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ORIGINAL INVESTIGATION

The influence of cost manipulation on water contrafreeloading induced by repeated exposure to quinpirole in the rat

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Abstract
Rationale Quinpirole (QNP), a D2/D3 dopaminergic receptor agonist, was found to elicit an apparently antieconomical drinking behavior called contrafreeloading (CFL). The perseverative operant responding observed may represent a compulsive-like behavior prompted by sensitization to the effects of QNP.
Objectives In the present study, we investigated the effect of different response costs on instrumental behavior and CFL in rats repeatedly treated with QNP (0.5 mg/kg i.p.). Moreover, we studied the consummatory components of ingestive behavior in no-choice paradigms and the role of learned operant conditioning in free drinking.
Materials and methods In experiment 1, rats were trained to perform under three different fixed ratio schedules of reinforcement (FR1, FR3, and FR10) and were given a choice between operant and free access to water. In experiment 2, rats were divided into four groups, each one resembling experiment 1 in one or more features, with no choice available and water consumption measured at an interval of 0–60 min.
Results (a) Increasing FR significantly reduced CFL % in saline—but not in QNP-injected groups; (b) under free-drinking conditions, QNP caused a progressive hypodipsic effect which was, however, contrasted by maintaining cues formerly contingent on operant access to water; and (c) under CFL conditions QNP-treated rats drank more than under free access conditions.
Conclusions QNP confers rigidity in responding for water, impeding adaptation to different contingencies for access to the resource. In QNP-treated rats, CFL behavior appears adaptive as far as it allows animals to partially circumvent the hypodipsic effect of the drug.

Keywords Quinpirole · Contrafreeloading · Water intake · Operant conditioning behavior

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Introduction

Contrafreeloading (CFL) is a behavioral strategy where animals continue to respond for a reward in an operant setting even after the same reward becomes available at no cost. This behavior seems to dispute the principle of selective preference for a response requiring the least effort. However, CFL is considered adaptive as far as it allows the animal to improve and update its foraging strategies in an uncertain environment (Inglis et al. 1997), a notion supported by the repeated observation that domestication reduces CFL performance (Schütz and Jensen 2001; Lindqvist et al. 2002, 2006). Although CFL is usually studied under conditions of access to food, we find that this behavioral strategy can also be observed in rats searching for water (Cioli et al. 2000). In particular, when mildly thirsty rats, kept on an FR3 schedule of access to water had contingent free access to the resource, they showed an initial 15–20% of CFL (the percent fraction of total fluid intake obtained via operant conditioning behavior), which then asymptotically declined across 9 days of observation. We found that, under such conditions, chronic administration of quinpirole (QNP), a D2/D3 dopaminergic receptor agonist, caused a spectacular increase in the CFL rate, eventually accounting for about 80% of total water intake

(Cioli et al. 2000; Amato et al. 2006, 2007). It should be noted, however, that QNP-induced CFL differed from spontaneous behavior in that the animals consumed only a portion of the water available; thus, the increase in the CFL rate depended on the steep decline of free water intake from the bottle and, in fact, the net effect was a progressive reduction of total water intake. Therefore, this QNP-induced behavior appears to be a complex phenomenon where exaggerated responding coexists with dissociation between the appetitive and consummatory components of water-reinforced behavior. It is not surprising that under our experimental conditions, QNP induced exaggerated responding in that the drug increased the excessive lever pressing obtained by post-training signal attenuation (Joel et al. 2001), elicited perseverative operant responding in the absence of reward (Kurylo 2004), caused rats trained to respond for food to remain focused on the response lever throughout the operant conditioning session (Bratcher et al. 2005), and induced repetitive checking of specific places (Szechtman et al. 1998). From an economic point of view, QNP-induced CFL appears quite dissipative, since under these conditions the animals work for a resource that they only partially use and which, in any case, they can have for free. Perseverative and dissipative are terms recurrent in the literature regarding obsessive–compulsive disorder and thus QNP-induced CFL may belong to the above-mentioned set of compulsive-like behaviors disclosed in different experimental settings by repeated administration of the drug (Szechtman and Woody 2004).

However, several aspects of QNP-induced CFL remain to be explored. First, we studied CFL under conditions of a single fixed ratio schedule of reinforcement (FR3). CFL for food has been found elastic, since responding markedly decreased as the ratio requirement increased (Rutter and Nevin 1990), but prolonged stimulation of dopaminergic D2/D3 receptors is expected to affect this spontaneous pattern of responding. Salamone et al. (2001, 2002) extensively examined the effect on food-reinforced behavior after striatal DA depletion or pharmacological inhibition in rats under different fixed ratio schedules of reinforcement, demonstrating that, while performance on a minimal work requirement was unaffected by these manipulations, instrumental lever pressing became sensitive to more costly schedules. On this basis, we foresee that, on the contrary, repeated stimulation of dopaminergic D2/D3 receptors by QNP confers inelasticity to allocation of instrumental responding.

Second, it is surprising that under QNP exposure, the increase of CFL rate is combined with a remarkable reduction in total water intake. We repeatedly found that daily administration of the same dose of QNP in normally hydrated rats produced a progressive increment in free water intake measured at 2, 5, and 24 h, with respect to controls (Fraioli et al. 1997; Cioli et al. 2000; Badiani et al. 2002). However, no data about the amount of water intake 1 h after QNP administration are available, but taking into account the hypodipsic effects of other dopaminergic agents (Carr and White 1986; Foltin et al. 1983; Wellman et al. 1982; Dourish and Cooper 1981; Stolerman and D'Mello 1978; Nielsen and Lyon 1973), we assume that this effect is shared by QNP. Hence, it is even possible that CFL protects animals from a possible initial hypodipsic effect of the drug.

Therefore, this study reports the results of two different experiments. The first experiment aimed to examine the influence of behavioral cost on QNP-induced CFL by adopting different fixed ratio schedules of reinforcement. The second experiment was conceived to examine two key points: (1) the effect of repeated administration of QNP on free water intake from a bottle measured 1 h after injection and (2) the role of learned operant conditioning on free drinking in no-choice paradigms.

Materials and methods

Animals

This study was performed using 140 male Sprague–Dawley rats (Harlan Nossan, Correzzana, MI, Italy) weighing approximately 150–175 g at the start of the experiment. The rats were individually housed at 23°C under a 12-h light/12-h dark cycle (lights on at 7:00 A.M.). During the first week, the animals had free access to food and water (standard rat diet, Harlan, Italy).

Apparatus

In this study, we used a set of operant conditioning chambers made of Plexiglas and stainless steel (28.5 cm long, 27 cm wide and 28 cm high). Each cage was equipped with a lever-controlled retractable dipper dispensing 0.1 mL of water. The dipper trough was located on the left wall of the cage, 1 cm above the floor. Two levers (Ralph Gerbrands) were mounted on the left and right of the dipper trough, 9 cm above the floor. A bottle was mounted on the front wall of the cage, either empty or filled with water, depending on the experimental conditions. The operant conditioning chambers were illuminated during the experimental sessions by a 6-W ceiling light. Each cage was located in a cubicle equipped with an exhaust fan. Custom-made software controlled the stimulus events and recorded the lever presses.

Experiment 1

Seventy-six rats were used in this experiment. To elicit a motivational state towards water, the animals were water-restricted during both the training and testing phases. Thus,

they were given free access to water for only 5 min a day in their home cage at the end of each session, with food available ad libitum.

The animals were initially trained to press the left lever for 0.1 mL of water on a fixed ratio 1 (FR1) schedule of reinforcement. Each training session lasted 20 min and was preceded by a 1-min pre-session with lights off. The sessions were conducted 7 days a week during the light phase (between 9:00 A.M. and 3:00 P.M.). Pressing on the inactive lever was taken as a measure of non-goal-directed behavioral activation. Rats were then randomly assigned to three groups. The first group was maintained on FR1, while the second and third were progressively shifted to FR3 and FR10 schedules of reinforcement, respectively. The training phase was considered over when a standard performance criterion (low inter-session variability) was achieved by all animals on the three different fixed ratios. One rat was excluded due to high inter-session responding variability.

The rats then underwent three baseline sessions on three consecutive days. Immediately before each of these 1-h sessions, all rats received an intraperitoneal (i.p.) injection of vehicle. After the establishment of baseline responding, each animal was randomly assigned to one of the two groups receiving either vehicle or 0.5 mg/kg QNP i.p. Thus, six groups were included in the experiment (vehicle FR1, N=12; vehicle FR3, N=12; vehicle FR10, N=12; QNP FR1, N=13; QNP FR3, N=12; QNP FR10, N=14), which lasted 15 days. All test sessions: (a) were conducted between 9:00 A.M. and 3:00 P.M., (b) consisted of one for each test day, and (c) lasted 60 min preceded by a 1-min pre-session with lights off. All rats were treated immediately before entering the operant conditioning chambers for the test session. On days 1–6 (operant conditioning phase), water was only available through lever pressing, while the bottle remained empty. On days 7–15 (choice phase), the bottle was filled with tap water available at no behavioral cost. Therefore, on these days, animals had access to two sources of water simultaneously. The amount of water ingested by the animals was measured by weighing the water receptacle and the bottle before and after each session.

Experiment 2

In this experiment, lasting 15 days, 64 rats were assigned to four groups: (1) *no-operant A* (N=16), (2) *no-operant B* (N=16), (3) *operant a* (N=16), and (4) *operant b* (N=16). Rats in the first two groups were not trained to lever press for water (non-operant), therefore the bottle was the only water source available in the test cage and was filled with tap water on days 1–15 (group no-operant A) and 7–15 (group no-operant B), respectively. Rats in the remaining two groups were instead trained to perform under an FR3 schedule of reinforcement (operant) to obtain water from the dipper. The bottle remained empty on days 1–6 and was filled with tap water on days 7–15. However, this phase (days 7–15) differed from experiment 1 in that the levers were either made inactive (group operant a) or the water was removed from the dipper receptacle (group operant b) beginning on day 7. This manipulation of the experimental conditions was introduced to evaluate the possible role of conditioned stimuli (such as the sound of the upcoming dipper) on the perseveration of operant conditioning behavior, since the reward was no longer available. A major difference from experiment 1 was that experiment 2 did not present a choice paradigm in any of the four groups. The lever presses of both groups were recorded. The light in the operant conditioning chambers remained on throughout the 15 daily sessions.

To produce a motivational state towards water, all animals were water-restricted throughout the experiment. Thus, in addition to the water obtained during the sessions, rats were given free access to water for 5 min a day, after being returned to their home cage at the end of the session. Group no-operant B, which did not have any access to water in the test cage until day 7, received an extra amount of water in the evening in the home cage, where food was available ad libitum.

All rats underwent three baseline sessions on three consecutive days, receiving an i.p. injection of vehicle immediately before entering the test cage. Group no-operant A had the bottle filled with water only on day 3 of baseline. Within each group, half the rats were assigned to i.p. vehicle treatment and the other to i.p. 0.5 mg/kg QNP treatment. Rats received the treatment immediately before entering the test cage. All test sessions (a) were conducted between 9:00 A.M. and 3:00 P.M. and (b) lasted 60 min preceded by a 1-min pre-session with lights off.

Table 1 summarizes and compares the four different experimental conditions.

Drugs

(−)-Quinpirole HCl, freshly dissolved in distilled water to a final volume of 1 mL/kg, was supplied by Research Biochemical International (Natick, MA, USA).

Data analysis

Baseline data (Fig. 1, triangles) were obtained by averaging the data over three baseline sessions for each animal. CFL data (Figs. 1 and 2b) indicate the percent fraction of total fluid intake obtained via operant conditioning behavior (i.e., dipper water intake / total water intake × 100). The data illustrated in Figs. 2 and 4 were analyzed using two-way analysis of variance (ANOVA) with one between-subject (treatment) and one within-subject factor (test session). Figure 1 shows data analyzed using three-way ANOVA with (treatment × FR × session).

Table 1 Summary of the experimental conditions relative to the four groups used in experiment 2

Experiment 2—15 days				
Groups/days	1–6		7–15	
	Operant	Bottle	Operant	Bottle
No-operant A	No	Yes	No	Yes
No-operant B	No	No	No	Yes
Operant a	Yes	No	No	Yes
Operant b	Yes	No	Yes (no reward delivery)	Yes

Operant stands for the possibility to gain water through operant conditioning behavior, *Bottle* means that the bottle inside the test cage is filled with water freely available

Results

Experiment 1

The animals remained healthy throughout the experiment. However, as already observed under conditions of free access to food and water (Fraioli et al. 1997), QNP-treated animals showed a reduced ponderal increment with respect to controls [+13.41±5.6 vs +75.39±3.7 (g ± SEM) from baseline to day 14].

Figure 1 summarizes the results of the entire experiment as a function of test session.

Operant phase (days 1–6)

Responding and water intake of vehicle-treated rats remained stable across the six daily sessions. Conversely, as already described in our previous works (Cioli et al. 2000; Amato et al. 2006, 2007), the first administration of QNP caused a complete suppression of responding in all treated animals. Lever pressing gradually recovered in the following sessions, but the number of rewards gained did not reach control levels in any FR group (ANOVA days 1–6: treatment, $F_{1,69}=13.63$, $P=0.0004$; session, $F_{5,365}=8.62$, $P<0.0001$; FR, $F_{2,69}=823.71$, $P<0.0001$; treatment × session, $F_{8,552}=15.48$, $P<0.0001$; treatment × FR, $P>0.05$). Moreover, QNP-treated rats did not consume all the water obtained, and thus, drinking appeared even more reduced than responding (ANOVA days 1–6: treatment, $F_{1,69}=971.77$, $P<0.0001$; session, $F_{5,345}=15.41$, $P<0.0001$; FR, $F_{2,69}=10.31$, $P=0.0001$; treatment × session, $F_{5,345}=1.18$, $P=0.31$; treatment × FR, $P>0.05$). The FR schedule of reinforcement statistically affected both rewards obtained and water intake, and data inspection clearly shows that these effects depended on the remarkable reduction in the number of rewards obtained by QNP-treated rats responding for water, according to an FR10 schedule of reinforcement.

Choice phase (days 7–15)

Once the bottle was filled with water, vehicle-treated rats shifted their attention to the freely available source. As a consequence, lever pressing and water intake from the dipper progressively declined throughout the nine test sessions, in a way that again depended on FR (ANOVA days 7–15: treatment, $F_{1,69}=1.54$, $P=0.21$; session, $F_{8,552}=0.92$, $P=0.49$; FR, $F_{2,69}=18.82$, $P<0.0001$; treatment × session, $F_{8,552}=15.71$, $P<0.0001$; treatment × FR, $P>0.05$). Thus, on day 15 only seven of the 12 rats in the FR10 group were still responding for water, and their CFL % accounted for as low as 3–4% of total water intake (Table 2).

As expected, treatment with QNP had a strong effect on these choice patterns. Initially, rats preferred to drink from the bottle, but thereafter the amount of water consumed from this source progressively declined in all FR groups to less than 2 g on day 15 (ANOVA days 7–15: treatment, $F_{1,69}=238.53$, $P<0.0001$; session, $F_{8,552}=28.42$, $P<0.0001$; FR, $F_{2,69}=16.17$, $P<0.0001$; treatment × session, $F_{8,552}=36.83$, $P<0.0001$; treatment × FR, $P>0.05$). Lever pressing was remarkably depressed on day 7, and in the FR10 group, only four of 14 animals actually gained at least one reward throughout the session. However, responding progressively increased till reaching, on day 15, the same level seen on day 6 of the operant phase for each FR group (Fig. 3). While control animals on days 7–15 progressively cut down to half the number of rewards gained, QNP-treated rats, in the same phase, doubled the number of rewards obtained (ANOVA days 7–15: treatment, $F_{1,73}=8.15$, $P=0.005$; session, $F_{8,552}=3.51$, $P=0.0006$; FR, $F_{2,69}=20.72$, $P<0.0001$; treatment × session, $F_{8,552}=15.48$, $P<0.0001$; treatment × FR, $P>0.05$) (Fig. 2a). As Table 2 shows, many more animals responded with respect to day 7 at FR10 (ten of 14), and the number of rewards gained was remarkably higher than in the respective vehicle-injected groups.

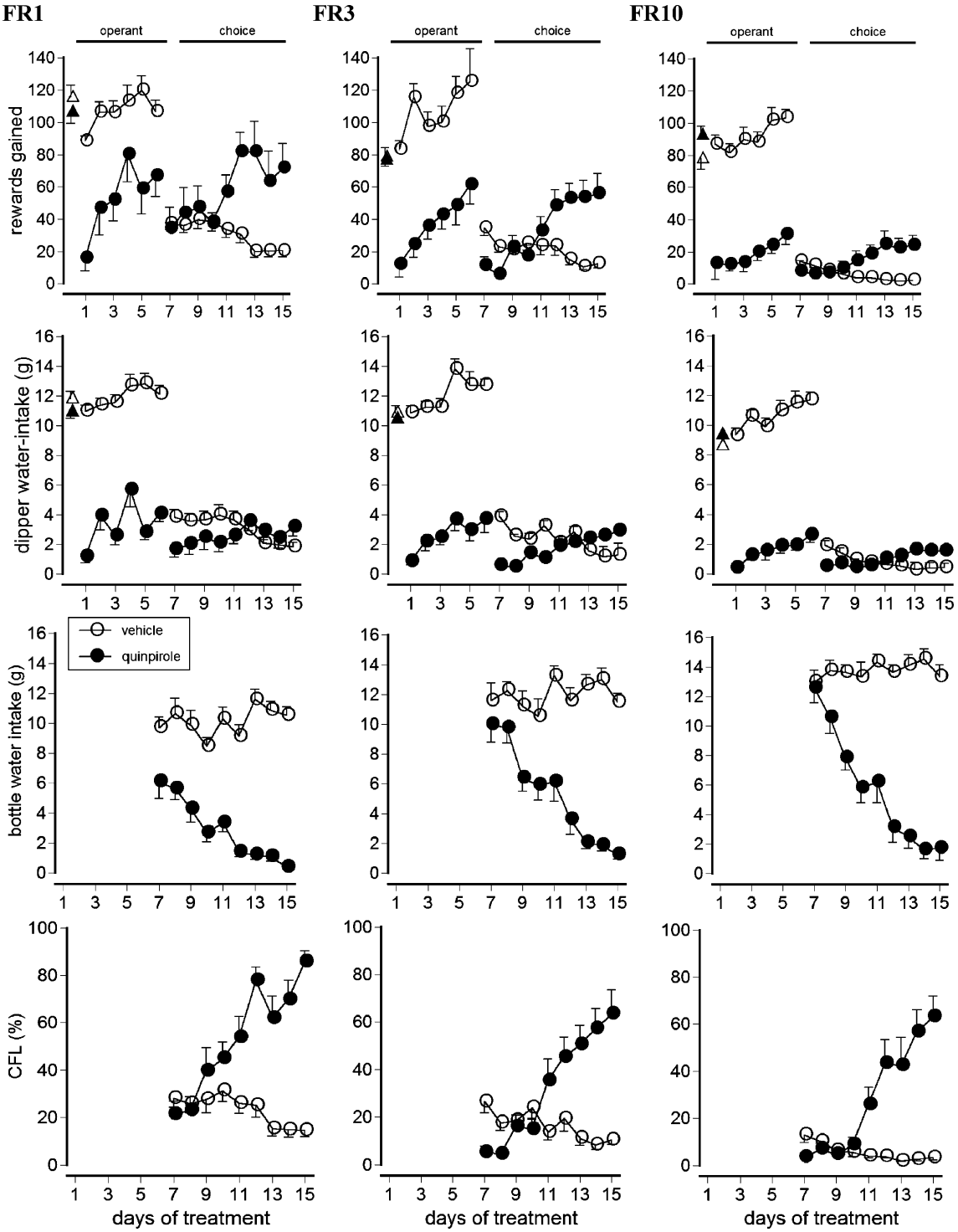

Fig. 1 Experiment 1. Effects of 15 daily i.p. injections of either vehicle or 0.5 mg/kg QNP administered immediately before a 60-min operant conditioning session (days 1–6) or a choice session (days 7–15). Rats were trained to perform under three FR schedules of reinforcement: FR1 (*left column*), FR3 (*middle column*) and FR10 (*right column*). Shown are the means ± SEM of rewards gained by lever pressing (*first row*), water intake from dipper (*second row*), water intake from bottle (*third row*), and % CFL (*fourth row*). *Triangles* illustrate baseline values

As a result, CFL increased throughout the 9 days, although in a way that was inversely related to the FR (ANOVA days 7–15: treatment, $F_{1,69}$=64.67, P<0.0001; session, $F_{8,552}$=20.33, P<0.0001; FR, $F_{2,69}$=19.28, P<0.0001; treatment × session, $F_{8,552}$=44.02, P<0.0001; treatment × FR, P>0.05). Indeed, among the three groups, rats trained on FR1 schedule exhibited a higher % of CFL (85% on day 15), while no differences appeared between the FR3 and FR10 groups

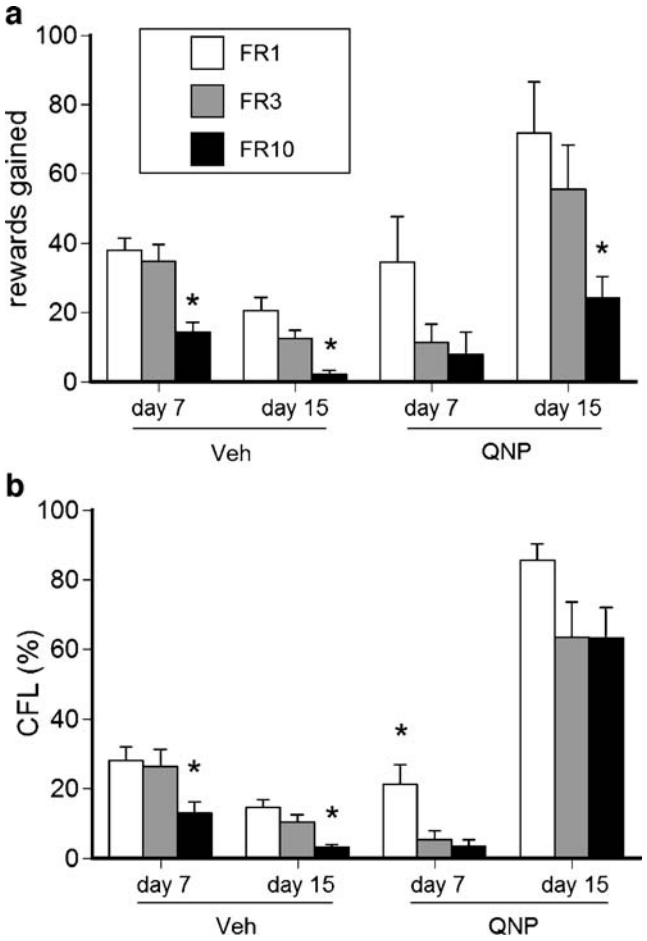

Fig. 2 Experiment 1. Effect of repeated i.p. injections of either vehicle or 0.5 mg/kg QNP on rewards gained (**a**) and CFL % (**b**) measured on the first and last days of choice phase (days 7 and 15, respectively). Shown are the means ± SEM. Statistical analysis revealed a significant effect of FR schedule on the number of rewards gained on days 7 ($P<0.05$) and 15 ($P=0.001$). Treatment had a significant effect on day 15 ($P<0.0001$), but no interaction with FR was found. FR and treatment produced a significant effect on CFL % on both days 7 (Ps<0.001) and 15 ($P<0.05$; $P<0.0001$), with no interaction effect. *Asterisks* ($P<0.01$) represent post hoc analysis within groups

(~63% on day 15) (Fig. 2b). However, when non-responding animals were excluded from statistical analysis, there were no more differences between FR groups on day 15 (Table 2).

Although animals persisted in performing instrumental behavior, they ingested only a fraction of the rewards delivered (50–60% on day 15). During the 9 days of choice, total water intake remained stable across the three vehicle-treated groups, while QNP-treated rats progressively decreased their intake, irrespective of the FR. On day 15, water intake was depressed to about 4 g in all QNP groups.

Experiment 2

The animals remained healthy throughout the experiment. Controls, but not QNP-treated animals, increased their body weight from baseline to day 14 [+63.01±2.3 vs −5.81±3.33 (g ± SEM)]. This experiment was focused on drinking behavior from the bottle during the first 60 min after drug administration. The four test conditions were modeled to resemble the procedures of experiment 1. Fig. 4 reports all data collected on bottle water intake.

Group no-operant A had free access to the bottle filled with water from day 1. Relative to controls, whose drinking remained high and stable throughout the 15 days, water intake of QNP-treated rats was already more than halved on the first day, and gradually decreased in the following sessions (ANOVA days 1–15: treatment, $F_{1,14}=612.6$, $P<0.0001$; session, $F_{14,196}=13.74$, $P<0.0001$; treatment × session, $F_{14,196}=5.02$, $P<0.0001$). On day 6, water consumption was practically nil and did not recover over the remaining 9 days.

Group no-operant B differed slightly from the previous one: the bottle was left empty on days 1–6, then filled with water on days 7–15. However, rats were treated with QNP from day 1, as in the previous group. Again, QNP had a dramatic effect on water intake, which was high on day 7, progressively declining to less than 1/2 on day 10 and 1/3 on day 11, and was no higher than 3 g on the remaining days (ANOVA days 7–15: treatment, $F_{1,14}=44.97$, $P<0.0001$; sessions, $F_{8,112}=16.91$, $P<0.0001$; treatment × sessions, $F_{8,112}=3.82$, $P=0.0005$).

In groups operant a and b (Fig. 4b), rats trained to lever press for water on an FR3 schedule of reinforcement were included in these two groups. They both underwent a first operant phase (days 1–6), which was identical to that adopted in experiment 1. Differences in lever pressing and water intake (Fig. 4b, inset) between vehicle and QNP-treated rats of both groups were statistically significant and comparable to the results obtained in the same phase of the first experiment.

On the following 9 days, levers were inactivated for group operant a, so that no reward was delivered, whereas group operant b had active levers but water was removed from the receptacle, so that no reward was available with contingent stimuli maintained (clicks of the dipper when activated). In both cases, the bottle was filled with water. In this context, there was no difference in instrumental behavior as a function of the treatment received. In fact, both groups extinguished their operant conditioning behavior (see cumulative lever presses across days 1–6 and days 7–15 in Fig. 4c. Operant a: one-way repeated measures ANOVA, $P=0.101$. Operant b: one-way ANOVA for repeated measure, $P=0.301$). However, the effect of QNP on water consumption from the bottle was once again remarkable, though the two groups differed. In group operant a, water intake abated according to the same time course observed in the no-operant groups (ANOVA days 7–15: treatment, $F_{1,14}=64.76$, $P<0.0001$; sessions, $F_{8,112}=$

Table 2 Effects of vehicle or 0.5 mg/kg quinpirole i.p. administration on drinking behavior on days 7 and 15 (first and last day of choice phase, respectively) in responder rats (rats which have gained at least one reward in the considered session day)

Experiment 1—responders' behavior

		Vehicle				QNP			
		Resp	Rewards	VT (g)	% CFL	Resp	Rewards	VT (g)	% CFL
Day 7	FR1	12/12	37.08±3.5	13.53±0.8	27.94±3.8	11/13	40.54±15	8.49±1.1*	23.32±6.4
	FR3	12/12	34.66±4.7	15.42±0.8	26.33±4.7	10/12	13.60±6.2*	10.38±1.3*	6.02±3.1*
	FR10	12/12	14.25±2.8	14.85±0.7	12.91±3.2	4/14	27.25±21.7	13.52±1.3	8.13±6.2
Day 15	FR1	12/12	20.41±3.7	12.32±0.6	14.39±2.4	13/13	71.53±15.0*	3.47±0.6*	85.61±4.6*
	FR3	12/12	12.41±2.4	12.73±0.4	10.35±2.0	10/12	66.60±12.5*	4.30±0.9*	73.75±9.0*
	FR10	7/12	3.57±1.5	13.15±0.9	3.71±1.1	10/14	33.60±6.6*	2.58±0.9*	80.08±5.5*

Data are expressed as mean ± SEM.
Resp Number of responder rats, *Rewards* number of water rewards gained through operant conditioning behavior, *VT* total water intake (dipper + bottle), *% CFL* percentage fraction of total water intake obtained through operant conditioning behavior
*Indicates significant drug effect ($P<0.01$)

3.01, $P=0.0042$; treatment × sessions, $F_{8,112}=2.69$, $P=0.0095$), whereas the persistence of conditioned stimuli in group operant b was associated with significantly higher water ingestion on the first day of bottle presentation ($P=0.01$) followed by a more gradual decrease on the remaining days (ANOVA days 7–15: treatment, $F_{1,14}=126.87$, $P<0.0001$; sessions, $F_{8,112}=10.83$, $P<0.0001$; treatment × sessions, $F_{8,112}=7.02$, $P<0.0001$).

When comparing water intake under conditions of free access (experiment 2) to total water intake under choice conditions (experiment 1), it becomes evident that, in QNP-treated rats, the presence of an operant access to water determines a four times higher water consumption over the last two days of treatment (Fig. 5).

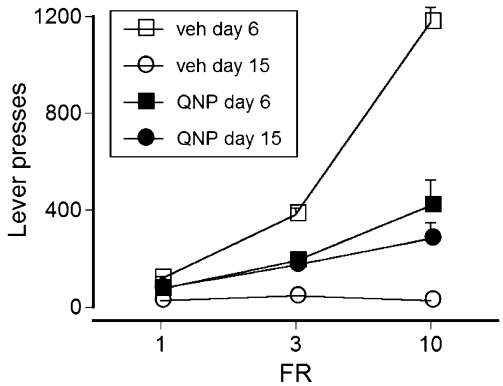

Fig. 3 Experiment 1. Effect of repeated i.p. injections of either vehicle or 0.5 mg/kg QNP on lever presses (mean ± SEM) measured on the last day of operant conditioning phase (day 6) and last day of choice phase (day 15) as a function of the FR schedule use. *X* axis is expressed in logarithmic scale. QNP-treated rats showed a clear inability to adapt their instrumental behavior to new environmental contingencies, represented here by the freely available water in bottle during choice phase. Instead, control rats showed higher levels of responding on day 6 and depressed responding on day 15 when bottle was available. This effect was independent of the FR adopted

Discussion

The present study confirms and extends our previous finding that daily QNP administration elicits an antieconomical behavior called CFL, measured as the fraction of total water intake gained through operant conditioning behavior, when free access to the resource is also possible. Three major findings were then provided: (1) QNP conferred rigidity in responding, impeding its adaptation to changed contingencies of access to the resource; (2) under free-drinking conditions, QNP caused a hypodipsic effect that progressively strengthened throughout the 15 days of the experiment; and (3) operant access to water contrasted the hypodipsic effect of QNP.

Appetition, consumption, and motivation

Although excessive, rigid and dissipative are adjectives often used in literature to describe compulsive-like behavior in animal models, no research is available on the influence of the variable "cost" on what appears to be just an "unmotivated" behavior. In the present work, we tested rats on different FR schedules: a continuous reinforcement schedule (FR1), the schedule we already used (FR3), and a higher schedule (FR10). These different schedules of reinforcement had a profound impact on the effects of QNP when access to water was only operant (days 1–6) (Fig. 1). Unlike control subjects, QNP-treated animals failed to cope with increasing behavioral costs and, accordingly, their cumulative water intake across the 6 days of this phase was an inverse function of FR. In turn, reduced water intake during the operant phase mirrored the drinking response in the first 2 days of choice access to the fluid, i.e., water intake from the bottle (free access) of QNP-treated rats was a direct function of FR. It is worth noting that on the first day of choice, all control

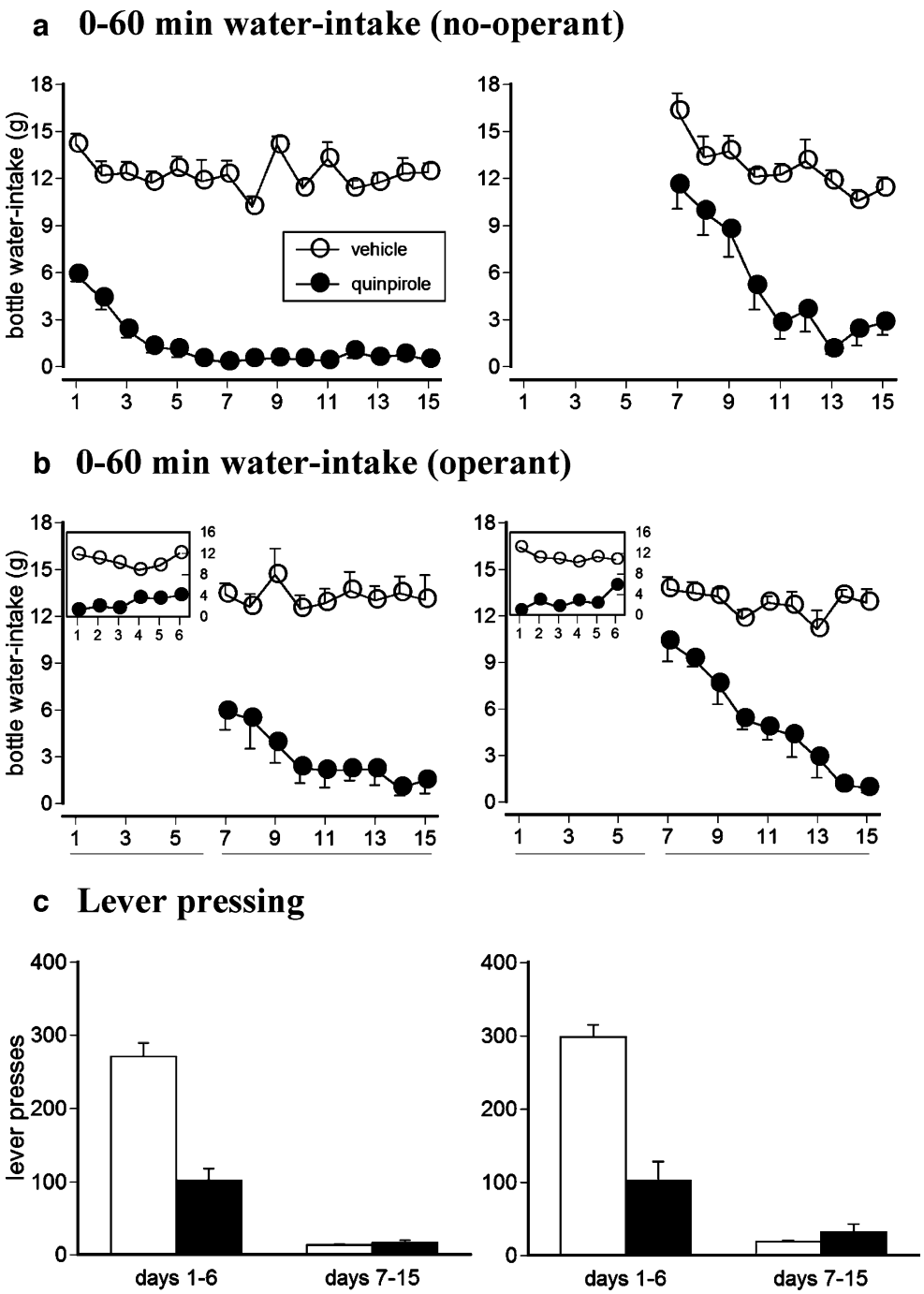

Fig. 4 Experiment 2. Effect of 15 daily i.p. injections of either vehicle or 0.5 mg/kg QNP on water intake (mean ± SEM) under four different experimental conditions. Table 2 summarizes the description of each condition. The **a** panels show bottle water intake of no-operant A (*left*) and no-operant B (*right*) groups. The **b** panels show bottle water intake of groups operant a (*left*) and *b* (*right*). *Smaller insets* display water intake from dipper across days 1–6. Histograms in **c** panels represent cumulative lever presses across days 1–6 and 7–15 of groups operant a (*left*) and operant b (*right*)

animals continued to respond for water in spite of the free access to the resource, while as many as ten of 14 subjects in the FR10 QNP-treated group did not gain any reward by lever pressing. This stands in sharp contrast to the situation registered on the last day of choice (day 15), when the number of FR10 controls that still lever pressed to gain little water was reduced to seven, whereas almost three quarters of FR10 QNP-treated rats responded on the lever to gain the majority of their water intake (Table 2). Summing up, vehicle-treated animals easily adapted to changes in the reinforcing contingencies; thus, under conditions of operant access (days 1–6), they increased lever pressing according to the FR in order to maintain asymptotic levels of water intake, whereas lever pressing dropped to minimal levels when water was also freely available (days 7–15). This finding is consistent with the observation that under CFL conditions, demand for food becomes elastic, and responding drops considerably when the FR increases (Rutter and Nevin 1990). Quite the opposite where, under QNP, lever pressing for water as a function of FR remained almost identical in

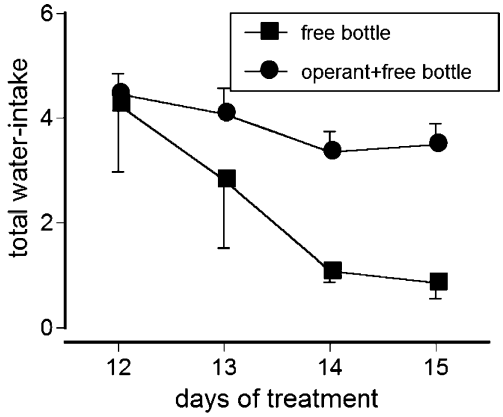

Fig. 5 Comparison between total water intake (operant + bottle) of QNP-treated rats in experiment 1 and water intake from bottle of operant b QNP-treated rats in experiment 2. Reported are data from the last 4 days of treatment (12–15). As shown, operant access to water attenuates the hypodipsic effect of QNP

both operant and choice contingencies (Fig. 3). In other words, rats responded less than necessary under conditions of operant access to water and excessively under conditions of choice between operant and free access to water. Therefore, under our experimental conditions, QNP conferred rigidity to responding for water, thus impeding adaptation to different contingencies. As already mentioned, lack of adaptation to changing contingencies of operant conditioning behavior was already observed during repeated exposure to QNP. Joel et al. (2001) found that QNP further increases the excessive lever pressing induced by post-training signal attenuation, whereas Kurylo (2004) found that the drug elicits perseverative operant responding in the absence of reward. Finally, Bratcher et al. (2005) observed that upon QNP administration rats trained to respond for food remained focused on the response lever for the entire operant conditioning session, whereas the selective D1 agonist, SKF 38393, increased locomotion and sniffing in the operant cage.

Perseveration of responding in the choice phase of the experiment led QNP-treated rats to gain many more rewards than vehicle-treated controls (Fig. 2a). Importantly enough, the amount of water earned fell off systematically as a function of the FR, showing an elastic demand for water in QNP-treated animals. However, response rates in this group were not influenced by the income supplement consisting of free access to water from the bottle (Fig. 3). This income supplement was substantially refused, and drinking from the bottle monotonically decreased throughout the 9 days of choice. In the meantime, only a fraction of the water gained by lever pressing was actually drunk, confirming our previous findings of dissociation between appetitive and consummatory components of drinking behavior (Amato et al. 2006). We do not know why QNP-treated rats continued to respond for a resource that they hardly ingested. One possibility is that repeated exposure to QNP makes rewarding the instrumental activity in the sense proposed by Premack (1959). In fact, delivery of reinforcers was required to maintain the instrumental activity of QNP-treated rats, even though they were only partially consumed, as demonstrated by the second experiment where responding extinguished upon interruption of water delivery (Fig. 4c). Another possibility is that QNP-induced dissociation of appetitive and consummatory components of ingestive behavior actually represents an extreme instance of hoarding, a spontaneous behavior whereby animals work for a resource not to consume immediately but at a future occasion. Although we are not aware of studies addressing the effects of QNP on hoarding, there is evidence that manipulation of the dopaminergic system affects this behavior. For instance, lesions of dopaminergic pathways or the administration of dopamine antagonists were found to reduce hoarding without affecting actual water or food intake (Blundell et al. 1977; Kelley and Stinus 1985; Whishaw and Kornelsen 1993).

QNP effect on free access to water

As a net effect, QNP reduced water intake and, in the meantime, elicited high levels of CFL (around 80%), an effect that was little influenced by the behavioral cost (FR) of operant access to water (Fig. 2b). Being insensitive to the increased behavioral cost of responding (increased FR), the antieconomic feature of QNP-induced CFL seems confirmed. The conclusion that QNP-induced CFL is a completely antieconomic behavior is, however, mitigated by comparing the total water intake under choice conditions with that observed under conditions of free access. Indeed, our data show that under the latter conditions, repeated treatment with QNP caused a progressive reduction of water intake during the hour that followed each injection (Fig. 4). Hypodipsia induced by QNP treatment is consistent with the dopaminergic agonist action of the drug. In fact, amphetamine, which is an indirect dopaminergic agonist, was repeatedly found to suppress drinking (Carr and White 1986; Foltin et al. 1983; Wellman et al. 1982; Stolerman and D'Mello 1978). Systemic administration of apomorphine and piribedil, two dopaminergic agonists, were able to inhibit 1-h water consumption as well (Dourish and Cooper 1981; Nielsen and Lyon 1973). Since we did not analyze the microstructure of water intake, we cannot say whether its progressive reduction was the result of sensitization towards the hypodipsic effect of the drug or the emergence of competing stereotyped behavior induced by QNP. It is important to note, however, that a similar increase in the hypodipsic effect was observed in the case of chronic administration of amphetamine (Camanni and Nencini 1994).

In the free-drinking experiment (Fig. 4), each group of rats was tested under different conditions, designed to resemble the previous CFL experiment in one or more features. Interestingly enough, these different conditions modulated the basic antidipsic effect of QNP. Thus, delaying to day 7 access to water in the test cage (group no-operant B) postponed the development of hypodipsia with respect to the matched group (no-operant A) that had access to water in the test cage from the very first day of the experiment. Because both groups received QNP from day 1, these differences in the development of hypodipsia may be easily interpreted in the framework of the contingent tolerance theory (Schuster et al. 1966). Differences in water intake also emerged between the two groups that experienced the same operant phase (days 1–6). Indeed, operant b rats drank approximately 50% more water than operant a rats when bottles were filled on day 7; thereafter, decreasing patterns of the two curves were statistically different, indicating that group operant b somewhat resisted QNP-induced hypodipsia. Persistence of conditioned stimuli (CS, i.e., click sounds related to the raising and lowering of the water dipper) previously linked to the instrumental behavior in group operant b, was probably the determining factor, since association between sounds related to dipper functioning and approaching drinking behavior represented the very first step in our training procedures. When CS, once related to the presence of water, are maintained, they may produce general arousal towards drinking behavior and, more precisely, an increased motivation for the reinforcer that they predict (Galarce et al. 2007; Rescorla and Solomon 1967). Since the filled bottle on day 7 represents both an environmental novelty and a source of the same reinforcer, water, it may not be just idle speculation to address the significantly higher water consumption in group operant b to the persistence of CS. This explanation is also supported by evidence that the absence of CS in group operant a determines a water ingestion identical to that observed in QNP-treated rats of group no-operant A in which those CS were never used.

When comparing the results of both experiments, intriguing differences in drinking behavior emerged between QNP-treated rats according to the conditions of water access. Comparing total water intake on days 14 and 15 in both experiments evidences that giving QNP-treated rats a choice between operant and free access to the resource preserved some drinking that, under free access conditions, was instead almost completely suppressed (Fig. 5). Moreover, as already stated, the persistence of conditioned cues once associated with operant conditioning behavior and not just pre-exposure to the operant conditioning testing on the preceding days somewhat prevented the progressive water intake suppression induced by QNP.

In previous studies, we stressed the analogies between the effects of QNP in the CFL model and the ability of QNP to maintain animals in perseverative checking and other ritual-like motor activity patterns reminiscent of human obsessive–compulsive disorder (OCD) (Szechtman et al. 2001, 1998). However, behavioral alterations induced by the drug in the present study do not bear complete similarity to specific characteristics of OCD diagnosed in humans (for a review, see Aouizerate et al. 2004): “repetitive”, “excessive”, “inappropriate”, “time-consuming”, and “unreasonable” are distinctive features of human compulsions that are not fully reproduced in our model. In fact, in the operant context, rats administered QNP work in excess to gain and consume a resource that, under the same pharmacological stimulus but under an open access condition, they tend to avoid. This responding for water was not basically counteradaptive, because QNP-induced CFL allowed animals to partially circumvent the hypodipsic effect of the drug. Indeed, there is a suggestion that CFL is adaptive in that it helps the animal to shape foraging strategies in an uncertain environment (Inglis et al. 1997). Likewise, Szechtman et al. (1998, 2006) also suggested that compulsive performances of QNP-treated rats indeed display a certain degree of “controllability” when appropriate stimuli are presented and may therefore not be purposeless. Hence, repeated stimulation of dopaminergic D2/D3 receptors by QNP seems to reduce behavioral flexibility in coping with environmental stimuli by exaggerating adaptive strategies, such as checking (see Szechtman’s studies) or CFL (this study).

A dysregulated dopaminergic transmission in brain areas involved in the occurrence of goal-oriented instrumental behaviors is likely implicated in the phenomenon described here. D1 and D2 receptor antagonists, directly infused in the orbitofrontal cortex, caused a significant reduction of the break point in a progressive schedule of reinforcement for food, but had no effect on the amount of food consumed or on food preference (Cetin et al. 2004). Intra-accumbens infusions of QNP increased both perseverative responding and, at higher doses, latency to make a correct response in the five-choice serial reaction time test (Pezze et al. 2007). These impairments may reflect an inability to correctly perform a behavioral sequence when initiating a trial, and to start a new trial after completing the previous response. Similar findings were provided with excitotoxic lesions of the prefrontal cortex (Chudasama et al. 2003). Interestingly, sensitization to chronic treatment with QNP was associated with decreased dopamine levels in the left prefrontal cortex (Sullivan et al. 1998). Moreover, O’Donnell and Grace (1994) showed that QNP administration is able to decrease nucleus accumbens excitatory response to prefrontal stimulation. Thus, perseverative behavior, which may provide a model of “compulsive” behavior, may directly result from D2 overstimulation

of the nucleus accumbens that presumably blocks the prefrontal inhibitory input to the accumbens via cortico-striatal projections.

Our results outline the importance of introducing variables of operant cost in the study of compulsive-like behavior and suggest that studies on pharmacological manipulation of economical aspects of behavior may be useful in interpreting specific OCD symptom clusters. As for our model, further research is necessary to establish the response to pharmacological treatments that were fruitful in ameliorating obsessive–compulsive symptoms in human patients, and to investigate brain areas likely to be involved in the development of the phenomenon.

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