Prefrontal cortex and reversion of atropine-induced disruption of the degraded contingency effect by antipsychotic agents and N-desmethylclozapine in rats

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Abstract

Interactive context processing is a cognitive ability that is altered in psychotic states, including schizophrenia. This deficit has been linked to prefrontal cortical dysfunction in humans. The degraded contingency effect (DCE) is a simple form of interactive context processing by which contextual information interferes with a target conditioned stimulus for control over conditioned responding. We have previously shown that the DCE was disrupted by the muscarinic receptor antagonist atropine and that this disruption was specifically restored by cholinergic drugs displaying an antipsychotic-like profile, such as physostigmine or xanomeline. The DCE was selectively associated with an increase in Fos immunoreactivity in the medial prefrontal cortex (mPFC), an increase that was not observed in the presence of atropine. Here, we set out to test the actions of typical, atypical and potential antipsychotics on atropine-induced disruption of the DCE and the related mPFC Fos-immunoreactivity profile. Low doses of haloperidol, olanzapine, clozapine and N-desmethylclozapine reversed atropine-induced disruption of the DCE, but with different dose-dependent curves (linear shapes for haloperidol and N-desmethylclozapine, inverted U shapes for olanzapine and clozapine). The level of Fos within the mPFC paralleled the pharmacological profile of the different drugs. Compared to contingent control groups, an increased level of Fos immunoreactivity within the mPFC was observed only with doses that reversed atropine-induced disruption of the DCE. These results suggest that the deficit of interactive context processing, which is a hallmark of psychotic states, might originate from a mere deficit of fundamental associative processes. This deficit might result from a cholinergic blockade of the PFC.

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Introduction

Context refers to the background or the surrounding circumstances in which a target event occurred. Two categories of contexts can be distinguished (Baddeley, 1982). First is the independent context, which does not interfere with the interpretation of the target event. In this case, contextual information is encoded independently of the target information. Second, in contrast, the interactive context is characterized by the fact that it affects the meaning, or the interpretation, of the target event, and is thus encoded in parallel with the target information. Baddeley’s distinction is relevant in several pathological conditions. Schizophrenia patients are unable to use interactive contextual information to disambiguate the meaning basis of a target event, whereas they normally process independent contextual information (Barch et al. 2003; Bazin et al. 2000; Cohen & Servan-Schreiber, 1992; Hemsley, 2005; Oberling et al. 1999; Rizzo et al. 1996). For example, Chapman & Chapman (1973) demonstrated a pronounced schizophrenia bias for dominant meanings of homonyms (e.g., ‘pen’ as a writing instrument) even...
when the sentential context called for secondary meanings (e.g. ‘pen’ as an enclosed fence). Recently, Holmes and colleagues (2005), using a measure that is a specific indicator of context processing, namely the associative AX-Continuous Performance Task (see Servan-Schreiber et al. 1996) demonstrated that a context-processing-related dysfunction in prefrontal cortex was specific to schizophrenia.

Interactive context processing is involved in many associative phenomena and can be explored both in humans and rodents. For example, the Pavlovian degraded contingency effect (DCE), first described in rats by Rescorla (1968) is a simple and fundamental phenomenon in which the addition of unsignalled unconditioned stimuli (US, e.g. a footshock) during the contingent pairing of a target event such as a tone (the so-called conditioned stimulus; CS) with the same US, results in the loss of control by that CS of the conditioned response (see Fig. 1). In the DCE, the signalling role of the target CS has been degraded by the experimental context because the experimental context has also gained a similar signalling role, and thus down-modulates the processing of the previously activated CS. The down-modulation of CS processing by the context cannot be evidenced during psychotic states as the patients cannot use any mental representation within the prefrontal cortex of that context (Cohen & Servan-Schreiber, 1992).

Using a fear-conditioning paradigm in the rat, we showed that the muscarinic receptor antagonist atropine selectively disrupted the DCE, with no influence on the processing of independent contextual information (see Fig. 1). Atropine-induced disruption of the DCE was fully reversed by administration of the anticholinesterase inhibitor physostigmine and the M₁/M₄ muscarinic receptor agonist xanomeline, but not by the non-selective agonists pilocarpine and oxotremorine (Carnicella et al. 2005a, b). This pharmacological profile was of particular interest for psychosis-related diseases such as schizophrenia, as certain alterations of the muscarinic system may underlie some cognitive deficits and psychotic symptoms present in these diseases (Raedler et al. 2007; White & Cummings, 1996). Positive symptoms in psychosis have been shown to be associated with alterations of the muscarinic function in the cortex (Ballard et al. 2000; Lai et al. 2001; Raedler et al. 2003). Moreover, muscarinic antagonists like atropine can produce psychotic symptoms in humans, as well as psychotic-like effects in animal models of schizophrenia (Barak & Weiner, 2007, 2009; Jones et al. 2005; Mathur et al. 1997; Shannon & Peters, 1990), and exacerbate positive symptoms and cognitive impairments in psychotic patients (Abood & Biel, 1962; Perry & Perry, 1995; Yeomans, 1995). Interestingly, stimulation of the cholinergic system with physostigmine and xanomeline, but not oxotremorine and pilocarpine (Davis et al. 2003). Moreover, muscarinic antagonists like atropine can produce psychotic symptoms in humans, as well as psychotic-like effects in animal models of schizophrenia (Barak & Weiner, 2007, 2009; Jones et al. 2005; Mathur et al. 1997; Shannon & Peters, 1990), and exacerbate positive symptoms and cognitive impairments in psychotic patients (Abood & Biel, 1962; Perry & Perry, 1995; Yeomans, 1995). 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et al. 1987; Mouradian et al. 1988; Sedman et al. 1995), was shown to reduce positive symptoms and cognitive deficits in psychotic patients (Bodick et al. 1997; Cummings et al. 1993; Mirza et al. 2003) and to exhibit antipsychotic-like profile in animal models (Barak & Weiner, 2007, 2009; Mirza et al. 2003).

Using the protein Fos expression as a marker of neuronal activity, we further found that, in more than 30 structures and sub-structures studied, the medial prefrontal cortex (mPFC) was the only structure specifically involved in the processing of the Pavlovian DCE and in its disruption by atropine. More precisely, we showed that degrading contingent conditioning by adding unsignalled US during training (the DCE) led to a substantial increase in Fos immunoreactivity during CS testing within the mPFC, compared to simple contingent conditioning or pseudo-conditioning. This Fos-activated pattern was specifically disrupted by atropine (Carnicella et al. 2006). Here again, the present results in rats were in parallel with an emerging set of data in human studies, which support the view that the PFC is a critical structure for the representation and the maintenance of interactive context information (Barch et al. 1997; Cohen et al. 1999; MacDonald et al. 2000). Moreover, functional imaging studies have reported a decreased level of activity within the PFC of schizophrenia patients that is related to a specific impairment of interactive context processing (Barch et al. 2001; Holmes et al. 2005; MacDonald & Carter, 2003; Perlstein et al. 2003), but which can be alleviated by antipsychotic treatment (Snitz et al. 2005).

Therefore, in order to further investigate the role of both antipsychotic medications and the mPFC in interactive contextual processing in rats, we set out to test the ability of subchronic administration of the prototype typical antipsychotic haloperidol, the atypical antipsychotics clozapine and olanzapine, and the principal active metabolite of clozapine, namely N-desmethylclozapine (NDMC) to block the atropine-induced disruption of the DCE, along with the alteration of Fos immunoreactivity in the mPFC that is associated with this deficit.

Materials and methods

Animals

Naive male Sprague–Dawley rats (CERJ, France), weighing 300–320 g at the beginning of the experiment were housed in a colony room (temperature at 22 ± 2 °C) maintained on a 12-h light/dark cycle (lights on: 08:00 hours). Food and water were initially provided ad libitum, and then water availability was limited to 10 min/d following a progressive deprivation schedule, which was imposed gradually over the week prior to the start of the behavioural study. All experiments were conducted in accordance with institutional guidelines complying with national (Council directive 87848, 19 October 1987, Ministère de l’Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale) and international guidelines (NIH publication, no. 86-23, revised 1985).

Apparatus

The apparatus has been described in a previous study (Carnicella et al. 2005a). Briefly, eight identical experimental rectangular chambers made of Plexiglas, with a clear front wall, were used. The floor consisted of stainless-steel rods connected to a grid shocker set to deliver, when needed, footshocks (US) that had been previously calibrated to the nearest threshold for which a reliable conditioned response could be observed in our conditions [0.27 mA, 1 s scrambled (20 ms on/140 ms off)]. Each enclosure was housed in a separate light- and sound-attenuated environmental isolation chest. Enclosures were each equipped with a water-filled lick tube. An infrared photobeam was projected horizontally across the recess. In order to drink from the lick tube, the rates had to break the photobeam with their tongue, making it possible to record the number of licks. Each enclosure was equipped with a speaker which was set to deliver a 3000-Hz tone of 30-s duration (CS), at ~6 dB(A) above the ambient background noise [72 dB(A)].

Behavioural procedure

The rats were handled gently for 2 min each day for 3 d during the week preceding the acclimatization sessions. They were weighed daily throughout the experiment to check that they were adapting correctly to the deprivation schedule and to the experimental treatments.

Acclimatization

On days 1 and 2, each rat was acclimatized to its respective enclosure for 45 min each day. During this time, they had access to water-filled lick tubes.

Conditioning

Conditioning was conducted on days 3–8 with the lick tubes removed. Removing lick tubes ensured that rats were not engaged in a drinking behaviour that could interfere with the conditioning sessions and/or the
current drug state. Each daily session lasted 45 min. During each conditioning session, the control groups [named Contingent (Cont)] experienced 3 CS-US trials pseudo-randomly interspersed with three CS-alone trials, while the experimental groups that experienced degraded contingency (Deg-Cont) experienced the same daily pattern of three CS-US trials and three CS-alone trials, but these trials were pseudo-randomly interspersed with 42 unsignalled US. When signalled by the CS, the US co-terminated with it. Pseudo-conditioning groups (Pseudo) received the same sequence of CS and interspersed US than Deg-Cont groups, but none of the six CS was reinforced (negative contingency training procedure; Oberling et al. 2000), thus nearly equating the stimuli (CS and US) densities. The Pseudo groups allowed controlling for any confounding activation of Fos expression by the tone presentation and/or the effects of atropine.

Independent context testing and reacclimatization

On days 9–12 the water-filled lick tubes were re-inserted into the enclosures and all animals were re-acclimatized to their enclosure for a 45-min session. The latency to complete the initial 30 licks was thereby recorded in the absence of any CS tone. Day 9 afforded the opportunity to gauge initial contextual fear, which reflects the associative strength between the independent context and the unsignalled US. Reacclimatization sessions were also necessary before testing for CS was possible, as groups receiving unsignalled footshocks produced a high level of lick suppression to background cues (independent contextual fear memory) that had to be extinguished first (Kasprow et al. 1987).

Pre-CS testing

On day 13, the latency to complete the initial 30 licks in the experimental enclosure was recorded for each rat in order to ensure that the drinking baseline prior to the CS presentation was similar across groups. When this drinking baseline was found unequal between groups, statistical analyses on CS testing were carried out using the pre-CS latency as the covariate.

CS testing

On the same day (i.e. day 13), all animals were tested for lick suppression in response to the CS. The tone was immediately presented to the rat in that trial, upon completion of its initial 30 licks (pre-CS testing). Thus, each rat was drinking at the onset of the tone, and the time required to complete the additional 30 licks (licks 31–60) in the presence of the tone was measured. All the animals were exposed to the tone during a 15 min period. The total amount of licks was recorded in order to ensure that the final level of hydration was similar across the different groups. This procedure ensured that modifications of Fos immunoreactivity within the mPFC were not the consequence of a different level of hydration or of any motor pattern.

Drugs and pharmacological procedures

Atropine sulfate was purchased from Sigma (St Quentin Fallavier, France), haloperidol from Janssen-Cilag (Issy-Les-Moulineaux, France) and olanzapine from Eli Lilly (Houten, The Netherlands). Clozapine and NDMC were synthesized by A.M and J.-J.B, according to a previous study (Capuano et al. 2002), and prepared as hydrochloride salts. Each compound was prepared daily in 0.9% saline. The drugs or control saline were injected intraperitoneally (i.p.) at a volume of 1 ml/kg.

Each rat was injected with 5 mg/kg atropine, 15 min prior to the behavioural session during conditioning (days 3–8), the first day of reacclimatization (day 9), and the day of the CS testing (day 13). The dose of atropine and the administration procedure used were previously shown to be effective in suppressing the DCE, without disrupting CS- and independent context-US conditioning (Carnicella et al. 2005a). Time of injection and doses for haloperidol (45 min before atropine, 0, 0.015, 0.03, 0.06 or 0.12 mg/kg), olanzapine (5 min before atropine, 0, 0.1, 0.2, 0.4 or 0.8 mg/kg), clozapine (5 min before atropine, 0, 0.75, 1.5 or 3 mg/kg) and NDMC (5 min before atropine, 0, 0.375, 0.75, 1.5 or 3 mg/kg) were carefully chosen to be pharmacologically and behaviourally effective (Aravagiri et al. 1999; Baldessarini et al. 1993; Kapur et al. 2000; Porter et al. 2000, 2005), but without inducing, in acute or in subchronic administration, an increase in Fos or Fos-like immunoreactivity per se (Gessa et al. 2000; Kontkanen et al. 2002; Kuroki et al. 1999; Li et al. 2005; Ohashi et al. 2000; Robertson & Fibiger, 1992; Vahid-Ansari et al. 1996; Verma et al. 2007; Young et al. 1998) and behavioural side-effects in combination with atropine (pilot experiments).

Fos immunohistochemistry procedure

Because of the daily time-consuming schedule, pharmacological experiments were run in duplicate and thus conducted in two separate but identical settings. The behavioural results of the initial setting was used to determine the doses of antipsychotic agents for
which it would be especially relevant to quantify Fos labelling within the mPFC at the end of the second setting.

Ninety minutes after the end of CS testing, the rats were deeply anaesthetized with sodium pentobarbital (60 mg/kg i.p.) and perfused through the ascending aorta with a 15–20 min perfusion of 0.1 M phosphate-buffered 4% paraformaldehyde at 4 °C. The brain was then removed, post-fixed for 2 h at 4 °C and cryoprotected in 0.01 M PBS with 30% sucrose. Cryostat-cut 40-μm-thick brain coronal sections were processed free-floating, using the avidin-complex method (Vector Laboratories, USA). Sections were rinsed in PBS (three washes, 10 min each) between each incubation step. After quenching the endogenous peroxidase (0.2% hydrogen peroxide in PBS, 10 min), the floating sections were incubated for 1 h in 1% BSA, 0.4% normal goat serum and 0.3% Triton X-100 (namely PBS+). Sections were then incubated for 24 h at 4 °C with anti-c-fos (SC-52 1/2000, Santa Cruz Biotechnology, USA). Thereafter, sections were incubated for 1 h with biotinylated goat anti-rabbit IgG (BA-1000, Vector Laboratories, 1/300 in PBS+) and then for 2 h with the avidin-biotin reagent in PBS. Revelation was realized using the DAB reaction (0.04% diaminobenzidine with 0.01% hydrogen peroxide, 5–10 min), and was intensified with nickel (0.03% nickel chloride). The sections were mounted on gelatine-coated slides, dehydrated, and coverslipped. To control for non-specific staining by the secondary antibody, additional slices were processed in a manner identical to that described above, but without the primary antibody. No immunoreactivity was detected in these slices.

Three adjacent sections of the mPFC were selected to count the amount of Fos-immunopositive nuclei, at about +2.70 mm according to the antero-posterior coordinates of Paxinos & Watson (1998) relative to bregma. Under bright-field microscopy, Fos-positive nuclei were counted manually by a researcher blind to the experimental conditions. The counting was carried out bilaterally on each of the three sections for the anterior cingular cortex (ACC), the infralimbic cortex (IL) and the prelimbic cortex (PrL), in the whole area of these subregions. This procedure resulted in a total of three bilateral quantifications of the amount of Fos-positive nuclei within each specific subregion per rat. The average of these three quantifications was used for the subsequent statistical analysis.

Data analysis

Latencies typically conform to a Poisson distribution. Suppression times were thus transformed to log₁₀ to approximate normal distributions of scores within groups, as assumed in the use of parametric tests. Data were then analysed using one- or two-way analysis of variance (ANOVA) or covariance (ANCOVA) when needed. Post-hoc analyses were carried out using the method of contrasts.

Results

Expt 1: effects of haloperidol on atropine-induced disruption of the DCE

In each experiment, 12 rats were used in each group, except where otherwise stated.

Independent context testing

Figure 2a shows that similar low levels of conditioned suppression to the context were observed in the Cont groups, whereas the Deg-Cont groups exhibited high levels of conditioned suppression. The absence of an effect of the atropine–haloperidol combination on independent contextual fear was supported by a 2 x 5 ANOVA which showed a significant effect of conditioning (F₁,₁₁₀ = 90.04, p < 0.001), no effect of pharmacological treatment (F₁,₁₁₀ = 0.26), and no interaction between the two factors (F₁,₁₁₀ = 0.27). Post-hoc analysis found a significant difference between Cont and Deg-Cont groups for each dose tested (t’s > 3.90, p’s < 0.001).

Pre-CS testing

Scores to complete the initial 30 licks on day 13 for the Cont groups were 0.93, 1.15, 0.82, 0.84 and 0.96, and scores for the Deg-Cont groups were 1.13, 0.82, 0.86, 0.94 and 1.00, for doses of 0.0, 0.015, 0.03, 0.06 and 0.12 mg/kg haloperidol, respectively. A 2 x 5 ANOVA found no effect of conditioning (F₁,₁₁₀ = 3.04), no effect of pharmacological treatment (F₁,₁₁₀ = 1.69), but a significant interaction between the two factors (F₁,₁₁₀ = 2.85, p < 0.05). Although there was no clear interpretation of this effect on pre-CS in regard to the conditioning procedure (Cont vs. Deg-Cont) or the doses of haloperidol used, the pre-CS was used as a covariate to further analyse the CS testing.

CS testing

Figure 2b shows that the Cont groups exhibited high and equivalent levels of conditioned suppression to the CS. The Deg-Cont group injected with 0 mg/kg haloperidol exhibited a similar level of conditioned suppression to the CS than the Cont groups, thus replicating our initial results (Carnicella et al. 2005a, b,

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Haloperidol dose-dependently suppressed the effect of atropine on the DCE, as the level of conditioned suppression in the Deg-Cont groups decreased as the dose of haloperidol increased. This was confirmed by a 2\(\times\)5 ANCOVA, which found a significant effect of conditioning (\(F_{1,109} = 37.18, p < 0.001\)), an effect of pharmacological treatment (\(F_{4,109} = 3.63, p < 0.01\)), and a significant interaction between the two factors (\(F_{4,109} = 3.65, p < 0.01\)). Post-hoc analysis found a significant difference between Cont and Deg-Cont groups for the haloperidol doses of 0.03, 0.06 and 0.12 (\(t\)'s > 3.62, \(p\)'s < 0.01).

**Ext 2: effects of olanzapine on atropine-induced disruption of the DCE**

**Independent context testing**

Figure 3a shows that low levels of conditioned suppression to the independent context were observed in the Cont groups, whereas the Deg-Cont groups exhibited high levels of conditioned suppression, but with a slight decrease of conditioned fear under olanzapine, compared to the group injected with saline. A 2\(\times\)5 ANOVA showed a significant effect of conditioning (\(F_{1,109} = 79.82, p < 0.001\)), but no effect of...
pharmacological treatment ($F_{4,109} = 1.89$), and no interaction between the two factors ($F_{4,109} = 0.19$). Post-hoc analysis found a significant difference between Cont and Deg-Cont groups for each dose tested ($t$’s > 3.55, $p$’s < 0.001).

Pre-CS testing

Scores to complete the initial 30 licks on day 13 for the Cont groups were 0.88, 0.92, 0.77, 0.87 and 0.99, and scores for the Deg-Cont groups were 0.87, 0.82, 0.87, 0.84 and 1.00, for the doses of 0.0, 0.1, 0.2, 0.4 and 0.8 mg/kg olanzapine, respectively. A $2 \times 5$ ANOVA found no effect of conditioning ($F_{1,109} = 0.02$), no effect of pharmacological treatment ($F_{4,109} = 1.13$), and no interaction ($F_{4,109} = 0.32$).

CS testing

Figure 3b shows that the Cont groups exhibited high levels of conditioned suppression to the CS, but with a slight decrease of conditioned fear under olanzapine, compared to the group injected with saline. The Deg-Cont groups expressed levels of conditioned suppression equivalent to those of their respective Cont group, except for the intermediate doses of olanzapine (0.2 and 0.4 mg/kg), for which the conditioned suppression in the Deg-Cont groups was reduced compared to their respective Cont group. A $2 \times 5$ ANOVA found a significant effect of conditioning ($F_{1,109} = 13.11$, $p < 0.001$), an effect of pharmacological treatment ($F_{4,109} = 6.71$, $p < 0.001$), but no interaction ($F_{4,109} = 1.63$). Post-hoc analysis found a significant difference between Cont and Deg-Cont groups only for the dose of 0.2 mg/kg ($t_{21} = 5.66$, $p < 0.001$) and a marginal effect for the dose of 0.4 mg/kg ($t_{22} = 1.97$, $p = 0.062$).

Expt 3: effects of clozapine on atropine-induced disruption of the DCE

Independent context testing

Figure 4a shows that low levels of conditioned suppression to the independent context were observed in the Cont groups, whereas the Deg-Cont groups exhibited similar high levels of conditioned suppression. A $2 \times 4$ ANOVA showed a significant effect of conditioning ($F_{1,87} = 53.44$, $p < 0.001$), no effect of pharmacological treatment ($F_{3,87} = 1.55$), and no interaction between the two factors ($F_{3,87} = 0.30$). Post-hoc analysis found a significant difference between Cont and Deg-Cont groups for each dose tested ($t$’s > 2.75, $p$’s < 0.02).

Pre-CS testing

Scores to complete the initial 30 licks on day 13 for the Cont groups were 1.04, 1.06, 0.98 and 1.10, and scores for the Deg-Cont groups were 1.01, 1.08, 1.18 and 1.15, for the doses of 0.0, 0.75, 1.5, and 3 mg/kg clozapine, respectively. A $2 \times 4$ ANOVA found no effect of conditioning ($F_{1,87} = 0.69$), no effect of pharmacological treatment ($F_{3,87} = 0.28$), and no interaction ($F_{3,87} = 0.42$).

CS testing

Figure 4b shows that the Cont groups exhibited high levels of conditioned suppression to the CS. The Deg-Cont groups injected with 0 or 3 mg/kg clozapine expressed levels of conditioned suppression to the CS equivalent to those of the Cont groups. However, for the doses of 0.75 and 1.5 mg/kg of clozapine, the Deg-Cont groups exhibited a lower level of conditioned suppression than their respective Cont groups. A $2 \times 4$ ANOVA found a significant effect of conditioning.
(\(F_{1,87} = 9.58, p < 0.01\)), an effect of pharmacological treatment (\(F_{3,87} = 5.42, p < 0.01\)), and a marginal interaction (\(F_{3,87} = 2.69, p = 0.051\)). Post-hoc analysis found a significant difference between Cont and Deg-Cont groups only for the dose of 1.5 mg/kg (\(t_{22} = 4.60, p < 0.001\)).

**Expt 4: effects of NDMC on atropine-induced disruption of the DCE**

**Independent context testing**

Figure 5a shows that low levels of conditioned suppression to the independent context were observed in the Cont groups, whereas the Deg-Cont groups exhibited high levels of conditioned suppression. A 2 × 5 ANOVA showed a significant effect of conditioning (\(F_{1,109} = 49.86, p < 0.001\)), no effect of pharmacological treatment (\(F_{4,109} = 0.48\)), and no interaction between the two factors (\(F_{4,109} = 0.95\)). Post-hoc analysis found a significant difference between Cont and Deg-Cont groups, for each dose tested (\(t's > 2.11, p's < 0.05\)).

**Pre-CS testing**

Scores to complete the initial 30 licks on day 13 for the Cont groups were 0.88, 0.99, 0.95, 0.98 and 1.02, and scores for the Deg-Cont groups were 0.88, 0.91, 0.98, 0.96 and 0.95, for the doses of 0.0, 0.375, 0.75, 1.5 and 3 mg/kg NDMC, respectively. A 2 × 5 ANOVA found no effect of conditioning (\(F_{1,109} = 0.17\)), no effect of pharmacological treatment (\(F_{4,109} = 0.25\)), and no interaction between the two factors (\(F_{4,109} = 0.08\)).

**CS testing**

Figure 5b shows that the Cont groups exhibited high levels of conditioned suppression to the CS. The Deg-Cont group injected with 0 mg/kg NDMC exhibited a similar level of conditioned suppression to the CS than the Cont groups, whereas the level of conditioned suppression in the other Deg-Cont groups decreased as the dose of NDMC increased. A 2 × 5 ANOVA showed a significant effect of conditioning (\(F_{1,109} = 15.59, p < 0.001\)), but no significant effect of pharmacological treatment (\(F_{4,109} = 2.14\)), and no significant interaction between the two factors (\(F_{4,109} = 1.33\)). Post-hoc analysis found a significant difference between Cont and Deg-Cont groups for the doses of NDMC of 0.75 and 3 mg/kg (\(t's > 2.10, p's < 0.05\)).

**Expt 5: effects of antipsychotic-atropine combination on pseudo-conditioning**

We tested whether effective dose of antipsychotics in combination with atropine would alter pseudo-conditioning, where the presentations of the CS and the US were never associated. This experiment included four groups (\(n = 8\)) according to pharmacological treatment (saline, 0.12 mg/kg haloperidol, 0.2 mg/kg olanzapine or 3 mg/kg NDMC).

**Independent context testing**

Figure 6a shows a high level of conditioned suppression as the unsignalled US were associated with the experimental context, although this level is reduced for the olanzapine-atropine combination. However, an ANOVA found no effect of pharmacological treatment (\(F_{3,28} = 0.54\)).

**Pre-CS testing**

Scores to complete the initial 30 licks on day 13 for the different groups were 1.39, 0.89, 0.73 and 0.99, for
saline, haloperidol, olanzapine and NDMC, respectively. An ANOVA found a significant effect of pharmacological treatment \( (F_{3,28} = 3.34, p < 0.05) \), therefore the pre-CS was used as a covariate to analyse the CS testing.

**CS testing**

Figure 6b shows a low level of conditioned suppression for all treatments. This was confirmed by an ANCOVA which found no effect of pharmacological treatment \( (F_{3,28} = 0.91) \).

**Expt 6: Fos immunolabelling of the mPFC after CS testing under antipsychotic–atropine combination**

After the first set of experiments, we determined the optimal doses for the different antipsychotics in order to test whether the reversal of atropine-induced disruption of the DCE is paralleled with an increase level of Fos immunoreactivity in the mPFC. Hence, immunolabelling was processed on six animals for each condition. The saline–atropine condition groups for each of the four treatments were pooled in order to control for the appropriateness of subsequent between-group comparisons across the different experiments \( (n = 24) \). A 2 (Cont vs. Deg-Cont) x 4 (experiments) ANOVA found no significant differences in the amount of Fos expressed across the different experiments for the saline–atropine condition, regardless of the sub-region (ACC, PrL and IL) under consideration \( (F’s < 2.28) \) or of the entire mPFC \( (F’s < 1.33) \) (data not shown). This suggests that the amount of Fos-positive nuclei counted within the mPFC was stable across time and experimental conditions.

**Hydration**

The total amount of licks recorded during the 15 min of CS testing (mean ± S.E.M.) for the different groups varied between 1629 ± 216 and 2797 ± 308. A 2 x 7 ANOVA showed no effect of conditioning \( (F_{1,104} = 0.78) \), no effect of pharmacological treatment \( (F_{6,104} = 2.00) \), and no interaction between the two factors \( (F_{6,104} = 0.09) \). This suggests that the level of water satiation was similar across the different groups and experiments.

**Immunolabelling**

The number of Fos-positive nuclei within the whole mPFC, along with the specific counting within each of the three subregions (ACC, PrL and IL), are shown in Table 1. Because the Fos immunoreactivity profile was similar in the three subregions, the amount of Fos-positive nuclei within the whole mPFC was analysed.

Figure 7 shows that for the doses of 0.12 mg/kg haloperidol, 0.2 mg/kg olanzapine, 1.5 mg/kg clozapine and 3 mg/kg NDMC, the Deg-Cont groups exhibited a higher level of Fos-immunoreactivity within the mPFC than their Cont counterparts. The Cont and Deg-Cont groups injected with saline, the dose of 0.8 mg/kg olanzapine, or the dose of 3 mg/kg clozapine, exhibited an intermediate but similar level of Fos-immunoreactivity. A 2 x 7 ANOVA found a significant effect of conditioning \( (F_{1,184} = 51.12, p < 0.001) \), no effect of pharmacological treatment \( (F_{6,184} = 1.37) \), and a significant interaction between the two factors \( (F_{6,184} = 8.47, p < 0.001) \). A post-hoc analysis found a significant difference between Cont and Deg-Cont groups for 0.12 mg/kg atropine–haloperidol, 0.2 mg/kg haloperidol, 0.2 mg/kg olanzapine and NDMC, respectively. An ANOVA found a significant effect of pharmacological treatment \( (F_{3,28} = 3.34, p < 0.05) \), therefore the pre-CS was used as a covariate to analyse the CS testing.
For each rat, values were summed within the whole mPFC (see Fig. 7). All the rats were treated with atropine (5 mg/kg). DCE, the dose-dependent profiles were different.

...chotics were due to a specific action on information processing during the DCE. Atropine alone at the dose used here to disrupt the DCE was also shown not to alter these conditions previously observed in the different subregions of the mPFC (ACC, PrL, IL) in the saline-treated Deg-Cont group, compared to the saline-treated Cont group (Carnicella et al. 2006).

The low doses of haloperidol, olanzapine, clozapine and NDMC used for subchronic administration in the present study were able to restore the DCE, without affecting the processing of the CS during conditioning and pseudo-conditioning, and the independent context. Atropine alone at the dose used here to disrupt the DCE was also shown not to alter these conditions (Carnicella et al. 2005a). Taken together, these data suggest that the restorative effects of the antipsychotics were due to a specific action on information processing during the DCE.

Although these drugs were found to restore the DCE, the dose-dependent profiles were different. Haloperidol and NDMC induced a linear dose-dependent effect, whereas olanzapine and clozapine exhibited a U-shaped dose–response curve. The present study cannot address the exact mechanism(s) by which haloperidol, olanzapine, clozapine and NDMC reverse the effects of atropine. However, haloperidol has no specific affinity for muscarinic receptors (Davies et al. 2005; Weiner et al. 2004) and is thought to display its antipsychotic effects via its potent dopaminergic antagonistic action. Some major interactions between the cholinergic and the dopaminergic systems have been evidenced in neurochemical and behavioural processes (Barak & Weiner, 2007; Day & Fibiger, 1992; Laplante et al. 2004). Dopamine can modulate the cholinergic transmission in the mPFC via a direct action of the substantia nigra/ventral tegmental area on the basal forebrain cholinergic neurons (Gaykema & Zaborsky, 1996) or via GABAergic interneurons in the mPFC (Laplante et al. 2004). Olanzapine and clozapine are potent dopaminergic receptor antagonists, but they also act as potent muscarinic antagonists, both in vitro and in vivo (Davies et al. 2005; Johnson et al. 2005; Porter et al. 2000, 2005; but see Bymaster & Falcone, 2000). The combination of these two pharmacological properties (dopaminergic/muscarinic antagonism) might account for the U-shaped response curves observed here with the atypical antipsychotic agents. Olanzapine and clozapine at low doses might reverse the effects of atropine by their dopaminergic antagonism activity, like haloperidol. At high doses, olanzapine and clozapine might also act as muscarinic receptor antagonists, thus counteracting the beneficial dopaminergic effect by potentiating the muscarinic effects of atropine. This hypothesis is supported by the dose-dependent linear effect obtained with NDMC which shares some common pharmacological properties with clozapine and

### Table 1

Mean number of Fos-positive nuclei (±S.E.M.) within the different subregions of the medial prefrontal cortex (mPFC)

<table>
<thead>
<tr>
<th>Subregion</th>
<th>Sal 0.12 Hal</th>
<th>0.2 Olz</th>
<th>0.8 Olz</th>
<th>1.5 Clz</th>
<th>3 Clz</th>
<th>3 NDMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC Cont</td>
<td>30±4</td>
<td>16±4</td>
<td>18±4</td>
<td>25±3</td>
<td>11±3</td>
<td>28±7</td>
</tr>
<tr>
<td>Deg-Cont</td>
<td>26±3</td>
<td>48±5</td>
<td>40±5</td>
<td>28±3</td>
<td>48±8</td>
<td>27±6</td>
</tr>
<tr>
<td>PrL Cont</td>
<td>51±6</td>
<td>23±5</td>
<td>29±7</td>
<td>44±3</td>
<td>10±1</td>
<td>38±9</td>
</tr>
<tr>
<td>Deg-Cont</td>
<td>44±7</td>
<td>86±7</td>
<td>74±6</td>
<td>49±6</td>
<td>80±11</td>
<td>39±8</td>
</tr>
<tr>
<td>IL Cont</td>
<td>34±4</td>
<td>22±4</td>
<td>20±3</td>
<td>39±4</td>
<td>7±2</td>
<td>22±4</td>
</tr>
<tr>
<td>Deg-Cont</td>
<td>34±5</td>
<td>56±8</td>
<td>67±5</td>
<td>49±6</td>
<td>61±13</td>
<td>22±6</td>
</tr>
<tr>
<td>mPFC Cont</td>
<td>114±12</td>
<td>61±10</td>
<td>67±11</td>
<td>108±6</td>
<td>28±4</td>
<td>88±18</td>
</tr>
<tr>
<td>Deg-Cont</td>
<td>105±13</td>
<td>191±14</td>
<td>182±15</td>
<td>126±13</td>
<td>189±31</td>
<td>88±18</td>
</tr>
</tbody>
</table>

ACC, anterior cingular; PrL, prelimbic cortex; IL, infralimbic cortex.

For each rat, values were summed within the whole mPFC (see Fig. 7). All the rats were treated with atropine (5 mg/kg).
olanzapine, but is, in contrast to these compounds, a potent muscarinic receptor agonist in vitro and in vivo (Davies et al. 2005; Li et al. 2005; Sur et al. 2003; Weiner et al. 2004). The agonistic activity of NDMC at the M\(_1\) muscarinic receptor might contribute to or account for its restorative effect on the DCE, as we have previously shown that the functional M\(_1\)/M\(_4\) agonist xanomeline reversed, in a linear dose-dependent relationship, atropine-induced disruption of the DCE (Carnicella et al. 2005b).

In a recent study focusing on the neural substrates involved in the Pavlovian DCE and in its disruption by atropine, we examined Fos expression within samples of the main cholinergic nuclei, auditory system, limbic structures (hippocampus, amygdala, nucleus accumbens) and the whole prefrontal cortex. We found that the subregions of the mPFC (ACC, PrL, IL) were the only structures studied in which the amount of Fos-immunoreactive neurons was differentially affected according to the conditioning procedure and the pharmacological treatment. Specifically, we showed that, in saline-treated groups, compared to pseudo-conditioning or contingent conditioning, degraded contingent conditioning induced a substantial increase in Fos immunoreactivity within these three subregions, an effect which was suppressed in atropine-treated groups (Carnicella et al. 2006). Here, we showed that the behavioural reversion of atropine-induced disruption of the DCE by low doses of haloperidol, olanzapine, clozapine and NDMC was associated with an increase in Fos immunoreactivity within the entire mPFC, as assessed by a higher level of Fos immunoreactivity in Deg-Cont groups than in Cont groups, which closely mimics what we previously observed in saline-treated rats. Interestingly, the effect of olanzapine and clozapine on the activity of the mPFC vanished for doses that no longer effectively alleviated the effects of atropine (0.8 mg/kg and 3 mg/kg, respectively), suggesting that the actions of these antipsychotic agents on the DCE and the mPFC are closely linked. Increasing evidence in rats suggests that the integrity of the mPFC is required not only for the selection of appropriate behavioural responses, but also for multiple computational functions (Brown & Bowman, 2002; Dalley et al. 2004; Heidbreder & Groenewegen, 2003). Although we cannot determine to what extent the pattern of Fos reactivity we observed here is a cause or a consequence of the behavioural pattern displayed by the rats, the present results highlight a major involvement of the mPFC in rats in atropine-induced disruption of the modulating role of the context in Pavlovian conditioning and in its recovery by antipsychotic agents.

Alteration of interactive context processing can be considered as a hallmark of psychotic states (Bazin et al. 1996; Cohen & Servan-Schreiber, 1992; Escobar et al. 2002; Hemsley, 2005; Oberling et al. 1999). Importantly, this deficit is associated with prefrontal cortical dysfunctions in schizophrenia (see Introduction), which can be reversed with antipsychotics (Snitz et al. 2005). The present series of experiments shows that atropine-induced disruption of the DCE, a fundamental and simple form of interactive context processing involved in associative mechanisms, involves the mPFC in rats and, furthermore, is sensitive to antipsychotic medications.

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Statement of Interest
None.

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Statement of Interest
None.
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