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ORIGINAL INVESTIGATION

Dopaminergic and serotonergic modulation of persistent behaviour in the reinforced spatial alternation model of obsessive–compulsive disorder

Dimitris Kontis · Vasileios Boulougouris · Vasiliki Maria Papakosta · Stamatina Kalogerakou · Socrates Papadopoulos · Cornelia Poulopoulou · George N. Papadimitriou · Eleftheria Tsaltas

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Abstract
Rationale We have proposed rewarded T-maze alternation as a model of obsessive–compulsive disorder (OCD): the serotonin agonist *m*-chlorophenylpiperazine (mCPP) increments persistence therein, while chronic pretreatment with selective serotonin reuptake inhibitor (SSRI fluoxetine) but not benzodiazepine or desipramine abolishes mCPP effects. However, we noted that acute SSRI administration also causes transient persistence increase, counteracted by mCPP pretreatment.
Objectives This study (a) further explores the cross-tolerance between fluoxetine and mCPP and (b) extends the model by investigating its sensitivity to dopaminergic manipulations ($D_{2,\ 3}$ agonism—quinpirole).
Materials and methods In both experiments, baseline and drug testing were carried out under daily T-maze alternation training. Exp. 1: Matched group (n=8) pairs of rats received one of the following 20-day pretreatments (daily intraperitoneal administration): (1) saline, (2) low-dose fluoxetine (2.5 mg/kg), (3) low-dose mCPP (0.5 mg/kg) or (4) combined fluoxetine + mCPP. One group per pretreatment then received a 4-day challenge with high-dose fluoxetine (10 mg/kg), the other with high-dose mCPP (2.5 mg/kg). Exp. 2**:** One group (n=12) of rats received 20-day treatment with saline, another with quinpirole (0.5 mg/kg).
Results Exp. 1: Saline and low-dose mCPP- or fluoxetine-pretreated animals showed significant persistence increases under both challenges, while combined low-dose fluoxetine + mCPP pretreatment afforded full protection from either challenge. Exp. 2: Quinpirole significantly increased directional persistence after 13 administration days.
Conclusions These results establish the sensitivity of the rewarded alternation OCD model to $D_{2,\ 3}$ receptor activation, thereby extending its profile of pharmacological isomorphism with OCD. Furthermore, they suggest a common mechanism of action of an SSRI and a serotonin agonist in the control of directional persistence.

Keywords Obsessive–compulsive disorder (OCD) · Animal model · Fluoxetine · mCPP · Quinpirole · Serotonin receptors · Dopamine receptors

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D. Kontis · V. M. Papakosta · S. Kalogerakou · E. Tsaltas (✉)
Experimental Psychology Laboratory, Department of Psychiatry,
Eginition Hospital, Athens University Medical School,
74, Vas. Sofias Ave.,
115 28 Athens, Greece
e-mail: tsaltasl@med.uoa.gr

V. Boulougouris
Department of Experimental Psychology
and the Behavioural and Clinical Neuroscience Institute (BCNI),
University of Cambridge,
Downing Site, CB2 3EB,
Cambridge, UK

D. Kontis · S. Papadopoulos
Psychiatric Hospital of Attica,
Athens, Greece

C. Poulopoulou
Department of Neurology, Eginition Hospital,
Athens University Medical School,
74, Vas. Sofias Ave.,
115 28 Athens, Greece

G. N. Papadimitriou
Department of Psychiatry, Eginition Hospital,
Athens University Medical School,
Athens, Greece

Introduction

Obsessive–compulsive disorder (OCD) is a high-prevalence psychiatric disorder affecting 2–5% of the population (Karno et al. 1988). Although its pathophysiology remains unclear, current evidence implicates contributions of the serotonergic and dopaminergic neurotransmitter systems (Barr et al. 1992; Denys et al. 2004a) and a neural circuitry that includes the orbitofrontal cortex, the thalamus and the striatum (Saxena and Rauch 2000).

The serotonergic involvement is mainly supported by the selective response of obsessive–compulsive symptoms to specific serotonin reuptake inhibitors (SSRIs; Goodman et al. 1990a; Baumgarten and Grozdanovic 1998; Hoehn-Saric et al. 2000), an effect which has been associated with increased 5-HT neurotransmission in the orbitofrontal cortex (Blier and de Montigny 1998). 5-HT2 receptors emerge as candidate mediators of this enhanced 5-HT release on the basis of preclinical data (Blier et al. 2000) and of clinical studies involving the administration of serotonin agonists to OCD patients (Hollander et al. 1992; Delgado 2000).

We have recently proposed that spontaneous persistence towards one direction during training in T-maze-rewarded alternation may provide an analogue of the human compulsive trait (Tsaltas et al. 2005). In this particular task, as well as in other forms of discrimination training, perseveration towards one response alternative is almost always noted in the early stages of training; hence, different strategies have been developed with the aim of distinguishing between ‘perseveration’ and ‘learning’ errors (Jones and Mishkin 1972; Hunt and Aggleton 1998; Chudasama and Robbins 2003; Boulougouris et al. 2007). We noted after training several rat cohorts in T-maze-rewarded alternation that this spontaneous persistence towards one direction gradually dissipates with vigorous behavioural training in all but a fraction (2–3%) of rats screened (Tsaltas et al. 2007a; Tsaltas et al. 2007b). On the basis of such screening, we formed groups of high and low spontaneous ‘persisters’ and subjected them to acute challenge with the non-specific serotonin agonist *m*-chlorophenylpiperazine (mCPP), which exacerbates obsessive–compulsive symptoms in unmedicated OCD patients, with no effect on patients treated with SSRIs or on controls (Zohar and Insel 1987; Hollander et al. 1992). We demonstrated that persistence towards one direction re-emerged upon mCPP challenge, but in initially persistent animals only. This exacerbation of persistence was counteracted by chronic pretreatment with the SSRI fluoxetine (but not with desipramine or diazepam), in accordance with the differential efficacy of these drugs in treating OCD patients (Tsaltas et al. 2005).

The rewarded alternation model therefore shows good correspondence with obsessive–compulsive symptomatology with respect to serotonergic manipulations. However, it has not yet been tested for sensitivity to dopaminergic manipulations. The dopaminergic system has been increasingly implicated in the pathophysiology of OCD from the observation that 40–60% of OCD patients are resistant to SSRI treatment (Hollander et al. 2002; McDonough and Kennedy 2002). In this population, particularly in patients with concurrent psychotic spectrum disorders or with comorbid chronic tic disorders such as Tourette's syndrome, the addition of dopamine antagonists has proved a useful therapeutic strategy (McDougle et al. 1994a, b). Certain atypical antipsychotics also augment SSRI effectiveness in resistant patients (Sareen et al. 2004). Additionally, increases in the activity of platelet sulphotransferase, an enzyme involved in the catabolism of phenolic compounds and of catecholamines such as dopamine, have been documented in OCD patients compared with controls (Marazziti et al. 1992), while abnormalities in the binding potential of the dopamine D_2 receptor in the striatum of OCD patients have also been reported (Denys et al. 2004b).

These data gave rise to the hypothesis that a putative serotonergic deficiency in OCD patients may result in increased dopamine release leading to postsynaptic D_2 receptor down-regulation in the striatum (Goodman et al. 1990b; Stahl 1998; Micallef and Blin 2001). Incisive exploration of this serotonin–dopamine interaction hypothesis of OCD pathogenesis is not feasible in clinical research, an alternative strategy being the use of animal models (Marek et al. 2005). With respect to the study of OCD pathophysiology, an ideal model would be one of established sensitivity to manipulations of both serotonergic and dopaminergic neurotransmission.

A recent OCD model has been based on the observation that prolonged but not acute administration of the $D_{2,\ 3}$ agonist quinpirole induces compulsive behaviour in rats, without evidence of stereotypy (Szechtman et al. 1998, 2001). It has been proposed that quinpirole sensitisation produces this effect by enhancing dopamine release (Eilam et al. 1989). Specifically, the quinpirole sensitisation condition has been associated with an up-regulation of subcortical dopamine activity (Sullivan et al. 1998). Although there is some evidence that quinpirole-induced compulsive checking is moderated (but not eliminated) by clomipramine administration (Szechtman et al. 1998), the quinpirole model has not been thoroughly examined with respect to serotonergic sensitivity. In contrast, the reinforced alternation model has been tested for serotonergic response (SSRIs, serotonergic agonists) and specificity thereof (noradrenergic antidepressants, benzodiazepines), but its responsiveness to dopaminergic manipulation remains to be examined. If it could be established that it also responds to quinpirole sensitisation by an increase in

its Persistence Index, it could then provide a useful tool for further investigation of the serotonin–dopamine interaction hypothesis of OCD pathophysiology.

A second issue which needs investigation in the pharmacological profile of the rewarded alternation OCD model is our observation (Tsaltas et al. 2005) that acute administration (<15 days) of the SSRI fluoxetine increased directional persistence, as did mCPP. This proved to be a transient phenomenon, dissipating by administration day 13–15, with restoration of drug-free baseline persistence levels. Furthermore, this acute SSRI effect was blocked by chronic pretreatment with mCPP. In brief, we noted cross-tolerance between the SSRI and the 5-HT agonist. Although this seemingly paradoxical finding is congruent with earlier findings suggesting a therapeutic potential of serotonin agonists in OCD (Pigott et al. 1992a, b; Martin et al. 1998), it deserves further exploration as it may provide a basis for investigating the therapeutic mechanism of SSRIs in OCD.

The present study therefore had two objectives. Experiment 1 examined the hypothesis that the cross-tolerance between fluoxetine and mCPP reported by Tsaltas et al. (2005) reflects a common pathway of action of the SSRI and the 5-HT receptor agonist on directional persistence. We approached this issue by testing for a synergistic interaction (Marek et al. 2005) between low doses of fluoxetine and mCPP that are, singly, relatively inactive in protecting against the increased directional persistence induced by acute challenge with high (behaviourally effective) doses of mCPP or fluoxetine. Experiment 2 investigated the effects of prolonged administration of quinpirole on directional persistence in the T-maze. We hypothesised that acute administration of quinpirole would not affect persistent behaviour, whereas chronic administration of this D_2–D_3 agonist would increase directional persistence in the T-maze.

Materials and methods

Animals

Eighty-five experimentally naïve adult male Wistar rats (Pasteur Institute of Athens) aged 1–2 months and weighting 120–190 g on delivery were housed in triads under stable environmental conditions (23–25°C, 12 h light–dark, lights on at 7:00 am) in the same animal room. After 10 days of habituation under ad libitum water and food (Standard Diet, 4RF18, Mucedola s.r.l, Italy), at which point the average weight was 290 g, they were put on a 23-h daily food deprivation schedule with freely available water. Animals were approximately 90% of free feeding weight at the onset of behavioural training.

Apparatus

Two identical wooden flat grey T-mazes were used. The mazes stood 120 cm above the floor surface. Their stem measured 90 cm long×10 cm wide. The first 20 cm of the stem acted as the start area, being separated from the main maze by a guillotine Plexiglas door. The cross arm measured 140 cm long×10 cm wide and had two reward cups fixed on the floor 2 cm from each end. The reward cups were opaque, 2 cm in diameter and 0.75 cm deep so that visual detection of reward (cereal puffs) from a distance was not possible. The maze was wiped clean with alcohol after each run. The two mazes were oriented at right angles to each other, to control for directional preferences due to extra-maze cues. Half of the animals of each experimental condition ran in each maze.

Drugs used

All substances used in experiments 1 and 2 were dissolved in physiological saline vehicle. They were injected daily (intraperitoneally, 28-gauge needle) at injection volumes constant for all conditions (6.67 ml/kg). Injections were made 30 min before onset of behavioural training.

Experiment 1 The substances used were fluoxetine (specific serotonin reuptake inhibitor, Eli Lilly SA Irish Branch, Dunderrow, Kinsale, Co., Cork, Ireland), *m*-chlorophenylpiperazine (mCPP; non-specific serotonin agonist, C-5554, Sigma Chemical Co., St. Louis, MO, USA) and physiological saline. The selection of high (behaviourally effective) doses of these substances was based on previous work (fluoxetine high dose=10 mg/kg: Tsaltas et al. 2005; Marek et al. 2005; mCPP high dose=2.5 mg/kg: Tsaltas et al. 2005). Low doses were initially set to 50% of the behaviourally effective high dose (fluoxetine low dose=5 mg/kg: Marek et al. 2005; mCPP low dose=1.25 mg/kg). At this dose, however, mCPP still appeared to have some behavioural impact in early pretreatment. Low doses of both substances were therefore further reduced on pretreatment day 4 (fluoxetine low dose=2.5 mg/kg; mCPP low dose=0.50 mg/kg). The dose reduction was applied concurrently to the groups treated with low doses of either substance and to groups receiving combined low doses of both.

Experiment 2 The substances used were physiological saline and the $D_{2,3}$ agonist quinpirole (quinpirole hydrochloride, Q-102, Sigma Chemical Co., St. Louis, MO, USA, 0.5 mg/kg). The dose of 0.5 mg/kg was selected on the basis of the Szechtman et al. (1998, 2001) observation that this dose produces a sensitisation effect representative of that induced by doses ranging from 0.25 to 2.5 mg/kg.

Quinpirole was administered daily, as the injection regime had to be identical to that of experiment 1, which ran concurrently and from which the saline control group of experiment 2 was drawn (see Experiment 2, "Method"). Szechtman et al (1994), comparing 2-, 4- and 8-day administration regimes, have shown that quinpirole sensitisation is controlled predominantly by injection number rather than by inter-injection interval. Furthermore, Foley et al. (2006) have shown that daily quinpirole administration (at the 0.5 mg/kg dose which we used) produced sensitisation similar to the regimes mentioned above.

Behavioural procedure

Behavioural training Animals were handled for 1 week, followed by a week of habituation to the loaded T-mazes in triads initially, individually thereafter. They were allowed to explore and eat freely for 5 min daily. At the end of the week, all animals ate the reward and anxiety signs (freezing, defecation) were minimised. Acquisition of rewarded alternation was then initiated. Each alternation trial included two runs through the T-maze, both food cups of which were baited. The animal was placed on the start point with its back towards the closed guillotine door. In the first (information) run, one arm of the maze was blocked according to a daily pseudo-random sequence (four left and four right forced runs daily, maximum two consecutive ones in the same direction). As soon as the animal reached the goal and consumed reinforcement, it was moved back to the start point; the obstacle was removed and the second (choice) run began immediately (0-s delay). The choice run was completed when all paws of the animal were in a lateral arm. Thereafter, change in choice was prevented. Choice of the arm opposite to the preceding forced arm was rewarded, choice of the same resulted in non-reward with 10-s timeout. Animals were run in squads of three in rotation, returning to the holding box after each trial. The resulting inter-trial interval was approximately 100 s. Initially, each animal received two daily trials, gradually incremented to four and then eight. Training continued for all until every animal had reached a criterion of 7/8 trials correct per day for five consecutive days.

Baseline phase Animals were subjected to drug-free alternation training until they all reached criterion (320 trials, 40 days), at which point all Persistence Index scores approached 0. Since the stringent criterion led to very low Persistence Index scores towards the end of baseline, spontaneous persistence screening and group matching for the subsequent chronic pharmacological pretreatment phase (see each experiment separately) was based on early baseline scores (trials 1–264). The two experiments reported here were run concurrently through baseline and chronic pharmacological pretreatment phase.

Quantification of directional persistence: the Persistence Index

The dependent variable recorded throughout the experiments was a simple estimate of persistence towards one of the two response alternatives available. This daily Persistence Index was the absolute value of the difference of daily right and left success rates [= |(daily LEFT correct choices/4)%−(daily RIGHT correct choices/4)%|]. A phase Persistence Index was also calculated for each experimental phase (see next section) on the basis of cumulative left and right errors per phase opportunities for left and right correct choices [= |(phase LEFT correct choices/phase LEFT opportunities)%−(phase RIGHT correct choices/phase RIGHT opportunities)%]. The phase Persistence Index offers more robust data since directional persistence is best documented if chance daily preference fluctuations are allowed to cancel out over time. For both indices, spontaneous values of near 0 reflect low persistence tendency. It can be argued that the Persistence Index score is relatively independent of individual differences in learning or memory capacity since errors due to those should be equally distributed to both directions, therefore cancel out if an animal shows no directional persistence (Tsaltas et al. 2005).

Experiment 1: Testing for synergy between the SSRI fluoxetine and the 5-HT receptor agonist mCPP on directional persistence

Method

Sixty animals were initially screened for spontaneous persistence and the 48 animals with the highest spontaneous persistence scores in the baseline phase were distributed across six groups, matched for drug-free Persistence Index scores. Twenty-five more animals were subsequently screened, to yield two additional groups (n=8 each) matched for drug-free Persistence Index scores with the initial six groups. This was essential as our labourious screening procedure limits the number of animals which can be run at once. Therefore, two control groups (a low-dose FLX-pretreated group challenged with high-dose FLX and a low-dose mCPP-pretreated group challenged with high-dose mCPP: see below) were ran at a latter date.

Exclusion of the least persistent animals was based on previous data showing that the effects of serotonergic agents (fluoxetine, mCPP) on directional persistence are

best expressed on a substrate of medium to high spontaneous persistence due to floor effects (Tsaltas et al. 2005). The design of the Experiment 1 is shown in Table 1).

Experimental phases

Chronic pharmacological treatment phase Following the baseline phase, the 20-day chronic pharmacological treatment phase was initiated. Two groups of animals (n=8 each) received daily saline; two had low-dose fluoxetine (n=8 each); two had low-dose mCPP (n=8 and n=7, respectively: one animal died) and a final two groups (n=8 each) received combined low doses of fluoxetine and mCPP (Table 1).

Pharmacological challenge phase Pretreatment was followed by 4 days of pharmacological challenge, with daily administration of full-dose fluoxetine or mCPP. One group from each pretreatment condition was included in each challenge condition (Table 1).

Statistical analysis

Analyses were carried out by the STATISTICA for Windows statistical package (2008, version 6.1 StatSoft Inc., Tulsa, OK, USA). Significant analysis of variance (ANOVA) effects were further explored through planned comparisons (contrast testing).

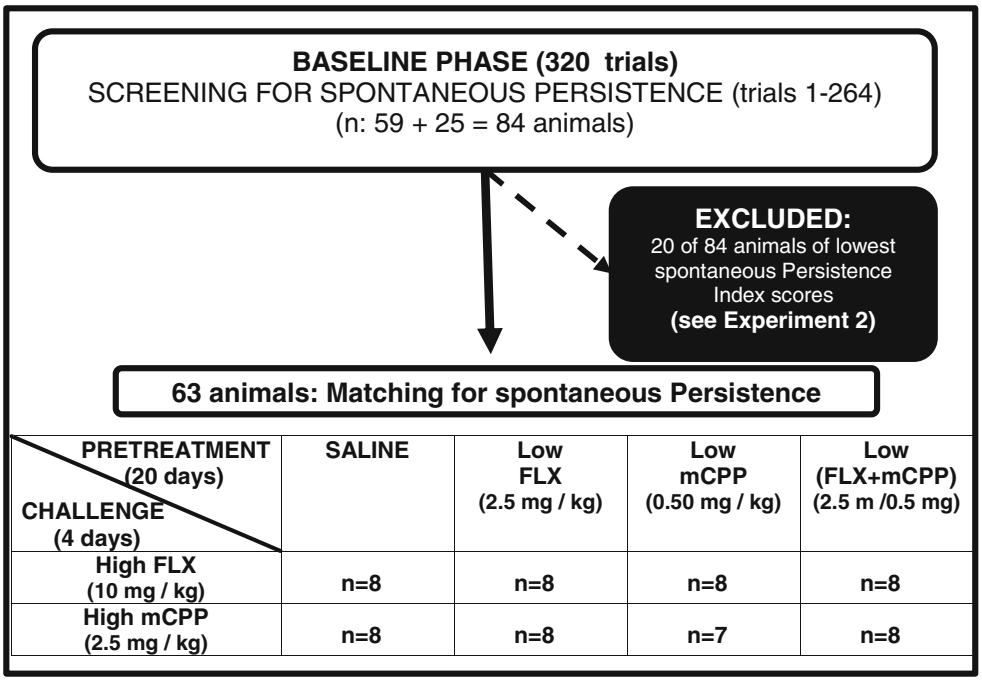
A mixed three-way ANOVA with repeated measures was carried out on phase Persistence Index scores after a square root-transform [SQRT(x+0.5)]. The independent variables were (1) mCPP pretreatment (two levels: low-dose mCPP, saline) and (2) FLX pretreatment (two levels: low-dose FLX, saline) and (3) challenge (two levels: high-dose fluoxetine or high-dose mCPP). The repeated measures reflected experimental phase and included three levels: baseline (BL: last 4 of 40 days), chronic pretreatment (Pretx: last 4 of 20 days) and challenge (4 days).

An additional two-way ANOVA (factors as 1 and 2 above) with repeated measures was carried out on the 20 pretreatment phase days in 4-day blocks (repeated measures). This was done (a) to ensure that our matching was successful across future challenge levels and (b) to detect the existence and examine the time course of any behavioural effects of the pretreatment conditions.

Results

Pretreatment phase analysis (Fig. 1) The main effect of days (in 4-day blocks) was significant ($F_{4, 236}$=12.74, p< 0.0001), as was the factor of mCPP pretreatment as a main effect ($F_{1, 59}$=14.54, p<0.0003) and in interaction with days ($F_{4, 236}$=4.36, p<0.002). When the effects of low-

Table 1 Experiment 1, flow diagram of the experimental procedure



PRETREATMENT (20 days) / CHALLENGE (4 days)	SALINE	Low FLX (2.5 mg / kg)	Low mCPP (0.50 mg / kg)	Low (FLX+mCPP) (2.5 m /0.5 mg)
High FLX (10 mg / kg)	n=8	n=8	n=8	n=8
High mCPP (2.5 mg / kg)	n=8	n=8	n=7	n=8

High-dose FLX, mCPP=10 and 2.5 mg/kg, respectively. Low-dose FLX, mCPP=5 mg/kg and 1.25 mg/kg, respectively. On pretreatment day 4, doses were further reduced to 2.5 and 0.50 mg/kg, respectively.
FLX Fluoxetine, *mCPP* *m*-chlorophenylpiperazine

dose mCPP were compared to those of saline with or without low-dose FLX through contrast testing, it emerged that mCPP significantly increased Persistence Index scores over baseline in the first three 4-day blocks of the pretreatment phase ($F_{1, 59}$=19.79, 16.49 and 11.10, respectively, p<0.0001). FLX pretreatment was not significant as a main effect or in interaction with mCPP. As reported by Tsaltas et al. (2005), the mCPP-induced increase in persistence was transient, gradually dissipating after approximately 12 administration days.

Main, three-phase analysis (Fig. 2) The main effect of phase was significant ($F_{2,110}$=64.48, p<0.0001), as was the mCPP pretreatment × phase interaction ($F_{2,110}$=4.91, p<0.009). The three-way interaction between mCPP pretreatment, FLX pretreatment and phase was also significant ($F_{2, 110}$=8.45, p<0.0004). Challenge type (full-dose FLX or mCPP) was not significant as a main effect or in interaction with pretreatment. Within-group contrast testing demonstrated that all pretreatment groups had returned to baseline Persistence Index scores in the last four pretreatment days (all baseline vs pretreatment contrasts: p>0.2). Furthermore, within-group contrasts showed that the groups pretreated with saline, low-dose FLX + saline or low-dose mCPP + saline showed significantly increased Persistence Index scores during challenge with either full-dose FLX or mCPP, compared to their baseline scores ($F_{1, 55}$=20.60, 36.30 and 30.50, respectively, p<0.0001). In contrast, groups pretreated with combined low-dose FLX + mCPP sustained baseline persistence levels under high-dose challenge with either full-dose FLX or mCPP (baseline vs challenge: $F_{1, 55}$=1.38, p=0.246). Between-group comparison of the saline-treated controls and the groups receiving combined low-dose FLX + mCPP during the challenge phase ascertained that the combined pretreatment resulted in significant protection from the effects of challenge ($F_{1, 55}$=5.72, p<0.020). As shown in Fig. 2, neither low-dose mCPP nor FLX pretreatment alone offered similar protection.

Experiment 2: Effects of prolonged administration of quinpirole on directional persistence in the T-maze

Method

Chronic pharmacological treatment phase Following the baseline phase, 12 of the animals excluded from experiment

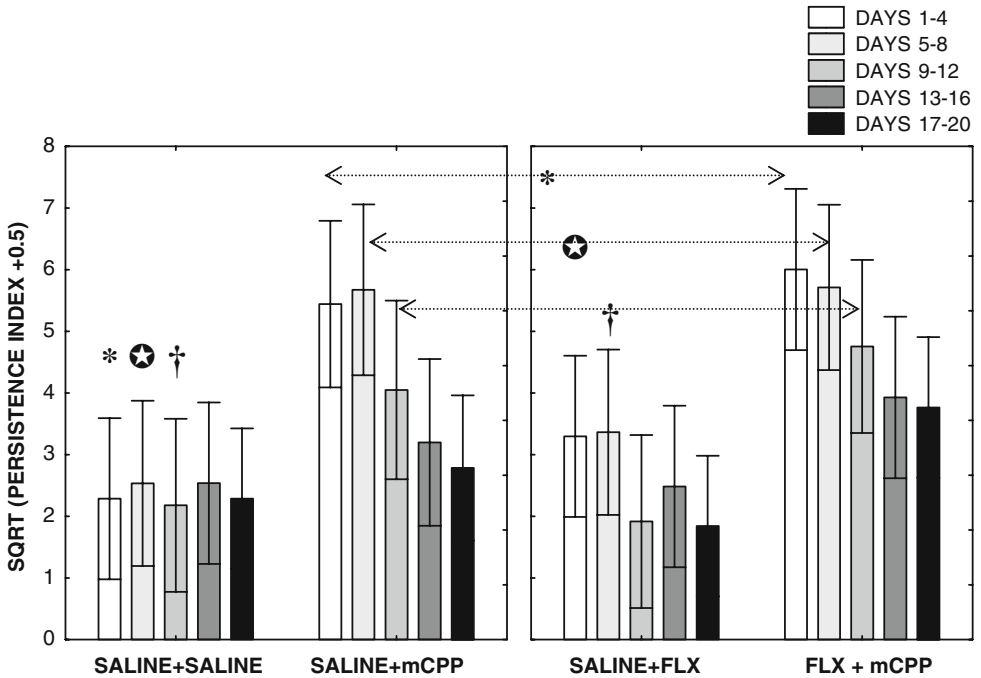


Fig. 1 From Experiment 1, effects of 20-day pretreatment with low-dose fluoxetine (FLX^+=2.5 mg/kg, FLX^- = saline), low-dose *m*-chlorophenylpiperazine ($mCPP^+$=0.50 mg/kg, $mCPP^-$ = saline) or combined low-dose FLX and mCPP (FLX^+=2.5 mg/kg, $mCPP^+$= 0.50 mg/kg) on directional persistence in T-maze rewarded alternation. Data are square-root-transformed Persistence Index scores (means and 95% confidence intervals are shown). The main effect of days (in 4-day blocks) was significant ($F_{4, 236}$=12.74, p<0.0001), as was the factor of mCPP pretreatment as a main effect ($F_{1, 59}$=14.54, p< 0.0003) and in interaction with days ($F_{4, 236}$=4.36, p<0.002). As shown by contrast testing, low-dose mCPP significantly increased persistence in the first three 4-day blocks of pretreatment ($F_{1, 59}$= 19.79 *, 16.49 ✪ and 11.10 †, respectively, p<0.0001). FLX pretreatment was not significant as a main effect or in interaction with mCPP

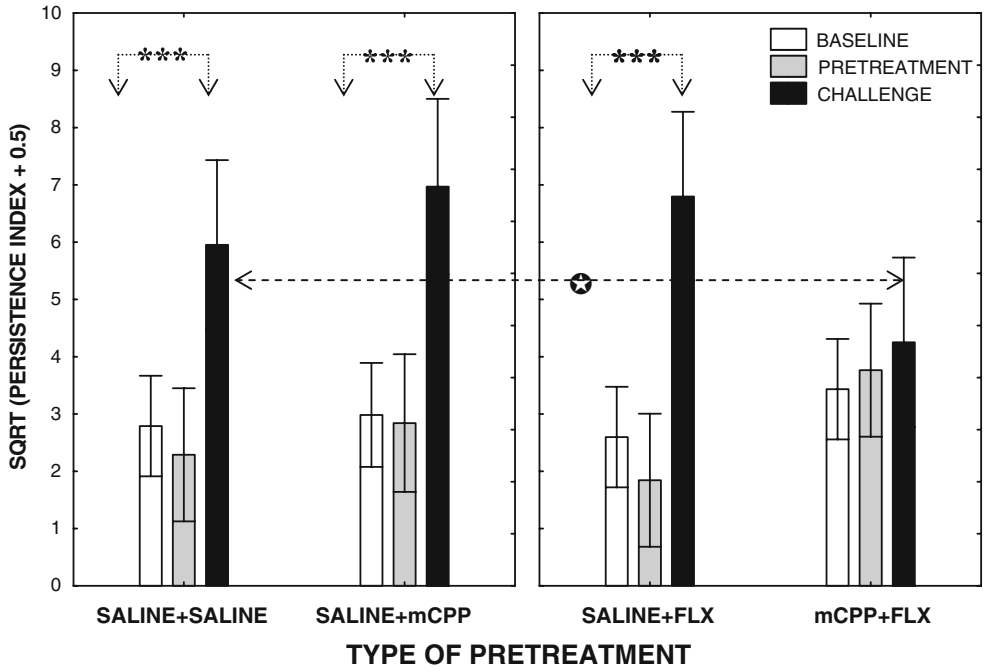


Fig. 2 From experiment 1, effects of fluoxetine (*FLX*) or *m*-chlorophenylpiperazine (*mCPP*) challenge (10 and 2.5 mg/kg, respectively) after chronic pretreatment (*ptx*) with saline, low-dose FLX (2.5 mg/kg) + saline, low-dose mCPP (0.50 mg/kg) + saline or combined low-dose FLX + mCPP on persistence in T-maze rewarded alternation. Data are square-root-transformed Persistence Index scores (means and 95% confidence intervals). Persistence was assessed through the phases of drug-free baseline (□, last 4 days), Pretreatment (▭, last 4 days) and challenge (■, 4 days). All pretreatment groups had recovered baseline Persistence Index scores in the last four pretreatment days. The main effect of phase was significant ($F_{2, 110}$= 64.48, p<0.0001), as were the mCPP ptx × phase interaction ($F_{2, 110}$= 4.91, p<0.009) and the mCPP ptx × FLX ptx × phase interaction ($F_{2, 110}$=8.45, p<0.0004). Challenge type (full-dose FLX or mCPP) was not significant as a main effect or in interaction with pretreatment. Within-group contrasts showed that the groups pretreated with saline, low-dose FLX + saline or low-dose mCPP + saline showed significantly increased persistence during challenge with either full-dose FLX or mCPP, compared to baseline ($F_{1, 55}$=20.60, 36.30 and 30.50, respectively, p<0.0001***). In contrast, groups pretreated with combined low-dose FLX + mCPP sustained baseline persistence levels under high-dose challenge with either substance (baseline vs challenge: $F_{1, 55}$=1.38, p=0.246). During challenge, these groups also showed significantly lower persistence than saline controls ($F_{1, 55}$= 5.72, p<0.020 ✪)

1 due to low spontaneous persistence received daily injections of quinpirole (quinpirole group) for 20 days, during which alternation training continued as in baseline. Given that the quinpirole group was run concurrently with experiment 1, its saline control group was drawn from the 16 saline-treated animals of that experiment. Specifically, the 12 saline animals exhibiting the lowest spontaneous persistence scores were used (saline control group). Therefore, the quinpirole group was compared to a saline group which initially during baseline (Fig. 3, days 1–20) had a higher mean spontaneous persistence score, although the two groups were indistinguishable in the latter part of the baseline phase (Fig. 3, days 20–40). It is therefore unlikely that any rate-dependent effects are operating here. Furthermore, this bias of (initial) higher spontaneous directional persistence in the control group is against our prediction that chronic quinpirole treatment should eventually increase directional persistence in the T-maze: a positive result would therefore be strengthened by this bias (design in Table 2).

Statistical analysis

Analyses were carried out by the STATISTICA for Windows statistical package (2008, version 6.1 StatSoft Inc., Tulsa, OK) and significant analysis of variance (ANOVA) effects were subjected to planned comparisons (contrast testing). Persistence Index scores were examined in 2-day blocks, in order to highlight the temporal pattern of the results. Square-root-transformed scores for the 40 baseline days (20 blocks) and the 20 days of drug administration (ten blocks) were subjected to separate one-way ANOVAs with repeated measures (independent variables: (a) drug treatment (saline vs. quinpirole) and 2-day blocks (repeated measures).

Results

Baseline phase The one-way ANOVA of baseline (20 2-day blocks) yielded a significant effect of days (F=5.67, df=19, 418, p<0.0001) and of (future) drug treatment (F=

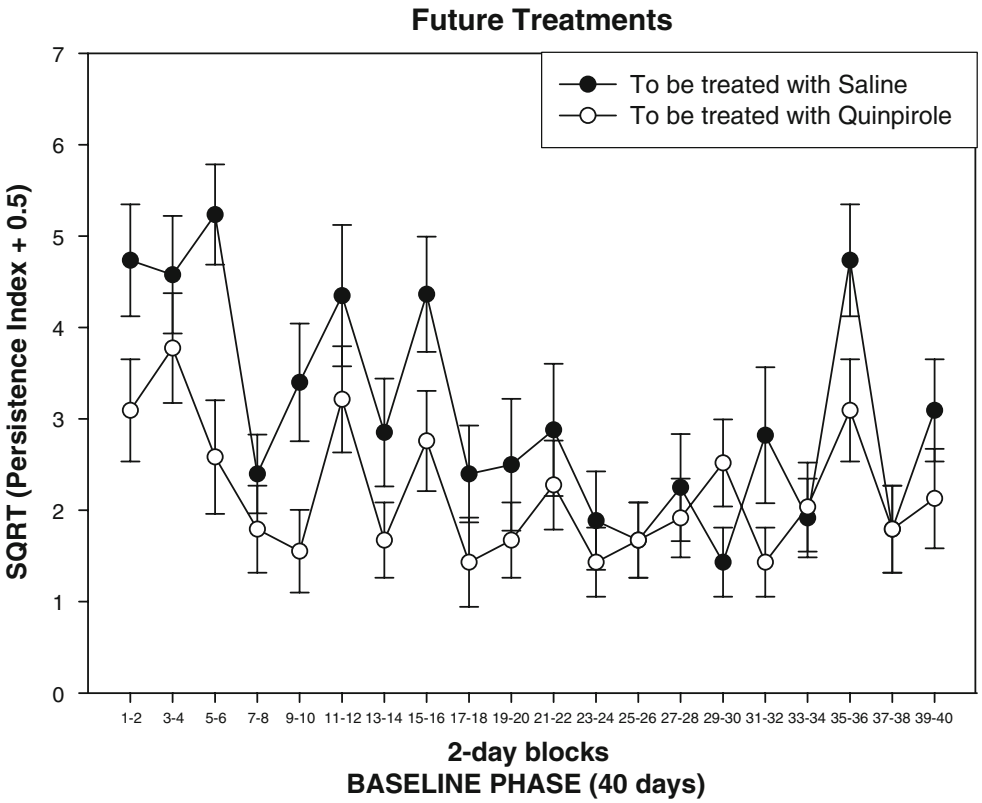
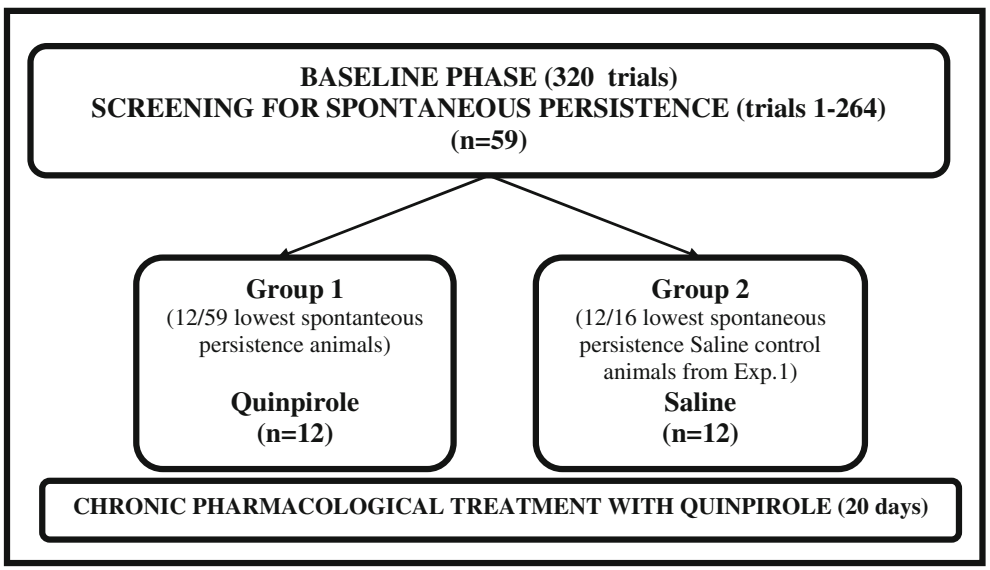


Fig. 3 From experiment 2, directional persistence during T-maze rewarded alternation through drug-free baseline training (40 days in 2-day blocks). Values are means and standard errors of square-root-transformed Persistence Index scores for 2-day blocks. The significant effect of days (F=5.67, df=19, 418, p<0.0001) and of (future) pharmacological treatment (F=10.5, df=1, 22, p=0.004) respectively reflect (a) a gradual dissipation of persistence as alternation training progresses and (b) overall lower spontaneous persistence in the quinpirole group than in the saline control group

10.5, df=1, 22, p=0.0038). As can be seen in Fig. 3, these differences reflect (a) a gradual dissipation of persistence as alternation training progressed and (b) lower spontaneous persistence in the quinpirole group, which was a result of the screening procedure (see Table 2). However, the future saline and quinpirole groups showed similar performance in the latter part of baseline (Fig. 3, days 20–40).

Chronic pharmacological treatment The one-way ANOVA of the pharmacological treatment phase × days (repeated

Table 2 Experiment 2, flow diagram of the experimental procedure



Quinpirole dose=0.5 mg/kg.

measure: ten 2-day blocks) yielded a significant main effect of days (F=2.78, df=9, 198, p=0.0043) and a significant days × pharmacological treatment interaction (F=2.28, df=9, 198, p=0.019). As can be seen in Fig. 4, this effect reflects a similar course of persistence scores in saline and quinpirole groups up to pharmacological treatment days 11–12, after which the quinpirole group showed increased persistence while saline values remained stable. Contrast testing showed that the two groups differed significantly on day blocks 13/14 and 19/20 (SAL vs. QUIN: F=6.2, p< 0.021 and F=12.57, p<0.0018 respectively, df=1, 22).

Discussion

Acute and chronic effects of low doses of fluoxetine and mCPP

In a previous study (Tsaltas et al. 2005), we reported a biphasic action of fluoxetine and mCPP (at doses of 10 and 2.5 mg/kg, respectively) on directional persistence in the rewarded alternation model of OCD. The effect consisted of an acute increase in persistence for the first week of administration. This increase gradually dissipated and persistence returned to pre-drug levels by administration days 13–15. We also reported cross-tolerance between these two substances: at the behaviourally effective ('high') doses

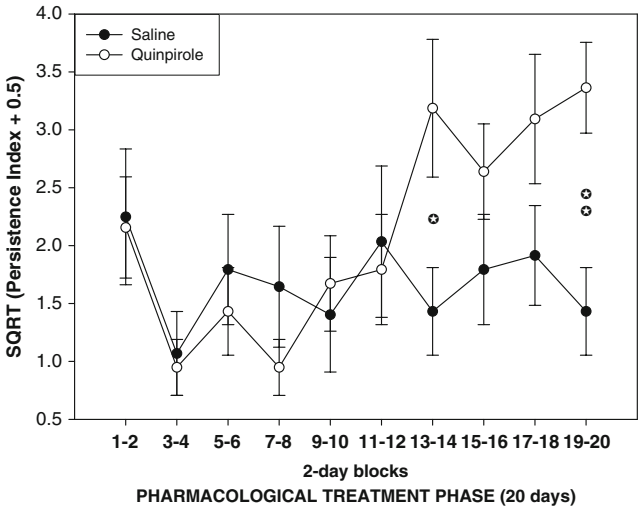


Fig. 4 From experiment 2, directional persistence during T-maze rewarded alternation through the pharmacological treatment phase (20 days in 2-day blocks). Values are means and standard errors of square-root-transformed Persistence Index scores for 2-day blocks. The significant days × pharmacological treatment interaction (F=5.12, df=9, 198, p=0.019) reflects increased persistence in quinpirole animals after treatment days 11–12 (contrasts, saline vs. quinpirole, day blocks 13/14 and 19/20, respectively: $F_{1,22}$=6.2, p<0.02 and $F_{1,22}$=12.57, p<0.002)

mentioned above, pretreatment with one substance offered protection from the acute, 'pathogenic' effect of the other. These results raise the possibility that the effects of fluoxetine and mCPP on persistence may be mediated by a common mechanism of action. Experiment 1 examined this hypothesis directly, by testing for synergy between low doses of fluoxetine and mCPP in the control of directional persistence. Our prediction was that chronic pretreatment with low doses of fluoxetine and mCPP (20–25% of the doses habitually used in the animal literature) would not offer protection against the pathogenic (persistence increasing) effect of acute challenge with high doses of either substance, whereas combined low doses of fluoxetine + mCPP would. The results of Experiment 1 are congruent with this prediction.

(a) The saline-pretreated animals sustained baseline levels of persistence throughout the pretreatment phase (Fig. 1). Subsequent challenge with high doses of either fluoxetine or mCPP produced a significant increase in persistence (Fig. 2). This acute effect of both the SSRI and the 5-HT receptor agonist constitutes a replication of our previous findings. From the point of view of congruence of this observation with the clinical literature, although some studies report no effect of mCPP on OCD symptoms (Charney et al. 1988; Goodman et al. 1995; Khanna et al. 2001), several others show symptom exacerbation in OCD patients after mCPP administration (Zohar et al. 1987; Pigott et al. 1991; Hollander et al. 1991, 1992; Broocks et al. 1998). Our finding concerning mCPP is in agreement with the latter studies. In contrast, symptom exacerbation after acute SSRI administration is not documented in the clinical literature. However, electrophysiological studies on the mechanism of action of the SSRIs show marked reduction in 5-HT neuron firing activity in the raphé dorsalis in the first days of SSRI administration, with partial recovery after 7 days and complete recovery of normal firing activity after 14 days of treatment (Chaput et al. 1986). These results, including their temporal pattern, are reminiscent of the acute, persistence-inducing effect of full-dose fluoxetine (10 mg/kg) and its gradual dissipation over 13–15 treatment days, which we observed in our model. The time course is compatible with the onset of emergence of SSRI therapeutic effects after 2–3 weeks of treatment and correlates with the desensitisation of 5-HT terminal autoreceptors and the resulting enhancement of 5-HT release observed in the orbitofrontal cortex with prolonged SSRI administration (Bergqvist et al. 1999). However, it is at odds with the reported significant enhancement of the evoked release of [3H]5-HT in the orbitofrontal

cortex after 8 weeks but not after 3 weeks of treatment in the guinea pig (El Mansari et al. 1995).

(b) The group pretreated with low dose of mCPP also displayed a transient increase in Persistence Index in the early part (days 1–8) of pretreatment phase (Fig. 1). This may indicate that the initial low dose we used (50% of the behaviourally effective, high dose) was not entirely without behavioural effect. For this reason, we further reduced the doses to 25% and 20% of the high dose for fluoxetine and mCPP on pretreatment day 4 (See "Materials and methods", "Drugs used"): greater dose decrease for mCPP was dictated by its more pronounced behavioural effect during pretreatment. Even so, the lowered dose of mCPP sustained significant persistence increase for a further 5 days. It is noteworthy that, in spite of the significant, transient persistence increase induced by low mCPP, this pretreatment failed to protect from high-dose challenge with either mCPP or fluoxetine (Fig. 2). This finding suggests that the acute increase and subsequent restoration of persistence levels during pretreatment is not a sufficient predictor of future 'treatment effectiveness' as we have earlier suggested (Tsaltas et al. 2005), although it may be a necessary one.

(c) The groups receiving the combination of low doses of fluoxetine and mCPP also showed a transient increase in persistence during pretreatment. This was more prolonged than the low mCPP pretreatment effect, remaining significant for a further 4 days (days 1–12, Fig. 1). In contrast to the saline and the low-dose pretreatment groups, the combined pretreatment animals demonstrated complete protection from high-dose challenge with either mCPP or fluoxetine. During the challenge phase, their persistence scores differed neither from their baseline nor from their late pretreatment scores (Fig. 2). Therefore, low doses of fluoxetine or mCPP which do not protect from high-dose challenge of mCPP or fluoxetine do synergise to offer protection corresponding to that achieved with high-dose pretreatment with either substance (Tsaltas et al. 2005).

Our earlier results (Tsaltas et al. 2005) have shown cross-tolerance between fluoxetine and mCPP on directional persistence, as well as non-involvement of the 5-HT1D receptor in this effect. The current results indicate synergy between the two substances. In combination, the two findings suggest a common route of action of fluoxetine and mCPP in the control of persistence in the rewarded alternation model. Given that mCPP is a non-selective 5-HT 2, 1D, 1A agonist, the possibility of 5-HT1A receptor involvement in our results cannot be excluded on the basis of present data. However, since mCPP does show preference for 5-HT2C receptors (Barnes and Sharp 1999), our results lend some support to the hypothesis that the SSRIs affect OCD symptoms by enhancing 5-HT transmission at 5-HT2 receptors, possibly in the orbitofrontal cortex (Blier et al. 2000). This possibility is currently under investigation in our laboratory, through use of specific 5HT2 receptor antagonists.

The extent to which our results can be extrapolated to OCD pathophysiology is of course subject to the limitation touching upon all extant OCD animal models, with the exception of the neuroethological ones (Rapoport et al. 1992; Nurnberg et al. 1997). Namely, model-produced data relate to persistence induced either by a pharmacological or by a behavioural challenge (usually frustration), whereas OCD patients show spontaneous symptomatology. In defence of the reinforced alternation model, it must be noted that, while it is expedient to use it in the capacity of pharmacologically induced persistence as we did here (used as a screening procedure, the model detects a 2–3% of rat samples screened as training-resistant 'persisters'), it in fact refers to spontaneously persistent behaviour which is rendered latent (as a tendency) after extensive alternation training, but readily re-emerges in response to mCPP administration (Tsaltas et al. 2005), as happens in OCD patients (Zohar et al. 1997, Hollander et al. 1992).

With the above reservations in mind, we will venture to note that our findings suggest a possible therapeutic role of 5-HT agonists. Although this proposition may appear controversial given that our own data and clinical reports show that mCPP administration can increase persistence–OCD symptoms, it must be noted that we observed this adverse effect after acute administration only, while clinical studies by necessity also administer mCPP acutely only. In contrast, we raise the possibility of therapeutic usefulness of mCPP (or more selective 5-HT2 agonists with fewer adverse side effects) in the context of prolonged, low-dose administration, as an adjunct to SSRI treatment. This proposition is congruent with the findings of Martin et al. (1998), who reported that 5-HT2 agonists improved persistent behaviour in a number of animal models of OCD. It is also consistent with the reported beneficial effects of hallucinogens with 5-HT2A–2C agonist properties (Delgado 2000) and mCPP (Pigott et al. 1992a) on obsessive–compulsive symptoms. To our knowledge, there are no data available on the use of 5-HT2 agonists as an add-on to SSRI treatment. The combination may deserve consideration, given that other add-on treatments based on the enhancement of serotonergic transmission, such as the addition of clonazepam, buspirone, *l*-tryptophan or fenfluramine to ongoing SSRI treatment, have not yet been conclusively evaluated (McDonough and Kennedy 2002).

Acute and chronic effects of quinpirole on the rewarded alternation model

Experiment 2 explored the possibility of a dopaminergic contribution to the mediation of persistence in the rewarded alternation model, by examining the acute and chronic effects of the $D_{2,3}$ agonist quinpirole. Our prediction was that chronic but not acute administration of quinpirole at a dose which has been shown to produce persistent behaviour without stereotypy in another animal model of OCD (Szechtman et al. 1998, 2001) should increase Persistence Index scores in the rewarded alternation model. As mentioned before (see "Materials and methods", "Statistical analysis"), the saline control group of this experiment was drawn from that of Experiment 1. As a result, it had higher spontaneous Persistence Index scores than the quinpirole group during the early part of baseline, although their scores converged after baseline days 20–21: rate-dependent effects are therefore unlikely (Fig. 3). Another issue raised by this initial difference between the quinpirole and saline groups is that the quinpirole animals might have undergone overtraining compared to the controls. To rule out this possibility, we compared the days to criterion for the two groups. There was no statistical difference between them ($F_{1,26}=2.53$, $p=0.12$), which shows that the two groups underwent the same amount of overtraining.

Despite the baseline differences in an unfavourable direction with respect to our predictions, chronic treatment with the drug significantly increased Persistence Index scores over those of the control group. During drug treatment, saline and quinpirole groups sustained comparable levels of persistence up until treatment days 11/12 (Fig. 4). Thereafter, the Quinpirole group showed an ascending trend in Persistence Index scores and differed significantly from the saline group on days 13/14 and 19/20.

These findings confirm our prediction that chronic, but not acute, quinpirole administration will increase compulsive behaviour as measured by the rewarded alternation model. It also confirms that the 'compulsive' behaviour of our model (and its Persistence Index) is sensitive not only to serotonergic (Tsaltas et al. 2005) but also to dopaminergic manipulations. Given that quinpirole is a $D_{2,3}$ agonist, the dopaminergic contribution to persistent behaviour in our model appears to involve either the D_2 or the D_3 receptor, or both.

Although several studies support a role for dopamine in OCD pathophysiology (Goodman et al. 1990b; Marazziti et al. 1992; McDougle et al. 1994a, b), there is only indirect evidence concerning specific receptor mediation of this contribution. Recent data implicate both the D_2 and the D_3 receptor, as well as the D_1 receptor. Clinical support for a D_2 receptor involvement in OCD pathophysiology stems from the finding that clomipramine possesses significant D_2 receptor blocking activity (Austin et al. 1991), as well as from the successful addition of D_2 receptor antagonists to SSRI treatment in refractory OCD patients (McDougle et al. 1994b). Additionally, Brambilla et al. (1997) attribute the blunted growth hormone response to apomorphine stimulation in OCD patients to subsensitive D_2 receptors. Finally, there is the observation that quinpirole causes compulsive checking behaviour (Szechtman et al. 1998, 2001; Tizabi et al. 2002; Eilam and Szechtman 2005). Sullivan et al. (1998) proposed that increased dopaminergic activity produced by quinpirole sensitisation results in a suppression of basal ganglia function. This latter effect, along with excessive cortical stimulation, has been suggested to underlie with compulsive behaviour (Modell et al. 1989). Our findings in experiment 2 are congruent with this view.

The possibility of D_3 receptor contribution to persistent behaviour cannot be excluded on the basis of the Szechtman et al. (1998, 2001) results or of our own from experiment 2. This receptor has also been implicated in OCD pathogenesis on the basis of an apparent genetic relationship between OCD and Tourette's syndrome (Pauls et al. 1986): Tourette's syndrome has been associated with a polymorphism in the dopamine D_3 receptor gene. However, after assessing the frequency of this polymorphism in OCD patients and controls, Catalano et al. (1994) concluded that there is no association between OCD and the D_3 receptor gene. This finding was recently supported by Billett et al. (1998) who, alternatively, proposed an association of OCD with the D_4 receptor gene. In conclusion, although the possibility of D_3 receptor contribution to directional persistence in our model and to OCD pathogenesis cannot be excluded at this point, it does not seem to be very likely. The issue can be conclusively explored by testing the effects of a specific D_3 antagonist on quinpirole-induced persistent behaviour.

Finally, some evidence also implicates the D_1 receptor in OCD pathophysiology (Campbell et al. 1999, Joel and Dolijansky 2003). Although involvement of this receptor is theoretically plausible (Saxena et al. 1998), the only clinical study using clozapine, an atypical antipsychotic with D_1 blocking properties, failed to provide support for this hypothesis (McDougle et al. 1995).

In summary, the results of experiment 1 demonstrate a synergistic action of mCPP and fluoxetine. In the light of previous data (Martin et al. 1998; Marek et al. 2005), this finding suggests 5HT2 contribution in the therapeutic mechanism of action of the SSRIs. This hypothesis is currently being tested by use of specific 5HT2 receptor antagonists in our model.

The findings of experiment 2, in accord with other preclinical studies and in combination with data available

on D_1 and D_3 receptor involvement in OCD pathogenesis, suggest a major role for the $D_{2/3}$ receptor.

Considered together, the results reported here suggest that the persistence behaviour of the rewarded alternation model is sensitive to both serotonergic and dopaminergic manipulation. The model therefore appears to offer a useful tool for further exploring the serotonin–dopamine interaction hypothesis of OCD pathogenesis (Goodman et al. 1990b; Stahl 1998; Micallef and Blin 2001), possibly through the investigation of the effects of specific dopamine antagonists on quinpirole-induced persistence behaviour. Further research is necessary to establish beyond doubt that the persistence noted after acute mCPP is the same phenomenon as persistence induced by chronic quinpirole. Ongoing work in our laboratory focusses on the direct exploration of SSRI effects on quinpirole-induced persistence.

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