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ARTICLE

Anti-anhedonic activity of long-term lithium treatment in rats exposed to repeated unavoidable stress

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Abstract

Behavioural and neurochemical responses to palatable food exposure represent an index of hedonic competence. In rats, a palatable meal increases extra-neuronal dopamine levels in the nucleus accumbens shell (NAcS) that confers to it incentive salience and reinforcing value. Repeated stress exposure decreases dopamine output and impairs the NAcS dopaminergic response to palatable food and the competence to acquire a vanilla sugar (VS)-reinforced instrumental behaviour [VS-sustained appetitive behaviour (VAB)]. Moreover, chronic stress exposure disrupts reactivity to aversive stimuli. A 3-wk treatment with lithium, the gold-standard treatment in bipolar disorder, tonically reduces NAcS dopamine output and the reactivity to aversive stimuli. However, it does not affect the dopaminergic response to VS and the competence to acquire VAB. This study investigated whether repeated lithium administration is endowed with anti-anhedonic activity. The NAcS dopaminergic response to VS and the competence to acquire VAB and sucrose self-administration (SA), in terms of fixed-ratio (FR)1, FR5 and progressive ratio schedules of reinforcement, were studied in saline or lithium-treated groups of non-food-deprived rats exposed or not to repeated unavoidable stress. Chronic stress exposure impaired the NAcS dopaminergic response to VS, acquisition of VAB and sucrose SA, in terms of FR1 and FR5 schedules of reinforcement and breaking point score. Repeated lithium treatment restored these parameters to control group values, even when treatment began in rats already showing an anhedonia-like condition. Since the breaking point defines the reinforcement efficacy of a hedonic stimulus, the present data suggest that lithium treatment is endowed with anti-anhedonic activity in rats.

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Introduction

Anhedonia, a key symptom of major depression, defines the loss of interest or inability to experience pleasure in previously rewarding daily activities. The term reward is complex to define and it has been proposed to connote the primary motivational and emotional effects of reinforcing stimuli (Everitt and Robbins, 2005; Salamone et al., 2005, 2007). Thus, anhedonia may also be defined as the inability to perceive a rewarding stimulus as a reinforcer. In animal models, palatable food may act as a reinforcer and exposure to palatable food is a validated index of hedonic responsiveness that elicits consistent behavioural and neurochemical responses (Willner et al., 1987). We use vanilla sugar (VS) as a palatable food and non-food-deprived rats acquire an instrumental behaviour based on the reinforcing properties of VS [VS-sustained appetitive behaviour (VAB)] (Ghiglieri et al., 1997). VS consumption induces a transient increase in extra-neuronal dopamine in discrete cortical-mesolimbic areas, such as the nucleus accumbens shell (NAcS; Gambarana et al., 2003). The behavioural and neurochemical responses to VS in fed animals are likely dependent on the novelty and hedonic components of the emotional value of VS representation (Di Chiara, 2002). Rats repeatedly exposed to mild unavoidable stressors develop an escape deficit to avoidable aversive stimuli, show a tonic decrease in dopamine output and no dopaminergic response to VS consumption in the NAcS (Gambarana et al., 1999b, 2001). Moreover, chronic stress exposure disrupts the rat's competence to acquire VAB that, like decreased glucose solution consumption (Willner et al., 1987), is considered an anhedonia-like condition (Ghiglieri et al., 1997). A 3-wk lithium treatment induces in rats a condition reminiscent of that observed in chronically stressed animals, since animals show a tonically decreased dopamine output in the NAcS

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and escape deficit (Gambarana et al., 1999a). However, while chronic stress exposure disrupts the NAcS dopaminergic response to VS consumption and the competence to acquire VAB, lithium-treated rats respond to VS and acquire VAB as efficiently as control rats (Masi et al., 2000). Even after a 3-wk administration, lithium does not induce an anhedonia-like condition, an outcome likely correlated with the preserved dopaminergic response to palatable food. Lithium is regarded as the gold- standard treatment for bipolar disorder (Coryell, 2009) and it is also used as augmentation therapy in resistant major depressive episodes (Bauer et al., 2000, 2010; Sugawara et al., 2010). The effects of repeated lithium treatment on VAB acquisition were compared to those of carbamazepine (Grappi et al., 2011), an antimanic compound used as a mood stabilizer in clinical practice (Baldessarini and Tarazi, 2007). Repeated carbamazepine administration disrupts the competence to acquire VAB, thus inducing an anhedonia-like condition (Grappi et al., 2011) and underscoring the peculiarity of long-term lithium effects. The aim of this study was to explore the possible anti-anhedonic activity of lithium. For this purpose, we examined the effects of repeated lithium treatment on the competence of non-food-deprived rats exposed to a chronic stress protocol to acquire VAB. Next, we tested the effects of chronic stress exposure on another palatable food-sustained instrumental behaviour, sucrose self-administration (SA). Stress exposure significantly impaired SA learning and performance; in particular, it decreased the breaking point (BP) score in a progressive-ratio (PR) schedule, which is considered to define the reinforcing efficacy of a pleasurable stimulus (Salamone et al., 2012). We then studied the effects of repeated lithium administration on sucrose SA in rats exposed to the chronic stress procedure.

Method

Animals

Experiments were carried out on male Sprague–Dawley rats (Charles River, Italy), weighing 200–225 g when the experimental procedures began, allowing 10 d habituation to the animal colony. Animals were housed 4–5 per cage in an environment maintained at a constant temperature and humidity with free access to food and water. A 12 h reverse light/dark cycle (lights off 07:00 hours) was used. Experiments were carried out from 09:00 to 17:00 hours under a red light and controlled noise conditions. In all the experiments, body weight did not significantly differ between groups at the beginning and at the end of experimental procedures. The procedures used were in accordance with the European legislation on the use and care of laboratory animals (EU Directive 2010/63) and the guidelines issued by the National Institutes of Health, and were approved by the University of Siena Ethics Committee. All efforts were made to minimize the number of animals used and their suffering.

Chronic stress protocol

The experimental procedure, previously described (Gambarana et al., 2001) and detailed in the Supplementary material, consisted of the induction of an escape deficit and its maintenance by exposure to minor unavoidable stressors. Rats were immobilized with a flexible wire net and administered about 80 tail shocks (1 mA × 5 s, one every 30 s). After 24 h, rats were exposed to a shock–escape test. Rats were then exposed on alternate days to unavoidable stressors, beginning 48 h after the escape test. Rats were exposed to stress sessions in the afternoon, 3–4 h after the end of SA sessions. The performance at the escape test in each experiment is reported in Supplementary Table S1.

Microdialysis procedure

Anaesthetized rats (50 mg/kg pentobarbital, 0.4 mg/kg scopolamine i.p.) were placed in a stereotaxic instrument and a concentric vertical probe was lowered into the NAcS (AP +1.7 mm, L ± 1.2 mm from bregma, V–8.0 mm from skull surface) according to Paxinos and Watson (1998), as described (Rauggi et al., 2005; Danielli et al., 2010) and provided in the Supplementary Material. After surgery, rats had 24 h recovery before the beginning of microdialysis. Water and standard food were available at this time and up to the end of the experiment.

VAB procedure

The experimental procedure, previously described in detail (Ghiglieri et al., 1997) and provided in the Supplementary Material, consisted of sessions of 10 trials with 15-min intervals administered daily between 09:00 and 12:00 hours for a total of 10 sessions, in a Y-maze (15 × 40 × 20 cm for each arm); a VS pellet used as reinforcer was placed at the end of one of the two divergent arms. If the empty arm was chosen (incorrect trial), the rat was returned to its cage before the next trial. When the baited arm was chosen (correct trial) the rat was allowed to consume the VS pellet and then returned to its cage before the next trial. A trial was defined as incomplete when the rat did not reach the end of one of the two arms. In each session, the variables recorded were number of correct, incorrect, incomplete trials and number of VS pellets consumed.

SA procedure

Experiments were conducted in operant response chambers (MED Associates Inc., USA). Chambers enclosed in a ventilated, sound-attenuating box contained two response levers located 6.5 cm above the floor; during SA testing, a lever-press response at the active lever delivered a sucrose pellet into the food receptacle and

produced no programmed consequence at the inactive lever. Inactive lever pressing was recorded and used to control for non-specific responding. A cue light was located 12 cm above the active lever. The house light was turned on at the start of each session. Experimental events and data collection were scheduled using MED Associates software (MED Associates Inc., USA). Before the beginning of the experimental procedure, rats were exposed to the apparatus for 15 min and the next day received 15 min magazine training, with pellets delivered with a 30 s interval independently of the animal's responses. Rats were given daily 30-min sessions between 09:00 and 12:00 hours and had free access to standard food in the home cage before and after each session. Rats were exposed to fixed-ratio (FR)1 sessions until a criterion of $\geq$50 lever presses was reached for 2 d consecutively by the control group; they were then switched to a FR5 schedule. When a criterion of $\geq$50 responses was reached by the control group for 2 d consecutively, rats were switched to a PR schedule, in which the number of lever presses required to receive a sucrose pellet was progressively raised in each test session. In particular, lever pressing was reinforced according to a PR schedule with a step size of 3. The schedule continued until 5 min had elapsed without a response (BP). BP is the conventional index of performance on the PR schedule of reinforcement and it was defined as the number of lever presses in the final completed (i.e. reinforced) ratio before session termination. Rats were exposed to three sessions of PR schedule and averages are reported. The number of responses on the inactive lever was similar between groups (Supplementary Figs. S1, S2).

Drugs

Lithium chloride was dissolved in deionized/distilled water and injected at a volume of 1 ml/kg rat body weight at a dose (0.8 mEq/kg i.p. twice daily) demonstrated to consistently yield plasma levels of 0.7–0.9 mEq/l (Gambarana et al., 1999a, 2000). Rats in the control groups received the same volume of saline. Pentobarbital (Sigma-Aldrich Co., USA) was dissolved in a mixture of 12% ethanol, 38% propylene glycol and 50% deionized/distilled water (vol/vol) containing scopolamine and this solution was injected at a volume of 4 ml/kg body weight.

Statistical analyses

Statistical analyses were performed on commercially available software (GraphPad Prism, GraphPad Software Inc., USA; IBM SPSS, SPSS Inc., USA). Escape test and BP results were analysed using one-way analysis of variance (ANOVA) or the unpaired *t* test. Microdialysis results were analysed using two-way, mixed factorial, repeated-measures ANOVA (r-ANOVA) with group as the between-subject variable and time as the within-subject variable. VAB and SA (FR1, FR5) results were analysed with three-way ANOVA with stress exposure, treatment and session as the three factors, or two-way mixed factorial r-ANOVA with the group as the between-subject variable and the session as the within-subject variable. *Post hoc* analyses were performed by Bonferroni's test when $p<0.05$.

Results

Expt 1: Effects of repeated lithium administration on the NAcS dopaminergic response to VS consumption in control and chronically stressed rats

Rats were divided into four groups: the saline ($n=9$) and the lithium ($n=6$) groups received saline (1 ml/kg) or lithium (0.8 mEq/kg) from day 3; the stress + saline ($n=9$) and the stress + lithium ($n=9$) groups underwent the sequence of unavoidable stress–escape test (days 1 and 2) and were then treated with saline (1 ml/kg) or lithium (0.8 mEq/kg) and exposed to the stress protocol from day 3. Treatments were administered i.p. twice daily. On day 13, rats underwent surgery in the morning, 18–20 h after a stress session, and received treatments in the afternoon and then the next day, 2 h before microdialysis began (Fig. 1*a*). At least four samples were obtained for basal levels determination. Each rat was then presented with five VS pellets and allowed 5 min to consume them; all rats ate the five VS pellets in 1–2 min. After consumption, samples were collected. Baseline dopamine values were different between groups (saline: 6.50 ± 0.7 pg/10 μl; stress + saline: 3.77 ± 0.5 pg/10 μl; stress + lithium: 6.26 ± 0.5 pg/10 μl; lithium: 7.22 ± 0.4 pg/10 μl; ANOVA: $F_{3,32}=7.07$, $p<0.001$); in particular, levels were lower in the stress + saline group compared to the other groups ($p<0.01$ *vs.* the saline and lithium groups; $p<0.05$ *vs.* the stress + lithium group, Fig. 1*c*). At variance with a 3-wk treatment, a 10-d lithium treatment was not associated with reduced dopamine baseline levels, as previously shown (Grappi et al., 2011). Increases in dopamine levels expressed as absolute increment after VS consumption were affected by group ($F_{3,290}=16.22$, $p<0.001$), time ($F_{10,290}=46.93$, $p<0.001$) and their interaction ($F_{30,290}=4.10$, $p<0.001$), as dopamine output increased in the saline, lithium and stress + lithium groups, but it was not significantly modified in the stress + saline group (Fig. 1*b*). These results indicate that lithium treatment restored the dopaminergic response to palatable food in rats exposed to repeated unavoidable stress.

Expt 2: Effects of repeated lithium administration on VAB acquisition in control and chronically stressed rats

Rats were divided into four groups: the saline ($n=6$) and the lithium ($n=7$) groups received saline (1 ml/kg) or lithium (0.8 mEq/kg) from day 3; the stress + saline ($n=6$) and stress + lithium ($n=6$) groups underwent the

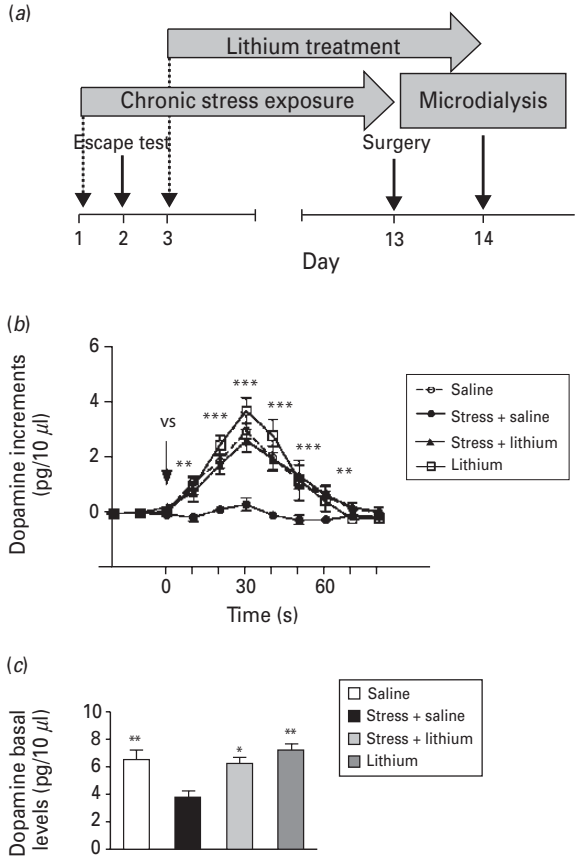

Fig. 1. Changes in the nucleus accumbens shell extra-neuronal dopamine levels in response to vanilla sugar (VS) consumption in control and chronically stressed rats treated or not with lithium. Rats were exposed to the unavoidable stress session on day 1 and were tested for escape on day 2. On day 3 they began treatment with saline (1 ml/kg, stress + saline) or lithium (0.8 mEq/kg, stress + lithium). Treatments continued for 10 d concomitant with exposure to the chronic stress protocol. Two groups of rats not exposed to the stress protocol received saline (1 ml/kg) or lithium (0.8 mEq/kg) for 10 d. Treatments were administered i.p. twice daily. After the assessment of baseline levels, five VS pellets were introduced in the microdialysis cage and samples were collected. (*a*) Outline of the experimental protocol. (*b*) Values represent the mean ± S.E.M. of dopamine increments above basal values. (*c*) Values represent the mean ± S.E.M. of dopamine basal levels. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ *vs.* stress + saline group values (Bonferroni's test).

sequence of unavoidable stress–escape test (days 1 and 2) and were then treated with saline (1 ml/kg) or lithium (0.8 mEq/kg) and exposed to the stress protocol from day 3 (Figs. 2*a* and 3*a*). Treatments were administered i.p. twice daily. On day 13, rats began Y-maze training alternating 1 d training and 1 d stress exposure, while continuing treatments (Fig. 2*a*). The saline group performed more correct trials and consumed more VS pellets with training, while the stress + saline group did not (Fig. 2*b, c*). Lithium treatment restored the performance of rats exposed to repeated stress to values similar to those of the saline group and did not affect performance in non-stressed rats (Fig. 2*b, c*). A stress × treatment × session ANOVA revealed significant main effects of stress exposure (correct trials: $F_{1,24}=9.75$, $p<0.01$; pellets consumed: $F_{1,24}=7.36$, $p<0.05$), treatment (correct trials: $F_{1,24}=5.67$, $p<0.05$; pellets consumed: $F_{1,24}=6.90$, $p<0.05$), session (correct trials: $F_{9,216}=16.4$ $p<0.001$; pellets consumed: $F_{9,216}=50.10$, $p<0.001$), as well as significant stress × treatment (correct trials: $F_{1,24}=24.40$, $p<0.001$; pellets consumed: $F_{1,24}=18.90$, $p<0.001$), treatment × session (correct trials: $F_{9,216}=5.82$, $p<0.01$; pellets consumed: $F_{9,216}=4.57$, $p<0.001$), stress × session (pellets consumed: $F_{9,216}=3.20$, $p<0.01$) and stress × treatment × session (correct trials: $F_{9,216}=2.14$, $p<0.05$; pellets consumed: $F_{9,216}=5.95$, $p<0.001$) interactions. Analysis of the number of incorrect trials indicated only an effect of session ($F_{9,216}=6.87$, $p<0.001$) and significant stress × session ($F_{9,216}=2.08$, $p<0.05$), treatment × session ($F_{9,216}=3.13$, $p<0.01$) and stress × treatment × session ($F_{9,216}=3.20$, $p<0.01$) interactions (Fig. 2*d*). The stress + saline group showed a number of incomplete trials higher than the other groups (Fig. 2*e*). A stress × treatment × session ANOVA revealed significant main effects of stress ($F_{1,24}=5.21$, $p<0.05$) and session ($F_{9,216}=7.89$, $p<0.001$), as well as significant stress × treatment ($F_{1,24}=11.30$, $p<0.01$), stress × session ($F_{9,216}=3.07$, $p<0.01$) and treatment × session ($F_{9,216}=3.08$, $p<0.01$) interactions. These results indicate that lithium administration restored the competence of rats exposed to repeated unavoidable stress to acquire VAB. Following 24 h after the last Y-maze training session, rats were exposed to the escape test. The number of escapes was different between groups (one-way ANOVA, $F_{2,23}=131.5$, $p<0.001$); *post hoc* analysis demonstrated a clear-cut escape deficit in the stress + saline group, while the stress + lithium and saline groups had similar performances; lithium treatment did not affect performance in non-stressed rats (Fig. 3*b*).

Expt 3: effects of repeated lithium administration on the performance of control and chronically stressed rats in the SA paradigm

Preliminary experiments indicated that exposure to the chronic stress protocol reduced lever pressing for sucrose pellets in non-food-deprived rats. The purpose of the experiment was to confirm this effect and investigate whether lithium treatment would antagonize the stress-induced deficit, similar to what was observed with the VAB paradigm. Rats were divided into four groups: the saline ($n=10$) and lithium ($n=12$) groups received saline (1 ml/kg) or lithium (0.8 mEq/kg) from day 3; the stress + saline ($n=10$) and stress + lithium ($n=12$) groups underwent the sequence of unavoidable stress–escape test (day 1 and 2) and were then treated with saline (1 ml/kg) or lithium (0.8 mEq/kg) and exposed to the

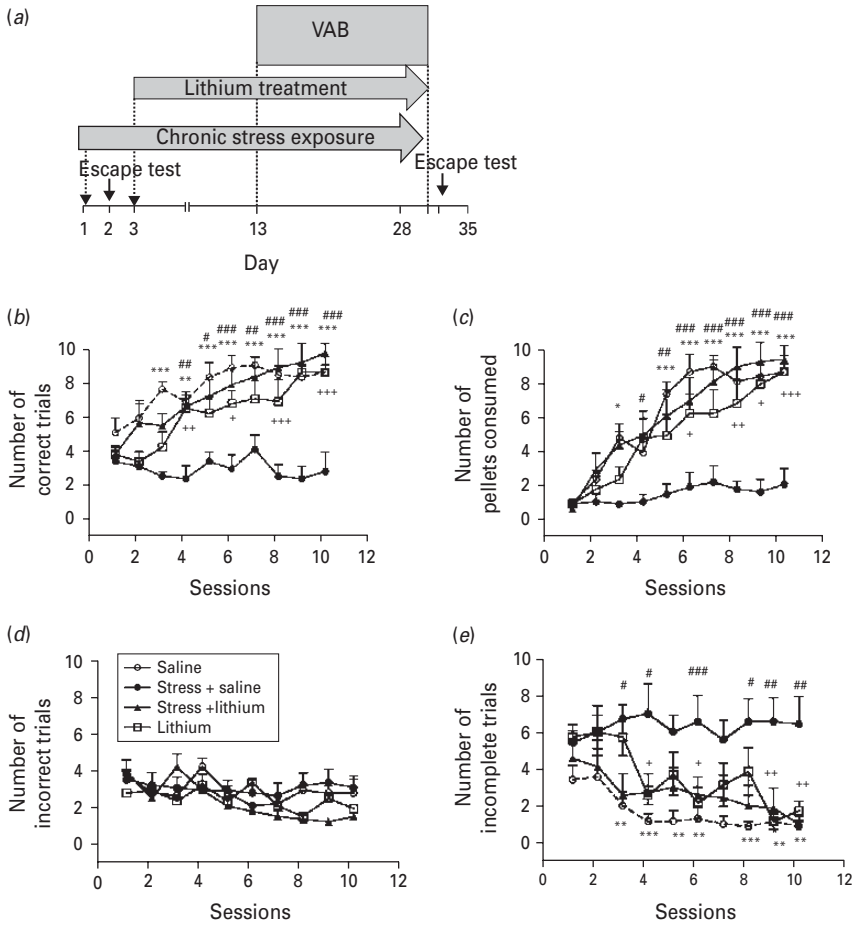

Fig. 2. Vanilla sugar-sustained appetitive behaviour (VAB) acquisition in control and chronically stressed rats treated or not with lithium. Rats were exposed to the unavoidable stress session on day 1 and were tested for escape on day 2. On day 3 they began treatment with saline (1 ml/kg, stress + saline) or lithium (0.8 mEq/kg, stress + lithium); treatments continued concomitant with exposure to the chronic stress protocol. Two groups of rats not exposed to the stress protocol received saline (1 ml/kg) or lithium (0.8 mEq/kg). Treatments were administered i.p. twice daily. After 10 d treatment, all groups began the Y-maze training. (*a*) Outline of the experimental protocol. Data are presented as mean ± S.E.M. of the number of correct (*b*), incorrect (*d*) and incomplete trials (*e*), and number of pellets consumed (*c*). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ saline *vs.* stress + saline group. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ stress + lithium *vs.* stress + saline group. $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ lithium *vs.* stress + saline group.

stress protocol from day 3. Treatments were administered i.p. twice daily. On days 11 and 12, after 8 and 9 d respectively treatment and stress exposure, rats received the two pre-training sessions and then on day 13 began daily sessions of SA training (between 09:00 and 12:00 hours) while continuing treatments and stress exposure (between 15:00 and 17:00 hours; Fig. 4*a*). Under the FR1 schedule, the saline, lithium and stress + lithium groups showed increased responses on the correct lever with training, while the stress group had a lower rate of responses (Fig. 4*b*). In particular, a stress × treatment × session ANOVA revealed significant main effects of session ($F_{6,26}=38.69$, $p<0.001$) and treatment ($F_{1,44}=14.15$, $p<0.001$), as well as significant stress × treatment ($F_{1,44}=13.28$, $p<0.001$) and stress × treatment × session ($F_{6,26}=2.65$, $p<0.01$) interactions. Similar results were observed for lever pressing under the FR5 schedule, as the saline group showed a clear-cut increase in lever presses with repeated exposure to sessions of operant behaviour, the lithium and stress + lithium groups showed an increase in responses and the stress group did not improve performance with progressive sessions (Fig. 4*c*). A stress × treatment × session ANOVA revealed significant main effects of session ($F_{6,26}=33.09$, $p<0.001$) and exposure to stress ($F_{1,44}=9.41$, $p<0.01$), as well as significant stress × treatment ($F_{1,44}=9.97$, $p<0.01$), stress × session ($F_{6,26}=7.69$, $p<0.001$) and stress × treatment × session ($F_{6,26}=6.21$, $p<0.001$) interactions. Under the PR schedule, analysis of the BP by one-way ANOVA showed a significant difference between groups ($F_{3,43}=4.90$, $p<0.01$). *Post hoc* analysis confirmed that chronic stress exposure strongly reduced motivation to lever pressing for sucrose pellets (stress compared to the saline group: $p<0.01$) and that lithium treatment restored the incentive motivation of stressed rats (stress + lithium compared to the stress group: $p<0.05$; Fig. 4*d*).

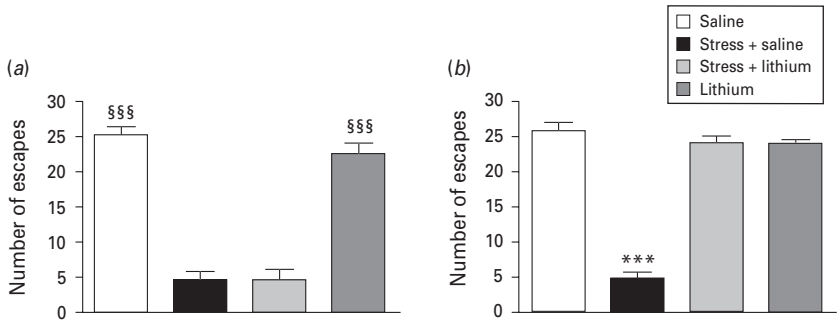

Fig. 3. Number of escapes scored by rats in the saline, lithium, stress + saline and stress + lithium groups at the end of Y-maze training. Rats were exposed to the unavoidable stress session on day 1 and were tested for escape on day 2. On day 3 they began treatment with saline (1 ml/kg, stress + saline) or lithium (0.8 mEq/kg, stress + lithium); treatments continued concomitant with exposure to the chronic stress protocol. Two groups of rats not exposed to the stress protocol received saline (1 ml/kg) or lithium (0.8 mEq/kg). Treatments were administered i.p. twice daily. After 10 d treatment, all groups began the Y-maze training. At 24 h after the end of Y-maze training they were tested for escape. (*a*) Scores at the initial escape test after induction; (*b*) scores at the final escape test after vanilla sugar-sustained appetitive behaviour training. Scores are expressed as mean number of escapes ± S.E.M. in 30 consecutive trials. (*a*) §§§ $p < 0.001$ stress + saline and stress + lithium *vs.* saline and lithium groups; (*b*) *** $p < 0.001$ stress + saline *vs.* saline, stress + lithium and lithium groups (Bonferroni's test).

Expt 4: effects of repeated lithium administration on performance in the SA paradigm of control rats and rats showing a stress-induced anhedonia-like condition

We investigated whether lithium treatment would reverse an established stress-induced anhedonia-like condition. Rats were divided into two groups: control rats (Con, $n = 6$) and rats exposed to the sequence of unavoidable stress–escape test (day 1 and 2) and then to the stress protocol (stress, $n = 12$). On days 8–9, rats received the two pre-training sessions; on day 10 SA training began under FR1 (five sessions) and FR5 (three sessions) schedules (09:00 to 12:00 hours), while stress exposure continued (15:00 to 17:00 hours; Fig. 5*a*). Analysis by two-way ANOVA of the number of lever presses revealed a significant effect of stress exposure (FR1: $F_{1,80} = 64.16$, $p < 0.001$; FR5: $F_{1,32} = 32.33$, $p < 0.001$), session ($F_{4,80} = 8.64$, $p < 0.001$; FR5: $F_{2,32} = 17.46$, $p < 0.001$) and their interaction (FR1: $F_{4,80} = 3.36$, $p < 0.01$; FR5: $F_{2,32} = 10.91$, $p < 0.001$). *Post hoc* analysis demonstrated that, under unavoidable stress exposure, rats did not acquire the operant behaviour (FR1: stress *vs.* Con group: $p < 0.01$ at session 3, $p < 0.001$ at sessions 4 and 5; FR5: $p < 0.001$ in all sessions; Fig. 5*b, c*). On day 21 rats exposed to the stress protocol were divided in two groups: six rats received saline (1 ml/kg, chronic stress + saline) and six rats received lithium (0.8 mEq/kg, chronic stress + lithium), while continuing stress exposure; six control rats received saline (1 ml/kg). Treatments were administered i.p. twice daily. After 7 d treatment (day 28), SA training with an FR5 schedule was resumed (Fig. 5*a*). Analysis by two-way ANOVA of lever presses revealed a significant effect of group ($F_{2,90} = 6.58$, $p < 0.01$), session ($F_{6,90} = 20.68$, $p < 0.001$) and their interaction ($F_{12,90} = 3.71$, $p < 0.001$). *Post hoc* analysis demonstrated that acquisition of the operant behaviour was reduced in the chronic stress + saline compared to the saline group ($p < 0.05$ at session 4, $p < 0.001$ at sessions 5, 6 and 7), while lithium treatment counteracted the effect of stress exposure since the performance of the chronic stress + lithium group differed from that of the chronic stress + saline group ($p < 0.05$ at sessions 6 and 7; Fig. 5*d*). Under the PR schedule, analysis of the BP by one-way ANOVA showed a significant difference between groups ($F_{2,17} = 14.90$, $p < 0.001$). *Post hoc* analysis confirmed that chronic stress exposure reduced motivation to lever pressing for sucrose pellets (chronic stress + saline *vs.* saline group: $p < 0.01$) and that lithium treatment restored the incentive motivation of stressed rats (chronic stress + lithium *vs.* the chronic stress + saline group: $p < 0.01$; Fig. 5*e*).

Discussion

This study examined the effects of repeated lithium treatment on the responses to palatable food in rats exposed to a chronic stress procedure that consistently disrupts the competence to avoid aversive stimuli and operate to obtain a positive reinforcer (Gambarana et al., 2001). Stress-induced escape deficit and the reduced competence to acquire VAB or sucrose SA mimic respectively the increased negative affect reactivity to stress (Wichers et al., 2009) and the reduced positive affect (anhedonia) in depressed patients (Heller et al., 2009; Wichers et al., 2009). In rats these two behavioural deficits can be dissociated, as animals that have acquired VAB develop a clear-cut escape deficit when exposed to unavoidable stressors, yet their discriminative performance is not affected (Ghiglieri et al., 1997). Thus, the two behavioural deficits likely depend on different mechanisms (Hikida et al., 2010). However, repeated lithium administration prevents the occurrence of this dissociation. A 3-wk lithium treatment induces escape deficit and tonic decrease in dopamine levels and output in the NAcS; yet,

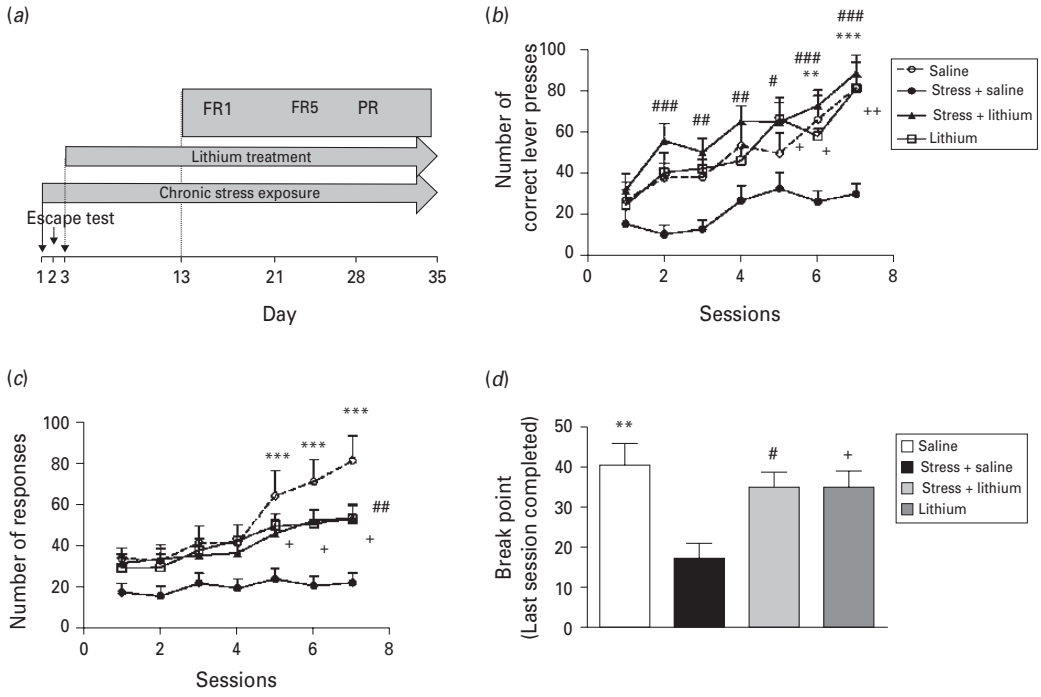

Fig. 4. Responding for sucrose pellets under fixed-ratio (FR)1, FR5 and progressive-ratio (PR) schedules in control and chronically stressed rats treated or not with lithium. Rats were exposed to the unavoidable stress session on day 1 and were tested for escape on day 2. On day 3 they began treatment with saline (1 ml/kg, stress + saline) or lithium (0.8 mEq/kg, stress + lithium); treatments continued concomitant with exposure to chronic stress protocol. Two groups of rats not exposed to the stress protocol received saline (1 ml/kg) or lithium (0.8 mEq/kg). Treatments were administered i.p. twice daily. After 10 d treatment, the four groups of non-food-deprived rats were trained to press a lever for sucrose pellets under FR1 and then FR5 schedules of reinforcement. When stable responses under FR5 reinforcement were established, rats were switched to an operant behaviour under a PR schedule with a step size of 3. (*a*) Outline of the experimental protocol. Data are presented as mean ± S.E.M. of the number of responses under FR1 (*b*) FR5 (*c*) and PR (*d*) schedules. (*b*, *c*, *d*) ** $p < 0.01$, *** $p < 0.001$ saline *vs.* stress + saline group; $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ stress + lithium *vs.* stress + saline group; $^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$ lithium *vs.* stress + saline group (Bonferroni's test).

lithium-treated rats easily acquire VAB and this behavioural modification is associated with a reinstatement of dopaminergic transmission and competence to escape an avoidable aversive stimulus (Masi et al., 2000). In agreement with previous results (Gambarana et al., 2001), rats exposed to the chronic stress protocol showed no dopaminergic response to VS consumption in the NAcS and a clear-cut deficit in the acquisition of VAB and sucrose SA in terms of FR1 and FR5 schedules of reinforcement. Lithium administration completely restored the dopaminergic response to palatable food consumption and the competence to acquire instrumental behaviours reinforced by palatable food, even when the stress-induced deficit was already manifested at the beginning of treatment. Since experiments were carried out in non-food-deprived rats, hedonic value was the prevalent emotional component of VS or sucrose representations. The acquisition of VAB and sucrose SA by rats exposed to chronic stress suggests that lithium treatment reinstated the competence to confer incentive value to the representation of palatable food, likely through an effect on dopaminergic transmission. Moreover, VAB acquisition by lithium-treated rats exposed or not to stress likely contributed to reinstating the competence to escape aversive avoidable stimuli through a common mechanism. The dopaminergic system plays a central role in the frontal-mesolimbic network and, although dopaminergic transmission does not directly affect the hedonic response to a rewarding stimulus (Berridge and Robinson, 1998), the phasic increase in dopamine output in these areas in response to a relevant stimulus is dependent on and quantifies the actual emotional value of its representation and confers incentive properties to it (Palmiter, 2007). This dopamine effect has been explained in terms of incentive salience (Berridge, 2007), sensorimotor arousal or activation (Salamone et al., 2005) or incentive arousal (Di Chiara, 2002). That is, the capacity to confer incentive properties to a stimulus and make a reinforcer out of it is dependent on the mesolimbic dopaminergic responsiveness. As a consequence of this hypothesis, it has been proposed that animals like the effects of dopamine signalling and that they will engage in natural activities that maintain elevated levels of dopamine (Palmiter, 2007). In this context, a condition of disrupted competence to acquire instrumental behaviours reinforced by palatable food associated with tonically decreased dopamine output and a lack of dopaminergic response to a palatable meal in the NAcS, as observed in chronically stressed

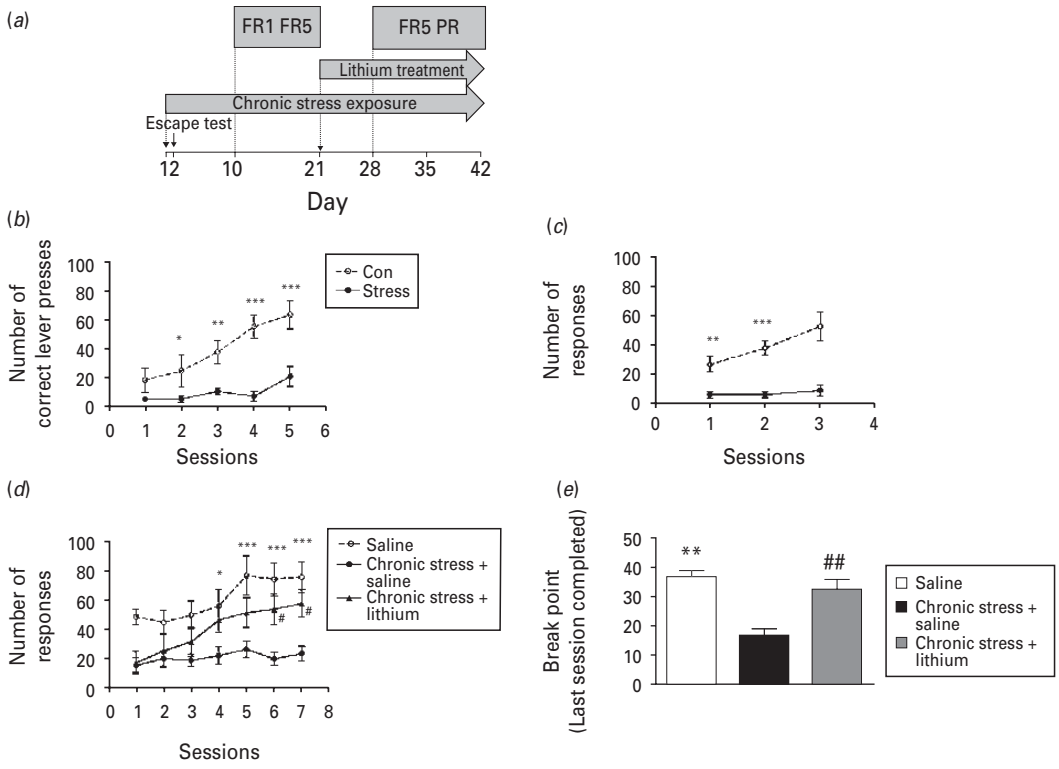

Fig. 5. Responding for sucrose pellets under fixed-ratio (FR)1, FR5 and progressive-ratio (PR) schedules in control and 'anhedonic' rats treated or not with lithium. Rats were exposed to the unavoidable stress session on day 1 and were tested for escape on day 2. On day 3 they began the chronic stress protocol (stress). A group of rats were not exposed to the stress protocol [control (Con)]. After 7 d stress exposure, the two groups of rats were trained to press a lever for sucrose pellets under FR1 (*b*) and FR5 (*c*) schedules of reinforcement. The stress group rats attained the criterion for anhedonia and were divided into two groups treated with saline (1 ml/kg, chronic stress + saline) or lithium (0.8 mEq/kg, chronic stress + lithium) for 7 d; then they resumed the FR5 (*d*) and PR schedules (*e*), while treatments continued concomitant with exposure to the chronic stress protocol. Treatments were administered i.p. twice daily. (*a*) Outline of the experimental protocol. Data are presented as mean ± S.E.M. of the number of responses. (*b*, *c*) * $p<0.05$, ** $p<0.01$, *** $p<0.001$ Con *vs.* stress group (Bonferroni's test); (*d*) * $p<0.05$, *** $p<0.001$ saline *vs.* chronic stress + saline group; # $p<0.05$, chronic stress + lithium *vs.* chronic stress + saline group (Bonferroni's test); (*e*) ** $p<0.01$ chronic stress + saline *vs.* saline group, ## $p<0.01$ chronic stress + lithium *vs.* chronic stress + saline group (Bonferroni's test).

rats, seems to conform to the face validity criteria for an anhedonia-like model. Moreover, a drug able to restore the tendency to engage in dopamine stimulating behaviours in a condition of decreased dopaminergic transmission can be proposed to have anti-anhedonic activity. Interestingly, rats that have acquired VAB show a tonically increased dopamine output in the medial prefrontal cortex and NAcS (Masi et al., 2001); that is, VAB acquisition models a behavioural pattern that maintains elevated dopaminergic transmission. Thus, the finding that lithium treatment restored the dopaminergic response to VS consumption and the competence to acquire VAB in rats exposed to repeated unavoidable stress before or during Y-maze training allows for the conclusion that lithium is endowed with anti-anhedonic activity. It would follow that stress-induced anhedonia appears to be mainly a deficit in conferring incentive salience to commonly pleasurable–rewarding stimuli.

Stress could, however, disrupt the competence to acquire an instrumental appetitive behaviour by directly acting on learning mechanisms. Indeed, a hypothesized effect of stress on learning mechanisms underlies the concept of learned helplessness (Overmier and Seligman, 1967) and impairments in learning in animals exposed to uncontrollable stress are readily demonstrated in a variety of paradigms, such as active avoidance (Foa et al., 1992) or the radial maze (Luine et al., 1994). Moreover, lithium effects on the acquisition of appetitive behaviours could also be related to a reduction in stress-induced cognitive deficits. Reports on the cognitive effects of lithium in humans (Ananth et al., 1987; Pachet and Wisniewski, 2003; O'Donnell and Gould, 2007) and animals (Hines and Poling, 1984; Hines, 1985, 1986; Cappeliez et al., 1989; Gallo et al., 1990; Pascual and Gonzalez, 1995; Nocjar et al., 2007; O'Donnell and Gould, 2007; Tsaltas et al., 2007a, b) are inconsistent. However, the finding that repeated lithium administration restores the analgesic effect associated with sweet taste pre-exposure, which had previously been abolished by chronic variable stress, led to the conclusion that lithium may counteract stress-induced anhedonia (Vasconcellos et al., 2006). This hypothesis is sustained by

the present finding that stressed rats showed a BP score markedly reduced compared to control animals and lithium treatment completely restored this parameter value. With a PR schedule, the ratio requirement increases as a successive ratio is completed, and the final ratio completed by the subject before the session ends is termed the BP, which is a measure of how much work an animal will exert in order to gain access to the reinforcing stimulus (Stewart, 1975; Salamone et al., 2012), which may be a useful measure of reinforcing value. Indeed, the BP, originally proposed as a measure of reward strength (Hodos, 1961), is presently considered to mainly define the reinforcement efficacy of a hedonic stimulus (Salamone et al., 2012). Thus, the condition of reduced reinforcement efficacy of pleasurable stimuli, as induced in rats by chronic stress exposure, conforms to the criteria of face validity for a model of anhedonia. Moreover, the fact that lithium treatment restored the BP scores to control values suggests that this compound is endowed with anti-anhedonic activity.

This body of experimental data raises the question of whether lithium anti-anhedonic activity may explain some of its unique clinical effects. This is a complex issue as there is not even complete agreement on the clinical significance of the term anhedonia. In fact, anhedonia is defined as mere inability to experience pleasure or as encompassing a deficit in dopamine-dependent responses to pleasurable stimuli (i.e. reduced competence to confer incentive value to a pleasurable stimulus; impaired decision making in the context of reward; Treadway and Zald, 2011). Depressed patients show increased sensitivity to negative daily life events with decreased sensitivity toward positive stimuli (Sloan et al., 1997; Dunn et al., 2004; Shankman et al., 2007) and altered reward-related, decision-making competence (Forbes et al., 2007). Pharmacotherapy with antidepressant medications decreases stress sensitivity and increases reward experience (Wichers et al., 2009; Geschwind et al., 2011). Early changes in positive affect, compared to early reductions in sensitivity to negative events, best predict the response to treatment (Geschwind et al., 2011) and the increase in positive affect discriminates between treatment responders and non-responders. Hence, anhedonia in depressed patients can be dissociated from stress resilience, similar to that observed in rats, and the persistence of anhedonia seems to impair the response to an antidepressant treatment. In this context, the anti-anhedonic activity of lithium, which in rats preceded the development of stress resilience, may account for its efficacy as augmentation therapy in drug-resistant depression. However, to the best of our knowledge, this specific issue has not yet been addressed in clinical studies.

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Statement of Interest

None.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145712001654.

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