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Original article

A panic experimental model: Validation of a complex operant behavioral method in undernourished rats, with desipramine to provide a template effect profile

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Abstract

Introduction: Clinical studies have shown that some antidepressants may be more efficient than benzodiazepines to alleviate anxiety associated with panic disorders; however, operant conflict procedures in rats developed so far seem not particularly able to model human anxiety sensitive to antidepressant treatments. Previous panic models with learned responses did not statistically subtract the effect of confounding factors from the variable of interest. **Methods:** Undernourished rats were selected due to their behavioral and neurobiological resemblance to human patients suffering from panic disorder. The Geller–Seifter paradigm represented the stressful environmental condition in adult life. Desipramine (10 mg/kg/day) or saline were administered IP during 7 days under a cross over design ($N=10$). Five daily 15 min-operant sessions were carried out on each experiment. Unpunished, unrewarded and punished operant behavioral periods were identical both in their duration and in their reward system (the FR1 schedule) in order to measure response suppression, which has not been considered in previous studies with the Geller–Seifter paradigm. The dependent variable was the difference between comparable unpunished and punished periods. **Results:** A significant Diet × Drug interaction was observed in the dependent variable, which represented the level of "suppression/suppression release" induced by treatments. **Discussion:** Compared to control rats, deprived rats showed a significant and selective anticonflict effect of desipramine on the stressful and complex operant performance. The animal model of perinatally protein-deprived rats along with the Geller–Seifter's operant behavioral paradigm may represent a more sensitive approach to model human anxiety sensitive to antidepressant treatments by considering the combined impact of both early biological trauma and adult learned experiences under the same design.

Keywords: Antidepressants; Conditioned anxiety; Desmethylimipramine; Geller–Seifter paradigm; Operant behavioral methods; Panic disorder; Perinatal protein malnutrition; Rat

1. Introduction

1.1. Geller–Seifter paradigm

There is a wide range of animal models and measures designed to assess anxiety or fearfulness. Many of these rely on the so-called approach–avoidance conflict paradigms. These tests have been extremely useful as initial screens for drugs affecting anxiety, but the components of anxiety assessed by these models remain poorly defined (Shekhar et al., 2001).

One of the most widespread animal models to assess anxiety–anti-anxiety effects is the Geller–Seifter paradigm (Beaufour, Ballon, Le Bihan, Hamon, & Thiébot, 1999; Geller & Seifter, 1960). It consists of a conflict operant procedure in which the feeding behavior (lever pressing) is suppressed by conditioned anxiety (an aversive stimulus associated with reinforcement). Researchers usually infer an anticonflict effect of drugs by measuring the difference between non-drug and drug responses during the punished period alone. They generally refer to this change as "release of the conditioned response suppression". But the response suppression itself is not actually measured because

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the reference criterion is generally the response rate under another schedule of reinforcement (assessed under another unit of measurement and under periods of different duration). In other words, the response suppression is generally assumed (but not statistically measured) by visually comparing the response rate under the punished period vs. the response rate under the unpunished one and by inferring that any difference is sufficient to be regarded as "suppression".

We consider that it could be more useful to incorporate also the information provided by the unpunished periods into the model of analysis particularly since an anticonflict effect of drugs should not affect normal activity in these periods. If a significant behavioral change in the unpunished period is accompanied by a simultaneous increase in the number of shocks received in the punished one, this could be interpreted as a cognitive deleterious effect of the drug as opposed to an anxiolytic or antidepressive one. In addition, the lack of a control group to test confounding appetitive, motor or analgesic effects, may also mask the final psychopharmacological effect. The use of animals predisposed to develop anxiety symptoms may help to better elucidate these questions. The analysis of the interaction between biological and environmental anxiogenic factors can provide a more realistic approach to the understanding of these psychopathological processes.

1.2. Deprived rats and panic disorder

Protein deprivation at perinatal age has long-lasting effects on morphological, neurochemical and behavioral parameters that persist in adulthood even after prolonged periods of nutritional recovery (Almeida, Tonkiss, & Galler, 1996; Morgane et al., 1986; Wiggins, Fuller, & Enna, 1984). As regards the catecholaminergic system, perinatally deprived rats showed alterations in noradrenergic neurotransmission (Keller, Munaro, & Orsingher, 1982; Marichich, Molina, & Orsingher, 1979; Nasif, Ramírez, Cuadra, & Orsingher, 2001) that resemble those of patients suffering panic attacks (Goddard & Charney, 1997; Laino, Córdoba, & Orsingher, 1993). Locus coeruleus activity is significantly higher in deprived rats than in controls; likewise, one week of desipramine (DMI) administration reduces the locus coeruleus activity of deprived rats to values comparable to controls, which were not affected after similar treatment (Nasif et al., 2001). Sodero, Valdomero, Cuadra, Ramírez, & Orsingher (2004) hypothesize that neuronal abnormalities observed in deprived rats may represent the neurobiological basis of the pathophysiology of panic disorder.

These abnormalities may account for some of the behavioral consequences observed in deprived rats such as increased avoidance performance, increased immobilization to a loud noise, impaired habituation to an open field after repeated exposures, and an increased number of ineffectual jumps in an active avoidance test (Brioni & Orsingher, 1988).

Considering the association observed between panic attacks and cocaine use in humans (O'Brien, Wu, & Anthony, 2005), it is interesting to note that an increased responsiveness to behavioral effects of cocaine and/or an enhancement of its reinforcement properties have also been observed in rats undernourished at perinatal age (Valdomero, Bussolino, Orsingher, & Cuadra, 2006; Valdomero, Isoardi, Orsingher, & Cuadra, 2005).

In the elevated plus-maze (Laino et al., 1993), drugs with therapeutic efficacy in panic disorders, such as diazepam and alprazolam showed a similar anticonflict effect in control and deprived rats, while buspirone, propranolol, desipramine and phenelzine induced a selective anxiolytic effect on deprived rats. Laino et al. (1993) affirmed that drugs that interact with noradrenergic and/or serotonergic systems exert a selective and anticonflict effect in deprived rats in the plus maze; consequently, deprived rats may represent a useful model for studying antipanic agents.

In previous studies with operant behavior, perinatally protein-deprived rats showed a significantly and gradually better performance than control rats under a variable ratio twenty (VR20) schedule of reinforcement as well as a worse performance under a differential reinforcement of low rate of five seconds (DRLR5) schedule of reinforcement (Brioni & Orsingher, 1988). These effects were attributed to the hyperreactivity of deprived rats to aversive or stressful situations. Under the Geller–Seifter test, the basal performance under FR1 schedule was not significantly different between groups. In the punished period, non-significant differences were observed under the non-drug situation. Nevertheless, 3 mg/kg of diazepam induced a lower anticonflict effect in deprived rats. The effect of this drug on the unpunished period performance was not evaluated (Brioni & Orsingher, 1988).

1.3. Antipanic drugs and desipramine

Models that emulate predisposing environmental events, such as early life stress or adult trauma have been useful for identifying brain circuits that are sensitized by exposure to adverse experiences (Shekhar et al., 2001). Punishment, exposure to novel stimuli and frustrative nonreward are considered as three major classes of anxiogenic environmental stimuli (Gray, 1982). Benzodiazepines, used in the clinic as anxiolytics, have been found in animal models specifically to attenuate behavioural suppression caused by these responses but it is probable that these drugs may alter decision-making by affecting the evaluation of the learned significance of the stimuli in the environment (Ljunberg, Lidfors, Enquist, & Ungerstedt, 1987).

Although in human anxiety disorders habit formation and conditioning of the anxious states will play roles in maintaining pathology, in animal models the cause for anxious behavior is usually acutely presented and, therefore, the attenuation of anxieties by drugs, such as benzodiazepines, may bring about immediate alleviation (Broekkamp, Berendsen, Jenck, & Van Delft, 1989). Interesting exceptions are the studies on the effect of long-term treatments in animal anxiety models. Results showed that drugs such as imipramine and desipramine are inactive with a single treatment but have an anticonflict effect after several weeks of treatments in normal rats (Broekkamp et al., 1989).

Clinical studies have shown that some antidepressants may be more efficient than benzodiazepines to alleviate anxiety associated with panic disorders (Ham, Waters, & Oliver, 2005;

Shekhar et al., 2001; Sheehan, 2002; Surany-Cadotte, Bodnoff, & Welner, 1990) but operant conflict procedures in rats developed so far do not seem particularly able to model human anxiety sensitive to chronic antidepressant treatments (Beaufour et al., 1999). (For a review of the effect of acute and chronic treatments with antidepressants in normal animals see Beaufour et al., 1999). Desipramine induced a gradual release of novelty-suppressed feeding (Bodnoff, Suranyi-Cadotte, Aitken, Quirion, & Meaney, 1988; Bodnoff, Suranyi-Cadotte, Quirion, & Meaney, 1989), the conditioned suppression of drinking (Fontana, Carbary, & Commissaris, 1989; Fontana & Commissaris, 1988), and the conditioned suppression of lever pressing in Geller–Seifter test (Beaufour et al., 1999) after several weeks of treatment in normal rats. As regards the studies dealing specifically with conditioned responses, DMI treatments also showed a parallel and significant reduction of water intake in the conditioned suppression of drinking (Fontana et al., 1989) as well as a significant reduction of the unpunished responding in the Geller–Seifter test (Beaufour et al., 1999), which were impossible to subtract from the variable of interest (the punished response) under such experimental conditions. Besides this, all cited studies about the effect of antidepressants on conflict paradigms with normal rats (Beaufour et al., 1999; Bodnoff et al., 1988, 1989; Fontana et al., 1989; Fontana & Commissaris, 1988) required more prolonged periods of treatment than the one-week periods required in deprived rats (Laino et al., 1993; Nasif et al., 2001; Sodero et al., 2004) to obtain similar anticonflict results. In human patients, Kats, Houston et al. (2004) observed that the onset of DMI antidepressant actions ranged from 3 to 13 days. Likewise, Frazer and Morilak (2005) suggested that the antidepressant-"therapeutic lag" might not be as long as has been commonly believed. Increasing speed of therapeutic action should be an aim in the development of antidepressant (Kats, Tekell et al., 2004).

1.4. Present experimental model

It is important to better define and extend existing models and behavioral measures related to specific processes that may be disrupted in anxiety disorders and to develop new models that consider the impact of combined factors in determining anxious behaviors (Shekhar et al., 2001). Pointing at developing more sensitive operant behavioral models in the study of panic disorders we decided to study the effect of both undernutrition and DMI on Geller–Seifter paradigm by evaluating the Diet × Drug effect on the punished and the unpunished periods simultaneously. The Geller–Seifter paradigm was selected not only as representative of the anxiogenic environmental milieu in adult life but also as a useful approach considering the possibilities that the operant procedure offers to control confounding variables. In the punished period we have preferred using a predictable aversive stimulus, in a similar way as Martin et al. (2002), in order to see if, under this condition, the test drug affects decision-making (Ljunberg et al., 1987) between the need for food vs. the need for avoiding punishment. Additionally, this approach represents a safer procedure for the animals. The role of stressful environmental factors on habit formation was also controlled by paying attention to the rest of the operant schedules of learning and its possible transference throughout the training sequence.

Undernourished rats were selected due to their behavioral and neurobiological resemblance to human patients suffering from panic disorder, i.e., due to their early-induced predisposition to develop, apparently, anxiety symptoms. Additionally, the effect of antidepressants has never been studied on the operant conflict behavior of such rats. A significant and selective anticonflict effect of desipramine was expected on the dependent variable, which was defined in this case as the difference between unpunished and punished periods. This difference lets us simultaneously assess the level of anxiety–anti-anxiety effects induced by treatments, by means of a unique variable, the level of "suppression/suppression release".

In addition, and considering the advantage of using a control group, the drug side effects on milk consumption, reactivity to the electric foot shock and unpunished response were also analyzed. The suppression effect was separately analyzed in order to test, with exploratory purposes and pointing at future studies with this model, if the simplest operant schedule of learning, i.e., the FR1 schedule, could be used to produce effective or significant response suppressions.

2. Methods

2.1. Materials

2.1.1. Animals

Adult rats subjected to a protein deprivation schedule at perinatal age, as previously described (Borghese et al., 1998), were used. Briefly, pregnant female Wistar rats at 14 days of pregnancy were divided into two groups, housed in individual polyethylene cages, and fed by isocaloric diets containing 24% or 8% casein for control and deprived rats, respectively (Borghese et al., 1998). Diet composition (%): For control rats: casein 24.0; sucrose 44.5; cornstarch 9.9; hydrogenated vegetable oil 15.0; corn oil 1.0; vitamin mix 1.25; minerals 4.0; DL-methionine 0.4. For deprived rats: casein 8.0; sucrose 57; cornstarch 13.4; hydrogenated vegetable oil 15.0; corn oil 1.0; vitamin mix 1.25; minerals 4.0; DL-methionine 0.4. Diets were prepared at the "Departamento de Farmacología de la Facultad de Ciencias Químicas de la Universidad Nacional de Córdoba" with rats of its own colony. Approval of animal experiments was given by the institutional Animal Care and Use Committee.

At birth, litters were culled to eight pups (male/female ratio close to 1). After weaning (30 days), pups continued consuming the same diet as dams until the end of the deprivation period (40 days of age). Thereafter, both groups were given balanced laboratory chow for at least 40 days prior to assays. Subjects came from different litters in order to avoid sibling replication. Animals were housed in standard cages (ten per cage) and maintained at 22 ± 2 °C in 12 h light–dark cycle (lights on at 7:00 AM) (Borghese et al., 1998). Food and water were available ad-lib except for the experimental days in which food restriction was necessary (see below).

Like previous studies, in which the effect of undernutrition and DMI was analyzed in the plus maze (Laino et al., 1993), female rats were employed in this case. Laino et al. (1993) affirmed that female and not only male rats had the same reactivity to the drugs assessed in the plus maze but also female rats showed a lower variability of the results, which were obtained most likely on account of their lower size which allowed a better displacement in the maze.

Body weights at the beginning of the experiment were (mean ± SD): 233.80±22.05 and 217.20±22.61 for control and deprived rats respectively (ANOVA: $F(1, 18)=2.97$, $p<0.10$). Ten rats were studied in each group.

During the operant behavior experiments, animals were maintained under food restriction. Water was always available ad-lib but not during the operant session itself. Food was available ad-lib only during weekends. Each operant session began after 24 h of food deprivation. Rats were weighed daily, and received water and standard solid food for 15 min after finishing the operant training session. Body weight loss on each experiment was always less than 5% (related to the free-feeding body weight assessed after the weekends).

2.1.2. Drug

Desipramine HCl (Lab. Montpellier, Buenos Aires, Argentina) was injected IP daily (10 mg/kg) for 7 days. The drug was dissolved in 0.9% NaCl solution. The dose was selected according to its effectiveness in previous behavioral and electrophysiological studies with deprived rats (Laino et al., 1993; Nasif et al., 2001). Control rats received saline in equal volume (1 ml/kg).

2.1.3. Apparatus: operant conditioning chamber

A standard operant chamber (Campden Instruments, Ltd.) of 22×22×22 cm was used. This chamber was fitted with a grid floor made of stainless steel bars (diameter 0.5 cm) spaced 1 cm apart, which was connected to a scrambled shock source. On a sidewall, the chamber had two response levers, placed 7 cm above the floor, and a lid leading to the liquid dispenser mounted between them. Reinforcement was a drop (0.1 ml) of sweetened condensed milk (Nestle product: 45% sugar) diluted 3:1 with tap water. The chamber was supplied with two 28 V lights located above each lever as well as a buzzer located in the middle of the ceiling. The test was performed in a dimly lighted room and the chamber was placed within a sound-attenuating box.

2.2. Procedures

2.2.1. Basal studies

2.2.1.1. Shaping or modeling. For two days, rats were trained to respond by pressing either of the two levers while both lamps were on and the buzzer off. During the first day, the lever-pressing response was modeled until animals achieved 30 responses under FR1 schedule. During the second day, each rat was placed in the chamber for 50 min or until 100 reinforcements were delivered (results not shown).

2.2.1.2. Milk consumption. Rats were habituated to drinking milk from a calibrated tube attached to an individual experimental cage for the three days before the consumption test. Milk drinking sessions in the individual cage were 15 min long after 24 h of food deprivation. Water was ad-lib in the homecage. The same procedure was carried out on the day of the test, and milk intake was registered. Data were analyzed by a one-way ANOVA with Diet as the independent variable and Milk Consumption as the dependent variable.

2.2.1.3. Reactivity to the electric foot shock. This study was carried out after the operant shaping (see above) had finished. On the day of the test the operant chamber had the levers and the milk dispenser covered so that these devices were inaccessible to the rats. The lights were off and the buzzer on. Rats were left in the chamber during 6 min for habituation. The shock intensity was initially set at 0.1 mA, which was the lowest level provided by the chamber (1 s long). Rats received the shock immediately after the habituation period had finished. If the rat responded positively with an escape response (running or jumping) it was removed from the chamber. If it did not respond, another shock was administered after 1 min of evaluation and recovery. The intensity was successively increased until the rat responded positively (maximal intensity used 0.25 mA). Data were analyzed by Chi-square (frequency of rats for each shock intensity).

2.2.1.4. FR1 response rate. Rats were trained daily, 5 days a week, on 12 min-sessions under FR1 schedule. During this phase of training, the operant chamber lights were on and the buzzer off. On the day of the test (the fifth day of the second week of training after stabilization) we studied the performance under the FR1 schedule during a session of 12 min divided in periods of 3 min. We did it that way considering that, on the complex operant program (see below), rats had to consume milk for only 9 min during the whole experimental session. Therefore, the performance on this FR1 session was taken as an index of the level of motivation/satiety on a period of 9 min (+3 min). Data were analyzed by a repeated measure ANOVA with Diet as the independent variable, and Period (four periods) as the dependent variable.

2.2.2. Complex operant behavioral model

2.2.2.1. Drug treatment. Pharmacological treatments (see above) were made under a cross over design (10 rats on the whole: 5 rats with DMI, 5 rats with saline per experiment) and 15 days of a drug washing period between the two experiments. As we carried out two experiments, the total number of rats on each drug condition was also ten. Each daily operant session took place 14 h after the injections. Each experiment lasted 7 days. During the washing period rats were trained to maintain baseline performance twice a week under FR1 schedule on sessions of 12 min long.

2.2.2.2. Behavioral design. Five daily 15 min-operant sessions were carried out on each cross over experiment. Rats were not trained on the weekends. Three different operant

contingencies were distributed throughout five −3 min-periods of training. The contingencies for each daily session were: a) unpunished response (FR1), b) time out or unrewarded response (UNR), and c) punished response according to the conflict paradigm of Geller–Seifter (PUN), specifically, milk reinforcement under FR1 schedule, and shock delivered under FR4 schedule. The sequence of the conditioned stimuli for the five periods was: 1) FR1 (lights on, buzzer off), 2) UNR (lights off, buzzer off), 3) PUN (lights on, buzzer on), 4) UNR (lights off, buzzer off), and 5) FR1 (lights on, buzzer off). All the periods were comparable not only in their duration but also in their reward system (the FR1 schedule). The stimuli were used in the same way as in the basal studies.

In an attempt to avoid as much as possible any transference from previous contingencies to the present ones, only the periods 3 and 5 were considered comparable since both of them were preceded by UNR periods. The primary dependent variable was then the performance difference between the second unpunished period and the punished period. UNR periods were considered just as "cleaning" or "break" periods since none of them were preceded by comparable schedules.

Stabilization took place after 15 days of training (three weeks) on the first cross over experiment and after 5 days of training on the second one. (Stabilization was defined as the lack of a day effect on the three schedules of learning during three consecutive days, analyzed by a repeated measure MANOVA (results not shown)).

The data obtained during the five training days were averaged and analyzed by a repeated measure ANOVA with Diet as the independent variable and Drug performances (Saline and DMI) as the dependent ones. Each rat served as its own control for the Drug effect since we were working with a cross over design. Consequently, the Diet × Drug interaction, which showed our hypothesis of study, could be analyzed and expressed in a simpler way by considering just Diet as the independent variable and Drug effect (Saline–DMI difference in performance) as the dependent one.

The intensity of 0.25 mA was selected as the aversive stimulus due to the basal study on the electric foot shock reactivity for these 20 rats (see above). (The selection was done this way to avoid the idiosyncratic regulation of the shock intensity for each rat, which has usually been employed in some of the Geller–Seifter studies, in which neither as independent nor as dependent variable the shock appears to have been clearly defined).

2.2.2.3. Additional measures. The escape response to each shock delivered was controlled. Likewise, the PUN response under saline for both groups and the PUN response under saline and DMI just for the control group were analyzed by ANOVA and MANOVA as a way to assess, respectively, the basal response to punishment (i.e., the basal response to the 0.25 mA electric foot shock) and the possible analgesic effect of DMI (Richeimer, Bajwa, Kahraman, Ransil, & Warfield, 1997; Rigal, Eschalier, Devoize, & Pechadre, 1983) in the control group. The maximum mean of lever presses observed during the PUN period and throughout both experiments in any group or situation was taken as an index of the maximum level of risk that rats were able to take in their decision-making between the need for feeding and the need for avoiding punishment.

Body weight of the five days was averaged and the effect of both treatments on weight was analyzed by a Diet × Drug repeated measure ANOVA with Diet as the independent variable and Drug (weight under Saline and DMI) as the dependent one.

The first training period under FR1 schedule was considered as an index of the initial cognitive and motor performance, i.e., as representative of the unpunished response before all the inhibitory or stressful contingencies appeared. The performance on this period was analyzed by a Diet × Drug repeated measure ANOVA in the same way as weight.

The first training period under FR1 schedule was also considered the reference period to test if the suppression effect was significant by comparing this period with all the subsequent inhibitory schedules (UNR and PUN). Suppression effect was analyzed by a repeated measure MANOVA after averaging all the days of training. In each analysis, two dependent variables were considered: the first FR1 period and one of the subsequent inhibitory schedules (UNR or PUN). For this purpose, Saline and DMI performances as well as control and deprived groups were separately evaluated.

2.2.3. Later study

2.2.3.1. Milk consumption on the eighth day of treatment. When the complex operant study had finished, rats were treated one additional day with DMI or saline according to the cross over design. As rats had already been habituated to drinking milk from a calibrated tube (see above) only one 15 min-session was carried out this time. The milk intake after 24 h of food deprivation (water ad-lib) was registered by following the same procedure described above in Basal Studies. Data were analyzed by a Diet × Drug repeated measure ANOVA with Diet as the independent variable and Drug response (milk intake under saline and DMI) as the dependent one.

3. Results

3.1. Basal studies

Control and deprived rats did not differentiate from each other on either their basal milk consumption (Control: 14.63 ± 2.57 ml; Deprived: 14.12 ± 1.99 ml; $F(1, 18) = 0.25$, $p < 0.63$) or their reactivity to the electric foot shock (0.1 mA: Control: 2 rats, Deprived: 3 rats; 0.25 mA: Control: 8 rats, Deprived: 7 rats; Chi square = 0.27; *df*: 1; $p < 0.60$).

Deprived rats showed a poorer but non-significant different performance than control rats in the FR1 schedule of reinforcement (Fig. 1). The Diet × Period ANOVA indicated either non-significant main effects (Diet: $F(1, 18) = 1.34$, $p < 0.26$; Period: ($F(3, 54) = 0.66$, $p < 0.58$)) or interaction between factors (Diet × Period ($F(3, 54) = 0.41$, $p < 0.75$).

As the mean rate of FR1 responses on each period for both groups was 13.2, 12.5, 12.7 and 11.1, the level of expected motivation, for each 3 min-period and during 9 min in the

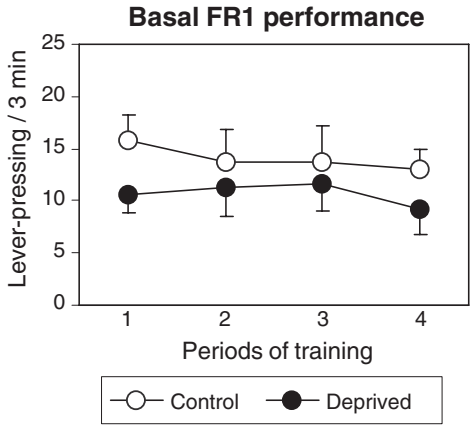

Fig. 1. Mean±SE of lever-pressing responses on each 3 min-period during a 12 min-session.

complex operant schedule, was established between 12 and 13 responses.

3.2. Complex operant behavioral model

The repeated measure ANOVA with Diet as the independent variable and Drug performances (Saline and DMI) as the dependent ones indicated non-significant effects of Diet ($F(1, 18)=0.43$, $p<0.52$) or Drug ($F(1, 18)=2.13$, $p<0.16$) and a significant Diet×Drug interaction ($F(1, 18)=6.49$, $p<0.02$). Taking into account these results, the Diet×Drug interaction was better described by considering just Diet as the independent variable and Saline–DMI difference in performance as the dependent one (Fig. 2). As can be seen in Fig. 2, which represents the level of suppression/suppression release induced by the drug treatment, control rats showed no change in performance (mean value near zero) while deprived rats showed not only a bigger change but also a change in the opposite direction than controls. A suppression release was not observed in control rats at all but rather the opposite pattern, i.e., a lightly higher suppression (towards the negative values) indicating a probable anxiogenic-like effect. In other words, deprived rats showed a significant and selective anticonflict effect of DMI, compared with control rats, on the performance difference between unpunished and punished periods.

All the rats escaped from the shock every time they were punished. Control and deprived rats did not differentiate from each other in their PUN responding under saline (ANOVA: ($F(1, 18)=0.03$, $p<0.86$) thus indicating a similar basal response to the electric foot shock (Control: 1.80±1.51; Deprived: 1.92±1.42). Control rats showed a non-significant tendency towards decreasing their punished responding under DMI compared to their own performance under saline (Saline: 1.80±1.51; DMI: 1.22±0.97; MANOVA Drug effect: $F(1, 9)=2.99$, $p<0.12$). In other words, control rats showed a change in the opposite direction to what would be expected from mediating an eventual analgesic effect of DMI on the electric foot shock. The maximum mean of lever presses observed during the PUN period and throughout both experiments was of 3.4 responses for deprived rats on the fifth day of DMI treatment. This result would indicate that, on average, the behavioral suppression release in this study never exceeded the limit of 4 responses established as the criterion to deliver the shock under the Geller–Seifter paradigm.

The effect of treatments on weight (Control/Saline: 240.16± 28.61; Control/DMI: 236.92±21.29; Deprived/Saline: 228.48± 17.60; Deprived/DMI: 220.84±19.57) did not produce any significant effect of Diet ($F(1, 18)=2.21$, $p<0.15$), Drug ($F(1, 18)=2.62$, $p<0.12$) or interaction between factors ($F(1, 18)=0.43$, $p<0.52$).

The effect of treatments on the first training period under FR1 schedule (Control/Saline: 10.6±4.0; Control/DMI: 7.22±2.84; Deprived/Saline: 11.94±4.11; Deprived/DMI: 7.02±4.17) indicated a non-significant main effect of Diet ($F(1, 18)=0.23$, $p<0.64$), a significant main effect of Drug ($F(1, 18)=11.35$, $p<0.003$), i.e., an inhibitory effect of DMI on unpunished responding, but independent of the Diet (Diet×Drug interaction ($F(1, 18)=0.39$, $p<0.54$)).

The comparison of the first unpunished training period under FR1 schedule with all the subsequent inhibitory schedules (UNR and PUN) indicated that this very simple operant schedule of learning, i.e., the FR1 schedule, was effective to produce significant response suppressions (suppression effect in all the comparisons: $F(1, 9)\geq 11.65$, $p\leq 0.008$ being the smallest mean differences those obtained with the first UNR period under DMI (Control: 4.76±3.73; Deprived: 4.92±4.56)).

Just for informative purposes, the analysis of either the individual components of the primary dependent variable or the other components of the complex model, isolated or combined, did not demonstrate any relevant effect regarding the hypothesis of study, i.e., any significant Diet×Drug interaction (results not shown).

3.3. Later study

Milk consumption on the eighth day of treatment (Control/Saline: 17.39±4.43 ml; Control/DMI: 13.83±3.60 ml; Deprived/Saline: 16.94±3.35 ml; Deprived/DMI: 10.81±4.62 ml) was reduced by DMI treatment (Drug effect $F(1, 18)=22.49$, $p<0.0002$). However, there was not a significant effect of either

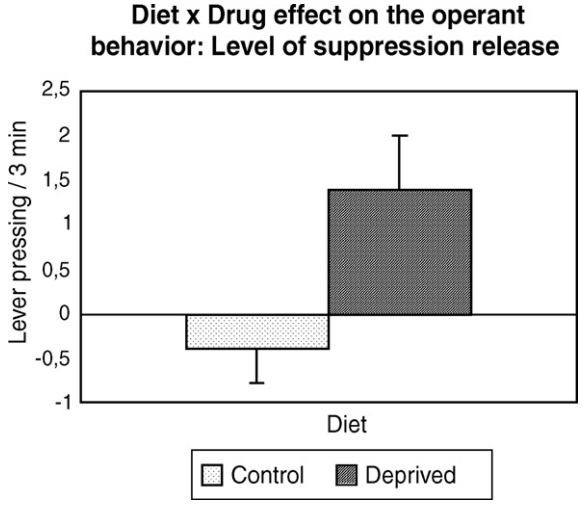

Fig. 2. Values indicate the mean difference (Saline–DMI treatments) of the difference (unpunished–punished periods) in performance±SE, $p<0.02$ (ANOVA).

Diet ($F(1, 18)=1.35$, $p<0.26$) or Diet × Drug interaction $F(1, 18)=1.58$, $p<0.22$).

4. Discussion

Present results support the hypothesis that the animal model of perinatally protein-deprived rats along with the Geller–Seifter's operant behavioral paradigm may represent a more sensitive approach to the study of the anticonflict effect of antidepressant drugs in panic disorders by considering the combined impact of both early biological trauma and adult learned experiences under the same design. Compared with control rats, deprived rats showed a significant and selective anticonflict effect of DMI on the stressful and complex operant performance. This effect was measured by the difference between comparable unpunished and punished periods, which had not been considered in previous studies with the Geller–Seifter paradigm.

Present results with DMI and learned responses were consistent with the neurobiological, pharmacological and behavioral findings of protein-deprived rats described in the Introduction (Keller et al., 1982; Laino et al., 1993; Marichich et al., 1979; Nasif et al., 2001; Sodero et al., 2004). Consequently, this study shows further evidence supporting the hypothesis that neuronal abnormalities observed in deprived rats may help to elucidate the pathophysiology of panic (Sodero et al., 2004). Besides, the present anticonflict effect was reached in less time of treatment compared with other conflict procedures in which only control rats were employed (Beaufour et al., 1999; Bodnoff et al., 1988, 1989; Fontana et al., 1989; Fontana & Commissaris, 1988).

Brioni and Orsingher (1988) stated that rats undernourished at perinatal age showed, in basal conditions, a hyperactivity to the stressful VR20 and DRLR5 operant schedules of learning, although in the same study the non-drug situation on the Geller–Seifter task did not produce significant differences between deprived and control rats. On the other hand, when evaluated just under saline in the plus maze test, deprived rats showed a non-significant tendency to spend less time than the control rats in the open arms (Laino et al., 1993). In the present study, the contribution of these non-significant or not so clear basal trends, if considered alone, acquired importance when they were incorporated in the complex model of analysis.

Frances et al. (1987) reported that the chronic administration of DMI produces a significant decrease in the spontaneous locomotor activity in rats. As well, Durcan et al. (1988) reported that food and water intake in the DMI treated rats (10 mg/kg/day, during 30 days), was initially and significantly decreased but progressively returned towards pretreatment levels over the course of the drug administration. In the present experiment an inhibitory DMI effect was observed on FR1 performance, which may have been caused by some of the motor or appetitive side effects of the drug. Nevertheless, this effect did not interact with Diet. Similarly, although on the eighth day of treatment a DMI effect on milk consumption was detected; this effect did not interact with Diet either. Consequently, the diet × drug effect observed on the primary dependent variable of the current complex model could be considered independent of these confounding effects.

As regards the probable analgesic effects of DMI (Richeimer et al., 1997; Rigal et al., 1983), control and deprived rats did not differentiate from each other in their basal response to the electric foot shock either on their reactivity in the basal studies, or in their operant PUN responding under saline. Under DMI, control rats showed a change in the opposite direction to what would be expected from an analgesic effect, i.e., control rats showed a non-significant decrease in their punished responding under DMI compared to their own performance under saline. Besides, as we were working with well-trained rats, and with a predictable punishment, the aversive response was produced by the conditioned signal rather than by the electric foot shock.

All these results stress the importance of using reference groups and other control parameters to test the side effects of the drugs on the variables of interest.

Beaufour et al. (1999), who evaluated the effect of antidepressant in normal rats by using the Geller–Seifter test did not report any significant effect of DMI on weight during the first week of treatment, which is consistent with our results. Besides, all previous studies about the effect of DMI on conflict paradigms with normal rats reported an initial (Beaufour et al., 1999) or acute (Bodnoff et al., 1988, 1989; Fontana et al., 1989; Fontana & Commissaris, 1988) anxiogenic-like effect in treated subjects, which coincided with the pattern observed in our experiment with control rats during one week of treatment.

In animal procedures devoted to the study of anxiety and anxiolytics, the effects of antidepressants are not unequivocal. This is probably due to several factors, among which is the fact that these experimental procedures were developed and optimized primarily for evaluating benzodiazepines and were further validated by their sensitivity to this class of compounds. Differences in pharmacological properties and pharmacokinetic characteristics of antidepressants can also play a crucial role in the observed variety of results (Beaufour et al., 1999).

Early malnutrition induced lower reactivity to the anxiolytic effect of diazepam either with the Geller–Seifter, light–dark transition (Brioni & Orsingher, 1988), or salt-suppressed drinking tasks (Almeida et al., 1990). Conversely, Laino et al. (1993) described similar anticonflict effects in control and deprived rats with diazepam and alprazolam in the elevated plus-maze test. Nevertheless, noradrenergic neuronal integrity appears to be required for the anxiolytic-like effects of chronic antidepressant DMI treatment, but not for the anxiolytic-like effects of acute treatment with benzodiazepines (Fontana, McMiller, & Commissaris, 1999).

From another point of view, it is probable that benzodiazepines in some way alter information processing and/or decision-making. Ljumberg et al. (1987), described an experimental test in which the rat decision-making was verified by the choice between two options. They found that diazepam caused strong impairment in the decision-making process. In human beings, it has been reported that diazepam impaired performance on tests of planning and risky decision-making (Deakin, Aitken, Dowson, Robbins, & Sahakian, 2004). In the present study, in which the animals had two clear options during the aversive condition, the behavioral suppression release during the PUN period never exceeded the limit of four

responses, which represented the criterion to deliver the shock. So, under the present operant behavioral model, DMI did not alter the rat's decision-making between the choice of feeding and the choice of avoiding punishment.

Beyond the present pharmacological results, some observations can be made with reference to the behavioral method, which can definitely be used with other drugs and with other groups of animals. The comparison of the first unpunished training period under FR1 schedule with all the subsequent inhibitory schedules (UNR and PUN) indicated that this very simple operant schedule of learning, i.e., the FR1 schedule, was effective to produce significant response suppressions. As demonstrated, this effect was not due to some appetitive factor, which may have interacted with the procedure since, compared with the basal FR1 performance study, rats were well motivated to drink milk throughout the whole session under the complex schedule. Likewise, and to be taken into account in future studies with this model, the use of the automated FR1 schedule of reinforcement for all the periods of training offers the possibility to study, in the operant chamber, the effect of drugs on alternative and spontaneous behaviors, such as ambulation, rearing, grooming, etc., which may interact with conditioned responses.

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