hal treatment significantly modulated the DA response in the CPu to TP. There was an effect of treatment ($F_{2,95} = 13.17; p < 0.001$), but no effect of time or interaction. The TP had no effect on DA levels in the CPu in the Veh group after 6-d Hal treatment. However, after 14-d Hal treatment TP induced a significant DA increase in all three treatment samples ($p = 0.001$, $p = 0.027$, $p = 0.004$). Hal treatment significantly modulated 5-HT response in the CPu to TP. There was an effect of treatment ($F_{2,95} = 5.76$, $p = 0.005$), but no effect of time or interaction. The TP had no effect on 5-HT levels in the CPu in the CPu in the control group. After 6-d Hal treatment, TP increased 5-HT levels in the CPu significantly in the second ($p = 0.006$) treatment interval. This effect was attenuated after 14-d Hal treatment.

**Hal effects on the locomotor responses to novelty, appetitive and aversive stimuli**

Hal inhibited the novelty-induced locomotor activity after 6 d ($t = 3.304$, $p = 0.005$) and 14 d ($t = 2.196$, $p = 0.044$) treatment, although overall this effect was reduced over 2 wk treatment (Fig. 6). Hal inhibited the food-induced locomotor activity significantly after 6 d ($t = 42.00$, $p = 0.045$), but not after 14 d ($t = 1.896$; $p = 0.077$) treatment. Hal inhibited the TP-induced locomotor activity significantly after 6 d ($t = 2.210$, $p = 0.043$), but not after 14 d ($t = 1.541$, $p = 0.144$) treatment.

**Expt 2**

**Hal effects on stimulated DA overflow**

Hal treatment modulated DA release in the NAc depending on the duration of treatment. The stimulation of the MFB induced frequency- and length-dependent release of DA in the NAc. The 2-d Hal treatment enhanced DA responses relative to Veh. This effect was abolished after 12-d Hal treatment (effects of treatment, frequency response: $F_{1,95} = 7.34$, $p < 0.01$; length response: $F_{1,95} = 12.64$, $p < 0.01$; Fig. 7).

**Discussion**

The present study showed that Hal induces a fall in DA, but not 5-HT baseline levels, depending on the time of treatment. After short-term Hal treatment, mPFC, but not NAc and CPu extracellular DA levels were decreased. After long-term treatment, DA levels in all three target areas were significantly reduced. These data may suggest that during short-term treatment, the Hal-induced adaptations in DA response dynamics in the NAc occur during preserved DA baseline levels. However, during long-term Hal treatment the DA systems not only respond differently to environmental challenges, but they do so at very much reduced DA baseline levels.

The detection of cortical and striatal DA levels has produced controversial results in previous studies (Hernandez & Hoebel, 1989; Ichikawa & Meltzer, 1990a, b; Moghaddam & Bunney, 1993; Zhang et al., 1989). Drug doses (Klitenick et al., 1996), anaesthesia (Mereu et al., 1995) and composition of the perfusion solution (Moghaddam & Bunney, 1993) were regarded as being responsible for these neurochemistry differences. Little significance was given to receptor occupancy (Kapur et al. 2003). Antipsychotics given daily by injection or orally by drinking fluid lead to a within-day transient kinetics, causing a lack of adequate drug receptor occupancy (Kapur et al., 2003) and different experimental outcomes (Samaha et al., 2008). Antipsychotic drugs can overcome these limitations, when delivered via osmotic mini-pumps. This method
allows achieving human D₂ receptor occupancy, and, as shown here, reveals different adaptations of DA and 5-HT baseline levels. It is currently unclear which mechanism underlies the DA baseline fall. As the depolarization inactivation (Lane & Blaha, 1987) was shown not to be directly involved (Ichikawa & Meltzer, 1990a; Moghaddam & Bunney, 1993), a D₂ up-regulation may underlie the decrease of DA levels by enhancing DA uptake (Benoit-Marand et al. 1991; Cass & Gerhardt, 1994; Mayfield & Zahniser, 2001; Meiergerd et al. 1993; Parsons et al. 1993). The effects of Hal on 5-HT baseline levels were less pronounced than effects on DA. Short-term Hal treatment significantly decreased 5-HT levels in the CPu. This might be a consequence of the dopaminergic modulation of 5-HT activity in the striatum (Sivam, 1995).

Salient stimuli increased extracellular DA and 5-HT activity in this study. Novelty was the weakest stimulus considering that we found only a modest DA increase in the NAc. This is line with other findings.

Fig. 5. Effects of 6-d and 14-d haloperidol treatment (0.5 mg/kg.d) by osmotic minipump on dopamine (a, c, e) and serotonin (b, d, f) responses to a tail pinch (grey bar). Results are expressed as percent of baseline (BL) (mean±S.E.M.; * p<0.05, ** p<0.01 vs. baseline).
Appetitive food led to consumatory behaviour and a significant DA increase in the NAc and mPFC in our study, which is in line with previous findings (e.g. Bassareo & Di Chiara, 1997). The short-term Hal treatment did not significantly modulate the DA response in the mPFC. However, it attenuated the DA response in the NAc, which may already reflect the beginning of a reduced hedonic responsiveness during antipsychotic treatment (Kapur, 2003). After long-term Hal treatment, the mPFC DA response to the food stimulus remained unchanged. However, the NAc DA response was completely abolished. Given the strong association of the NAc DA response with the incentive properties of a stimulus (Berridge, 2007), these adaptations suggest a loss of the incentive salience of the appetitive stimulus after long-term Hal treatment. In line with the neurochemical response adaptations was the behavioural response to food, which was significantly reduced by short-term Hal treatment. This effect was only slightly reversed after long-term treatment suggesting that the locomotor activity inhibition induced by Hal that was diminished after 2 wk treatment, might have only partially affected the consumatory behaviour.

Palatable food has also been shown to stimulate the 5-HT system (Erlanson-Albertsson, 2005; Hoebel, 1985). In our study, food stimulus induced a 5-HT increase in the NAc, but not in the mPFC. The short-term Hal treatment did not change the NAc 5-HT response. A preserved 5-HT response in the NAc suggests that 5-HT might not contribute to the antipsychotic effects of Hal. After long-term Hal treatment, however, the NAc 5-HT response to the food stimulus was attenuated. A loss of 5-HT responding in the NAc, may reduce the emotional significance of an appetitive stimulus and contribute to a less beneficial neurochemical response profile.

In our study, TP significantly increased extracellular DA levels in the NAc, but not in the mPFC and CPu. This suggests different sensitivities of the mesolimbic, mesocortical, and nigrostriatal DA projections to aversive stimulation. Our findings are consistent with previous studies showing that TP and other stressors lead to DA activation (Abercrombie et al. 1989; Imperato et al. 1991; Robinson et al. 1987; Thierry et al. 1976). Short-term Hal treatment reduced the DA response in the NAc, but enhanced it in the mPFC, while not changing it in the CPu. In the mPFC it was repeatedly shown that heavy stressors stimulate mPFC DA, which was not affected by Hal treatment (Dazzi et al. 2004). It should be noted that the TP applied in this study was only mildly aversive. Importantly, the present results suggest that reciprocal

(Badiani et al. 1998; Bardo et al. 1990; Feenstra & Botterblom, 1996; Noguchi et al. 2001; Piazza et al. 1991). However, long-term treatment with Hal induced a de-novo DA response in the mPFC. The exposure to novelty may, while on Hal treatment, have acquired properties of a stressful stimulus which is characterized by this response (Bardo et al. 1990). This effect might have contributed to reducing the arena exploration and consequently the locomotor activity. There was no effect of novelty on 5-HT activity. Nevertheless, Hal induced a mPFC 5-HT response after short-term treatment. It is likely, that the reciprocal relationship between DA and 5-HT systems (De Deurwaardere & Spampinato, 1999; Ichikawa & Meltzer, 2000; Kilpatrick et al. 1996) may account for the reciprocity found in this study.

Fig. 6. Effects of 6-d and 14-d haloperidol treatment (0.5 mg/kg.d) by osmotic minipump on the locomotor activity induced by salient stimuli. Results are expressed as units (mean ± S.E.M.; * p < 0.05, ** p < 0.01 vs. Veh).
DA response adaptations occur in mPFC and NAc during exposure to an aversive stimulus after short-term Hal treatment.

After long-term Hal treatment, the DA responses to TP largely resembled those of the Veh condition. The TP did not induce a DA response in the mPFC any longer, and the NAc DA increase re-occurred (see also Klitenick et al. 1996). This reversal of an initial DA response adaptation to an aversive stimulus, together with the reversal of the locomotor activity inhibition after 14-d treatment, might contribute to the loss of therapeutic efficacy of Hal during long-term treatment, as is observed in the clinic and in animal models.

The TP induced a 5-HT increase in the NAc and mPFC, which is in line with the role of 5-HT in general arousal and sensorimotor activation (Müller & Jacobs, 2010). Short-term Hal treatment abolished the 5-HT response in the mPFC, but did not change the response in the NAc. In the CPu, a new response was observed. After long-term Hal treatment, the 5-HT response in the mPFC and CPu reversed and was similar to those of the Veh condition. No adaptations were seen in NAc. These data suggest that in particular the sensorimotor activating effects of mPFC 5-HT (Pum et al. 2008) may be attenuated during short-term Hal treatment, but no longer during long-term treatment.

DA D₂ receptor supersensitivity has been shown during long-term, but not during short-term Hal treatment (Seeman et al. 2005). Here we report that the DA release in the NAc after MFB stimulation increased in response to short-term Hal treatment, but was reversed to Veh treatment levels after long-term Hal application. These data suggest that the observed changes in DA responsiveness during short-term Hal treatment can be elicited by a potentiated dopaminergic activity in the NAc. The observed reversal of DA dynamic changes during long-term Hal treatment, in turn, may be accounted for by a reversal of the changes in dopaminergic activity, coinciding with the reduced baseline activity found in our study and D₂ supersensitivity (Seeman et al. 2005).

Evidences from the clinic shows that antipsychotic drugs are discontinued by 74% of patients diagnosed with schizophrenia. While tolerability accounts for a small part of it, a large number of patients discontinue the treatment because of lack of efficacy. The present study, using a treatment regimen which resembled human D₂ receptor occupancy for short- and long-term treatment, shows that the DA and 5-HT responses to salient stimuli changed, along with the behaviour, during chronic Hal treatment. In particular, after short-term Hal treatment, the NAc DA response to aversive stimulation was attenuated, while the NAc DA response to appetitive stimulation was largely preserved. This neurochemical pattern was associated with a substantial inhibition of the locomotor activity. After long-term Hal treatment, both the neurochemical and behavioural patterns were reversed. In the case of novelty we have not observed a reversed drug effect pattern. Actually, the Hal effect did not change over time neither at neurochemical nor at the behavioural level. Novelty is able to increase the psychomotor activating effects of amphetamine without stimulating DA release (Badiani et al. 1998) and this might be related to the relatively stable Hal effect during the 2 wk of treatment. It is possible that the exposure to a novel environment delays the failure

**Fig. 7.** Dopamine overflow in the nucleus accumbens following burst stimulation of the medial forebrain bundle. Frequency (a) and lengthdependent (b) dopamine overflow in the nucleus accumbens following 2-d (–■–) or 12-d (–▲–) treatment with haloperidol as percentage of the vehicle-treated rats.
of antipsychotics. As far as the cortical DA response is concerned we found that the mPFC DA response to salient stimuli showed two different patterns according to the treatment. With Hal it showed a sort of reciprocal relationship with the NAC DA response. However, it appeared independent from the NAC DA when saline was given. It might be the case that the mPFC DA observed in the two treatment groups was produced by two different sources such as DA neurons (when on Hal) and noradrenergic neurons (when on saline – see Devoto & Flore, 2006 for review). When on Hal, a similar reciprocity was also found between mPFC 5-HT and mPFC DA.

Although, only a range of patients relapse due to treatment failure, we have shown that all rats went through this critical stage. Nevertheless, it should be mentioned that we plotted group averages. However, we also found a response variability to the drug. The reduced genetic and environmental variance in the rats investigated in a laboratory setting may be responsible for the reduced inter-individual drug-response differences. The generalization of the Hal failure to the other categories of antipsychotics might be a limitation of this study. However, a comparable time-course of failure was reported for the atypical antipsychotic, olanzapine, by Samaha et al. (2007).

Conclusion

Our findings provide evidence that reversible adaptations, in particular, in the DA responses to salient stimuli in interaction with baseline activity occur during chronic Hal treatment. These changes might be important mechanisms contributing to antipsychotic efficacy and treatment failure. Targeting maladaptive responses adaptations with supplemental treatments may be a valuable strategy to maintain antipsychotic therapeutic efficacy.

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Statement of Interest

None.

References


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