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Antidepressant and pro-motivational effects of repeated lamotrigine treatment in a rat model of depressive symptoms

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Abstract

Background: The antiepileptic lamotrigine is approved for maintenance treatment of bipolar disorder and augmentation therapy in treatment-resistant depression. Previous preclinical investigations showed lamotrigine antidepressant-like effects without addressing its possible activity on motivational aspects of anhedonia, a symptom clinically associated with poor treatment response and with blunted mesolimbic dopaminergic responsiveness to salient stimuli in preclinical models. Thus, in rats expressing a depressive-like phenotype we studied whether repeated lamotrigine administration restored behavioral responses to aversive and positive stimuli and the dopaminergic response to sucrose in the nucleus accumbens shell (NAcS), all disrupted by stress exposure.

Methods: Depressive-like phenotype was induced in non-food-deprived adult male Sprague-Dawley rats by exposure to a chronic protocol of alternating unavoidable tail-shocks or restraint periods. We examined whether lamotrigine administration (7.5 mg/kg twice a day, i.p.) for 14–21 days restored a) the competence to escape aversive stimuli; b) the motivation to operate in sucrose self-administration protocols; c) the dopaminergic response to sucrose consumption,

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evaluated measuring phosphorylation levels of cAMP-regulated phosphoprotein Mr 32,000 (DARPP-32) in the NAcS, by immunoblotting.

Results: Lamotrigine administration restored the response to aversive stimuli and the motivation to operate for sucrose. Moreover, it reinstated NAcS DARPP-32 phosphorylation changes in response to sucrose consumption.

Limitations: The pro-motivational effects of lamotrigine that we report may not completely transpose to clinical use, since anhedonia is a multidimensional construct and the motivational aspects, although relevant, are not the only components.

Conclusions: This study shows antidepressant-like and pro-motivational effects of repeated lamotrigine administration in a rat model of depressive symptoms.

Keywords: Neuroscience, Psychiatry, Pharmaceutical science

1. Introduction

Lamotrigine is a widely used antiepileptic agent with a broad spectrum of anticonvulsant efficacy that is approved by the FDA and EMA for maintenance treatment of bipolar disorder without an indication for acute mania [1, 2, 3]. It is particularly effective for the prevention of bipolar depression relapse [4, 5, 6, 7, 8, 9] and recent guidelines recommend lamotrigine as the first-line treatment for mild to moderate bipolar depression [10]. Moreover, it is also used in treatment-resistant unipolar depression as an augmentation drug and in post-traumatic stress disorder [11, 12, 13].

When the first clinical studies indicated lamotrigine efficacy in the treatment of bipolar depression [4, 9, 14], rodent studies were carried out in order to verify its effects in tests for antidepressant activity and models of affective disorders. Antidepressant-like effects of lamotrigine have been reported after acute treatment in the forced swimming test (FST) in mice and rats [15, 16] or after sub-chronic administration in the learned helplessness model [16]. After long-term administration, lamotrigine shows antidepressant-like activity in rats in the maternal deprivation-induced model [17], and in the chronic unpredictable stress model, where it positively affects performance in the FST and tail suspension test, the sucrose preference test, and the novelty-suppressed feeding test [18].

Although the anticonvulsant effects of lamotrigine are most likely related to the inhibition of voltage-sensitive sodium channels [19, 20], cellular and animal studies suggest that it may modulate monoaminergic systems [15, 21, 22, 23] and may exert neuroprotective effects by reducing oxidative stress [24]. However, it is still unclear which of these molecular mechanisms are relevant for lamotrigine efficacy in the treatment and prevention of bipolar depression [25]. Some studies support an

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involvement of the brain-derived neurotrophic factor (BDNF) signaling pathway in the behavioral antidepressant-like effects of lamotrigine [16, 17, 18]. Other evidences suggest that lamotrigine's mood stabilizing efficacy may be related to the enhancement of the hyperpolarization-activated inward current I_h [26]. This current plays an important role in modulating the excitability of ventral tegmental area (VTA) dopamine neurons, specifically the VTA dopaminergic neurons projecting to the nucleus accumbens (NAc) [27]. In the chronic social defeat stress model the antidepressant-like effects of lamotrigine have been ascribed to its ability to regulate VTA dopamine neuron excitability through an increase in I_h current [27].

Anhedonia, defined as loss of interest or pleasure in previously pleasurable activities, is a core symptom of depression [28] that occurs in half of the patients with bipolar depression [29] and has been associated with poor treatment response [30, 31]. Anhedonia may be considered as a composite symptom that can be resolved in different components: a reduction in the experience of pleasure (liking), a deficit in the motivation to obtain a reward (wanting), and, possibly, a disruption in reward learning [32]. The lack of motivation (motivational anhedonia) may reflect an underlying dysfunction in dopaminergic reward circuit that originates in the VTA and projects to the NAc, a key brain region in the ventral striatum that integrates different excitatory and inhibitory inputs to signal the salience of stimuli [33]. Previous studies of lamotrigine effects in animal models of depression have only explored the consummatory aspect of anhedonia by the sucrose preference test. Thus, we aimed to investigate whether long-term lamotrigine administration affected the responses to aversive and positive stimuli, and specifically reinstated the motivation to operate for a natural reward in a rat model of depression. To this end, we used a well-validated model of depressive behaviors induced by exposure to an unavoidable chronic stress protocol, that is characterized by decreased reactivity to aversive stimuli (escape deficit) and reduced motivation to operate to earn a palatable food, sucrose (motivational anhedonia) [34, 35]. Motivational anhedonia, assessed in rats by sucrose self-administration, is accompanied by decreased dopaminergic responses to a natural reward (sucrose) in the shell of the NAc (NAcS) [35]. The dopaminergic responses are assessed by analyzing the levels of extraneuronal dopamine and measuring the dopamine D_1 receptor-dependent signaling, in terms of cAMP-dependent protein kinase (PKA)-dependent phosphorylation of the threonine (Thr) 34 residue of dopamine and cAMP-regulated phosphoprotein Mr 32,000 (DARPP-32) [36]. Pharmacological treatments that reinstate motivation to operate for a natural reinforcer in self-administration protocols (lithium, imipramine, clozapine, aripiprazole, and fenofibrate), also restore the dopaminergic response to sucrose consumption in the NAcS of stress-exposed rats, regardless of the acute molecular mechanism [35, 37, 38, 39]. Thus, considering the reported effects of lamotrigine on the VTA dopaminergic neurons projecting to the NAc [27], we investigated whether repeated lamotrigine treatment could reverse the stress-induced

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disruption of the dopaminergic transmission in response to sucrose consumption, evaluated in terms of Thr34 DARPP-32 phosphorylation levels in the NAcS. Moreover, the study aimed to further validate the hypothesis that the re-established dopaminergic response to sucrose is accompanied by a restored motivation to operate for the reward disrupted by stress exposure, using a sucrose self administration protocol [35, 37, 38, 39]. The results of this study may have a translational value as they could suggest possible clinical uses of lamotrigine to address specific symptoms in unipolar or bipolar depression, in particular the symptom domain of motivational anhedonia.

2. Materials and methods

2.1. Animals

Experiments were carried out on male Sprague-Dawley rats (Charles River, Calco, Italy), 9−10 week old and the experimental procedures began after 10 days of habituation to the animal colony. Rats were housed in stable social groups of 4−5 animals per cage (bedding Lignocel® 3/4S, Harlan Laboratories, San Pietro al Natisone, Italy) in a room maintained at constant temperature and humidity, on a 12 h reverse light/dark cycle (lights on from 7 am to 7 pm) with free access to food (4RF21, Mucedola, Settimo Milanese, Italy) and water. All rats were manipulated daily by experimenters. Locomotor activity tests, escape tests and sucrose self-administration protocols were performed between 9 and 12 am under red light and controlled noise conditions, while stress exposure was carried out between 3 and 4 pm. The procedures used were in compliance 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.

2.2. Acute escape deficit induction and chronic stress protocol

Acute escape deficit was induced by exposure to an unavoidable stress session (pretest) [34, 40]. Rats, immobilized with a flexible wire-net, were administered 80 tail shocks (1 mA × 5 s, 1 every 30 s) in 50 min and 24 h later they were exposed to a shock-escape test. The escape deficit criterion was an escape number from 0 to 6 in 30 trials.

The condition of escape deficit was chronically maintained by repeated exposure to minor unavoidable stressors (10 min tail-shocks or restraint) on alternate days, as described [34]. Rats were restrained by immobilizing them in a flexible wire net for 10 min. Exposure to unavoidable stress sessions were performed 3−4 h after the end of self-administration sessions.

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2.3. Locomotor activity

Locomotor activity was measured as previously described in an apparatus (Imetronic, Pessac, France), composed of eight compartments, each of them equipped with a transparent Perspex motility cage (23 × 33 × 19 cm) and a system of photocell beams that detected horizontal and vertical activity [41]. On the test day rats were placed in motility cages for 35 min and total motility counts were recorded in the last 30 min.

2.4. Self-administration procedure

Responding for sucrose was performed in rat operant chambers equipped with two retractable levers (MED Associates Inc., St. Albans, VT, USA). Pressing on active lever delivered a sucrose pellet (Bio-Serv, Frenchtown, NJ, USA) into the food receptacle, while pressing on the inactive lever had no programmed consequences [35]. Experimental events and data collection were scheduled using MED Associates software (MED Associates Inc.). Experiments were carried out daily between 9 and 12 am in 30-min sessions in non food-deprived rats that had free access to food and water in the home cage before and after each session. Rats were exposed to a fixed-ratio 1 (FR1) schedule until the control group reached a criterion of 50 lever presses for 2 consecutive days, then they were switched to a fixed ratio 5 (FR5) schedule. When the control group reached a criterion of 40 responses, rats were switched to a progressive ratio (PR) schedule, in which the number of responses required to receive a sucrose pellet was progressively increased with a step size of 3 until 5 min had elapsed without a response (breaking point, BP). BP was defined as the number of lever presses in the final completed ratio. The criterion for a deficit in appetitive motivation, induced by exposure to the chronic stress protocol, was a lever-pressing rate lower than 60% of the control group rate in FR1 and FR5 schedules [37].

2.5. Immunoblotting

NAcS was identified using the Rat Brain Atlas as corresponding to plates 10−12 [42] and excised using rapid head-freeze technique [38, 43]. Tissues were solubilized in boiling 1% sodium dodecyl sulfate (SDS) and 50 mM NaF, and protein content was determined by a modified Lowry protein assay method (DC protein assay, Bio-Rad Laboratories, Hercules, CA, USA). Western blot analysis was performed as previously described [44, 45]. Briefly, proteins (30 μg) were resolved into 10% SDS−PAGE gels and electro-transferred onto nitrocellulose membranes. Membranes were incubated with anti-phospho-Thr34 DARPP-32 (Thr34), anti-DARPP-32 (Cell Signaling Technology, Beverly, MA, USA) and anti-β-actin antibodies. To control for equal loading, membranes incubated with anti-DARPP-32 were stripped and reprobed with anti-β-actin, and membranes incubated with anti-phospho-Thr34 DARPP-32 were stripped and reprobed with anti-DARPP-32 antibody. Detection

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of proteins was performed using a chemiluminescence system (Pierce Biotechnology Inc., Rockford, IL, USA) and quantified with the Versa Doc 1000 Imaging System (Bio-Rad Laboratories). Total DARPP-32 levels were normalized to those of β-actin, phospho-protein levels were normalized to those of DARPP-32.

2.6. Drugs and chemicals

Lamotrigine (Shreeji Pharma International, Vadodara, India) was dissolved in 0.5% methylcellulose and 0.4% Tween 80 in deionized/distilled water and administered at the dose of 7.5 mg/kg i.p. twice a day or 15 mg/kg i.p. twice a day [16, 18]. Rats in the control groups received vehicle (0.5% methylcellulose and 0.4% Tween 80 in deionized/distilled water). All treatments were administered in a volume of 1 ml/kg body weight. All other chemicals were purchased from commercial sources.

2.7. Statistical analysis

Statistical analyses were performed on commercially available software (GraphPad Prism statistical package, GraphPad, San Diego, CA, USA). Differences between two groups were determined by unpaired Student's *t*-test. Self-administration experiment data (FR1 and FR5) were analyzed by 2-way repeated measures analysis of variance (RM ANOVA) with group as main factor and sessions as repeated factor. BP results and escape numbers were analyzed using one-way ANOVA or two-way ANOVA, as appropriate. Data on DARPP-32 phosphorylation levels after sucrose consumption were analyzed by two-way ANOVA. Post-hoc analyses were performed by the Bonferroni's test, when $p < 0.05$.

3. Experimental

3.1. Lamotrigine effects on reactivity to aversive stimuli

a) Response to acute avoidable stress. First we investigated whether repeated lamotrigine administration impaired the escape response to avoidable aversive stimuli. Rats, divided into 3 groups, were tested for escape without exposure to the unavoidable stress session: one group received the vehicle (1 ml/kg i. p. twice a day, Naive vehicle group, $n = 6$); one group received lamotrigine, 7.5 mg/kg i. p. twice a day (LTG 7.5 group, $n = 8$); one group received lamotrigine, 15 mg/kg i. p. twice a day (LTG 15 group, $n = 8$; Table 1). Lamotrigine or vehicle were administered for 14 days. In order to evaluate whether lamotrigine treatment, at the dose and regimen used, modified motility, and thus confounded the results of escape tests, locomotor activity was evaluated 24 h before the escape test.

b) Prevention of the consequences of acute stress exposure. We then investigated whether a 14-day lamotrigine administration prevented the acute consequences

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Table 1. Outline of experimental protocols.

Experiment	Experimental groups	*n*	Treatment	Test
Acute avoidable stress	*Vehicle Naive*	6	Vehicle 1 ml/kg i. p. twice a day for 14 days	Escape test
	LTG 7.5	8	Lamotrigine 7.5 mg/kg i. p. twice a day for 14 days	Escape test
	LTG 15	8	Lamotrigine 15 mg/kg i. p. twice a day for 14 days	Escape test
Prevention	*Vehicle Naive*	6	Vehicle 1 ml/kg i. p. twice a day for 14 days	Escape test
	Vehicle + Acute Stress	6	Vehicle 1 ml/kg i. p. twice a day for 14 days	Pre-test and escape test
	LTG Naive	6	Lamotrigine 7.5 mg/kg i.p. twice a day for 14 days	Escape test
	LTG + Acute Stress	6	Lamotrigine 7.5 mg/kg i.p. twice a day for 14 days	Pre-test and escape test
Reversal	*Vehicle Naive*	6	Vehicle 1 ml/kg i. p. twice a day for 21 days	Escape test
	Chronic Stress + Vehicle	6	Chronic stress, Vehicle 1 ml/kg i. p. twice a day for 21 days	Escape test
	Chronic Stress + LTG	8	Chronic stress, lamotrigine 7.5 mg/kg i.p. twice a day for 21 days	Escape test
Performance in sucrose SA	*Vehicle*	5	Vehicle 1 ml/kg i. p. twice a day for 21 days	Sucrose self-administration (FR1, FR5, PR)
	LTG	6	Lamotrigine 7.5 mg/kg i. p. twice a day for 21 days	
Effect on motivational anhedonia	*Vehicle*	8	Vehicle 1 ml/kg i. p. twice a day for 21 days	
	Chronic Stress + Vehicle	8	Chronic stress, Vehicle 1 ml/kg i. p. twice a day for 21 days	Sucrose self-administration (FR1, FR5, PR)
	Chronic Stress + LTG	8	Chronic stress, lamotrigine 7.5 mg/kg i.p. twice a day for 21 days	
Phosphorylation levels of THR34-DARPP-32 in the NACS	*Vehicle*	12	Vehicle 1 ml/kg i. p. twice a day for 14 days	Immunoblotting
	Chronic Stress + Vehicle	12	Chronic stress, vehicle 1 ml/kg i. p. twice a day for 14 days	
	LTG	12	Lamotrigine 7.5 mg/kg i. p. twice a day for 14 days	Every group was sacrificed at baseline or after sucrose consumption
	Chronic Stress + LTG	12	Chronic stress, lamotrigine 7.5 mg/kg i. p. twice a day for 14 days	

of unavoidable stress exposure (Fig. 1a). Rats were divided into 4 groups: one group received the vehicle (1 ml/kg i. p., twice a day) and was exposed to the escape test without exposure to the unavoidable stress session (Naive vehicle group, $n = 6$); one group received the vehicle (1 ml/kg i. p., twice a day), was exposed to the unavoidable stress session and 24 h later to the escape test (Vehicle + Acute Stress group, $n = 6$); one group received lamotrigine (7.5 mg/kg i. p. twice a day) and was exposed to the escape test without exposure to the unavoidable stress session (LTG Naive, $n = 6$); one group received lamotrigine (7.5 mg/kg i. p. twice a day), was exposed to the unavoidable stress session and 24 h later to the escape test (LTG + Acute Stress group, $n = 6$; Table 1).

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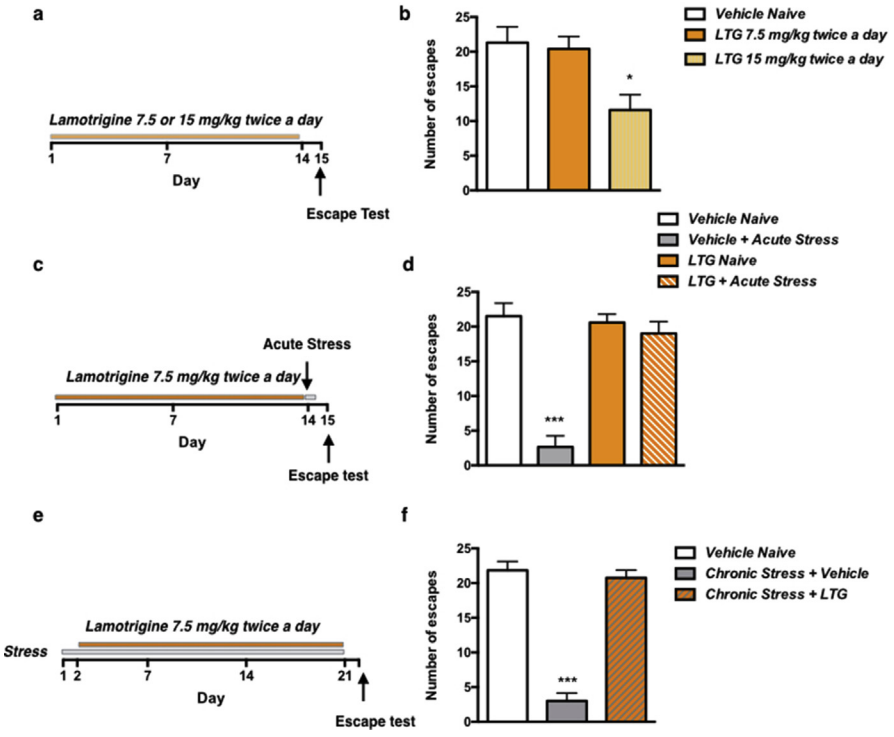

Fig. 1. Lamotrigine reinstated the reactivity to aversive stimuli. (a, b) A 14-day lamotrigine treatment at the dose of 7.5 mg/kg twice a day did not modify the competence to escape avoidable aversive stimuli, while at the dose of 15 mg/kg twice a day reduced avoidable stress reactivity. Rats, divided into 3 groups, received vehicle (1 ml/kg, i. p.) or lamotrigine 7.5 mg/kg (LTG 7.5) or 15 mg/kg i. p. (LTG 15), twice a day. After 14 days, rats were tested for escape without exposure to the unavoidable stress session. (c, d) A 14-day lamotrigine administration prevented the development of stress-induced escape deficit. As outlined in (c), two groups of rats were administered vehicle or lamotrigine 7.5 mg/kg i. p. twice a day for 14 days. Then they were exposed to unavoidable stress (Vehicle + Acute Stress and LTG + Acute Stress) and 24 h later to the escape test, or they were tested for escape without unavoidable stress exposure (Vehicle Naive and LTG Naive). (e, f) Lamotrigine repeated treatment reverted the condition of escape deficit induced by chronic stress exposure. As outlined in (e), two groups of rats were exposed to unavoidable stress session and 24 h later they were tested for escapes. Four-five hours later, they began treatment with vehicle (Chronic Stress + Vehicle), or lamotrigine 7.5 mg/kg i. p. twice a day (Chronic Stress + LTG) for 3 weeks, concomitantly with exposure to the stress protocol. The Vehicle Naive group received vehicle for 3 weeks, without stress exposure. At the end of 3 weeks, all rats were tested for escape. Scores are expressed as mean ± S.E.M. of escape numbers in 30 consecutive trials. *$p < 0.05$, ***$p < 0.001$ versus the other groups (post hoc Bonferroni's test).

c) Reversal of the consequences of chronic stress exposure. Next, in order to evaluate the possible antidepressant-like activity of lamotrigine, we examined whether a repeated treatment reverted the escape deficit induced by exposure to unavoidable stress and maintained by the chronic stress protocol (as outlined in Fig. 1e). To this end, 20 rats were divided into 3 groups: vehicle-treated control rats (1 ml/kg i. p., twice a day) never exposed to stressors (Vehicle Naive, $n = 6$); vehicle-treated rats (1 ml/kg i. p., twice a day) first exposed to the pre-test and escape test sequence, and then to the 21-day chronic stress protocol

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(Chronic Stress + Vehicle, $n = 6$); lamotrigine-treated rats (7.5 mg/kg i. p. twice a day) first exposed to the pre-test and escape test sequence, and then to the 21-day chronic stress protocol (Chronic Stress + LTG, $n = 8$; Table 1). Treatments began on day 2 after the Chronic Stress + Vehicle and Chronic Stress + LTG groups had been exposed to the escape test and continued for the 3 weeks of exposure to the stress protocol. Rats were then tested for escape, 18 h after the last treatment and stressor exposure.

3.2. Lamotrigine effects on performance in sucrose self-administration protocols

a) Effect of lamotrigine administration on sucrose self-administration. A preliminary experiment was carried out to verify that repeated lamotrigine administration did not affect the performance in sucrose self-administration schedules. Rats received vehicle (1 ml/kg i. p., twice a day, Vehicle, $n = 5$) or lamotrigine (7.5 mg/kg i. p. twice a day, LTG, $n = 6$) for 8 days, Table 1; then, they began the self-administration training while continuing treatment. When rats attained consistent responding in FR1 and FR5, the schedule of reinforcement was changed to a PR with a step size of three.

b) Lamotrigine effects on stress-induced motivational anhedonia. Rats were divided into a control group (CTR, $n = 8$) and a group exposed to the sequence of unavoidable stress-escape test (day 1 and 2) and then to the chronic stress protocol (Chronic Stress, $n = 16$) (Fig. 3a). At day 10 self-administration training began under FR1 and FR5 schedules. When the chronic stress group attained the criterion for appetitive motivation deficit, rats were allocated in two different subgroups, one treated with vehicle (1 ml/kg/day, i. p., twice a day, Chronic Stress + Vehicle, $n = 8$) and the other with lamotrigine (7.5 mg/kg, i. p., twice a day, Chronic Stress + LTG, $n = 8$) (day 21), while continuing to be exposed to the stress protocol. The CTR group received the vehicle (1 ml/kg/day, i. p., twice a day, Vehicle, $n = 8$; Table 1) (Fig. 3a). On the 8^{th} treatment day (day 28), self -administration training with an FR5 schedule was resumed for 6 sessions, then rats were switched to a PR schedule with a step size of three (14^{th} treatment day, Fig. 3a).

3.3. Lamotrigine effects on phosphorylation levels of Thr34-DARPP-32 in the NAcS of rats exposed to the chronic stress protocol

In order to study whether repeated lamotrigine administration restored the DARPP-32 response to sucrose, in rats exposed to chronic stress and administered

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lamotrigine, the phosphorylation pattern of DARPP-32 in the NAcS was examined by immunoblotting at baseline and after the consumption of a 10% sucrose solution. Forty-eight rats were divided into Control (CTR, $n = 24$) and Chronic Stress ($n = 24$) groups. Rats in the Chronic Stress group were exposed to the sequence of pretest and escape test (day 1 and 2) and then to the stress protocol until the end of the experiment (Fig. 4a). From day 21 half of the animals in the Control and Chronic Stress groups received the vehicle (1 ml/kg/day i. p. twice a day, Vehicle, $n = 12$; Chronic Stress + Vehicle, $n = 12$), and half of the animals received lamotrigine (7.5 mg/kg i. p. twice a day, LTG, $n = 12$; Chronic Stress + LTG, $n = 12$; Table 1), (Fig. 4a). During the last week of treatment, all rats were habituated to receive an oral administration of 500 μl of water. After 14 days of treatment, 6 rats in each subgroup were sacrificed at baseline and the other 6 rats were sacrificed 30 min after oral administration of 500 μl of a 10% sucrose solution.

4. Results

4.1. Effects of repeated lamotrigine administration on the reactivity to aversive stimuli

Repeated lamotrigine administration at the dose of 7.5 or 15 mg/kg i. p. twice a day did not modify locomotor activity (motility counts: Vehicle = 50.50 ± 10.75; LTG 7.5 = 42.00 ± 8.8; LTG 15 = 35.13 ± 5.2; one-way ANOVA, $F_{2,19} = 0.821$, $p = 0.45$). Moreover, the competence to escape avoidable aversive stimuli was not modified by a 14-day lamotrigine treatment at the dose of 7.5 mg/kg i. p. twice a day, while at the dose of 15 mg/kg i. p. twice a day, lamotrigine reduced avoidable stress reactivity (one-way ANOVA, $F_{2,19} = 6.72$, $p < 0.01$; Fig. 1a,b). Post hoc analysis demonstrated that the LTG 15 group showed a number of escapes lower than the LTG 7.5 or Vehicle Naive groups ($p < 0.05$, both comparisons). Thus, a dose of lamotrigine of of 7.5 mg/kg twice a day was used in the following experiments.

We then investigated whether a 14-day lamotrigine administration prevented the acute consequences of unavoidable stress exposure (Fig. 1c). Analysis of the number of escapes by two-way ANOVA showed a significant effect of stress ($F_{1,120} = 39.95$, $p < 0.001$), treatment ($F_{1,20} = 22.79$, $p < 0.001$), and their interaction ($F_{1,20} = 28.42$, $p < 0.001$). Post hoc comparisons revealed that acute stress exposure induced a clear-cut escape deficit, while lamotrigine treatment prevented this deficit (Vehicle + Acute Stress group versus Vehicle Naive, LTG Naive and LTG + Acute Stress groups: $p < 0.001$, Fig. 1d).

Next, in order to evaluate the possible antidepressant-like activity of lamotrigine, we used a more stringent criterion and examined whether a repeated treatment reverted the escape deficit induced by exposure to unavoidable stress and maintained by the chronic stress protocol (as outlined in Fig. 1e). Lamotrigine treatment completely

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reverted the stress-induced escape deficit (one-way ANOVA, $F_{2,17} = 75{,}98$, $p < 0.001$). In particular, post hoc analysis showed that the Chronic Stress group exhibited a clear condition of escape deficit in comparison to the Naive and Chronic Stress + LTG groups (Chronic Stress group versus Naive and Chronic Stress + LTG groups: $p < 0.001$, Fig. 1f).

4.2. Effects of repeated lamotrigine administration on performance in sucrose self-administration protocols

A preliminary experiment was carried out to verify that repeated lamotrigine administration did not affect the acquisition of an operant behavior maintained by sucrose, a natural reward. Lamotrigine administration did not modify the ability to acquire sucrose self-administration as the only factor that affected the performance was the training session (two−way RM ANOVA: FR1: treatment $F_{1,9} = 0.019$, $p = 0.89$, training session $F_{5,45} = 12.25$, $p < 0.001$, interaction $F_{5,45} = 0.42$, $p = 0.83$; FR5: treatment $F_{1,9} = 0.35$, $p = 0.56$, training session $F_{4,36} = 10.15$, $p < 0.001$, interaction $F_{4,36} = 0.20$, $p = 0.93$; PR: unpaired Student's t test, $p = 0.37$; Fig. 2).

Next, to further study the antidepressant-like effects of lamotrigine, we examined whether lamotrigine treatment was able to reinstate the motivation to operate in a sucrose self-administration protocol impaired by chronic stress exposure (Fig. 3a). As expected, the chronic stress protocol affected the acquisition of sucrose self-administration [two-way RM ANOVA, stress (FR1: $F_{1,22} = 25.10$, $p < 0.001$;

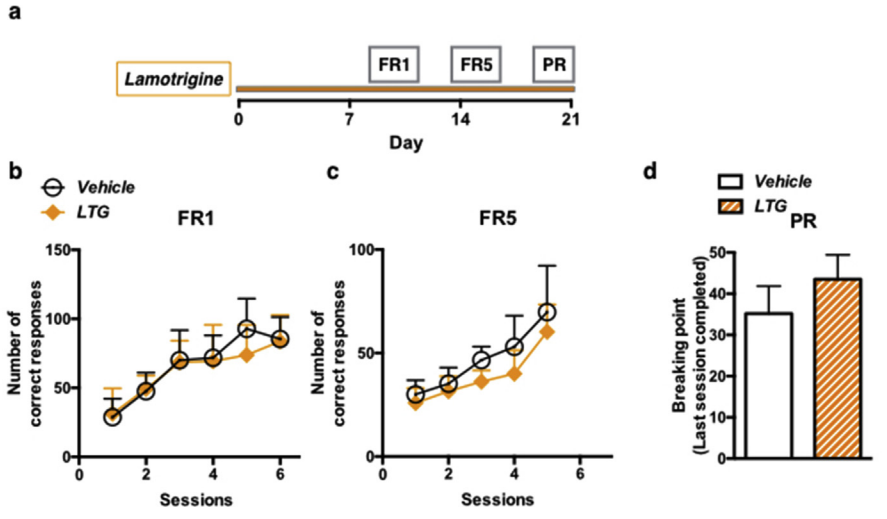

Fig. 2. Lamotrigine *per se* did not affect sucrose self-administration. As outlined in (a), rats were administered lamotrigine 7.5 mg/kg i. p. (LTG) or vehicle (Vehicle) twice a day for 8 days and they were then trained to lever press for sucrose under Fixed Ratio 1 (FR1) schedule (b), while continuing treatments. When stable responses under Fixed Ratio 5 (FR5) schedule (c) were obtained, rats were switched to a Progressive Ratio (PR) schedule (d) with a step size of three. Data are presented as mean ± S.E.M.

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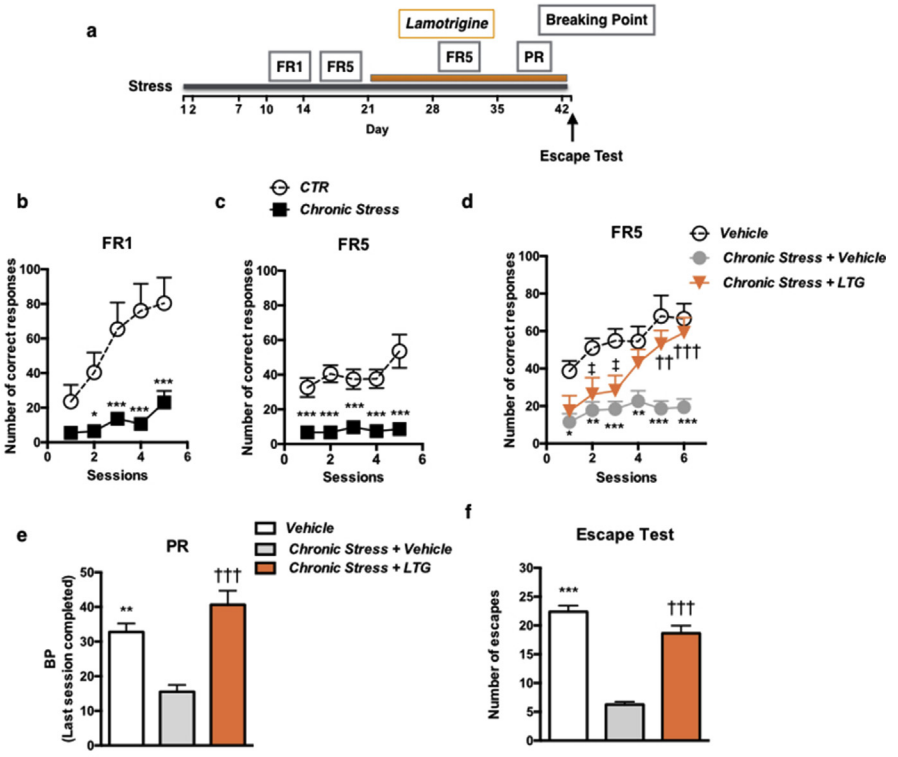

Fig. 3. Lamotrigine counteracted stress-induced motivational anhedonia and escape deficit. As outlined in (a), rats, exposed to the chronic stress protocol (Chronic Stress) or not exposed (CTR), were trained for sucrose self-administration and when motivational anhedonia was established (c), rats in the Chronic Stress group started to receive vehicle (Chronic Stress + Vehicle) or lamotrigine (7.5 mg/kg i. p., Chronic Stress + LTG) twice a day. After 8 days of treatment (day 28), they resumed the FR5 schedule (d) and at the 14th day of treatment were shifted to the PR schedule (e), while continuing treatment and stress exposure. (f) 24 h after the last PR session, rats were tested for escape. Scores are expressed as mean ± S.E.M. of escape numbers in 30 consecutive trials. (b, c) $^{*}p < 0.05$, $^{***}p < 0.001$, Chronic Stress group versus CTR group; (d) $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Chronic Stress + Vehicle group versus Vehicle group; $^{‡}p < 0.05$, Chronic Stress + LTG group versus Vehicle group; $^{††}p < 0.01$, $^{†††}p < 0.001$, Chronic Stress + LTG group versus Chronic Stress + Vehicle group (post hoc Bonferroni's test).

FR5: $F_{1,22} = 37.33$, $p < 0.001$), session (FR1: $F_{4,88} = 20.89$, $p < 0.001$; FR5: $F_{4,88} = 7.256$, $p < 0.001$), and their interaction (FR1: $F_{4,88} = 8.25$, $p < 0.001$; FR5: $F_{4,88} = 5.76$, $p < 0.001$)]. Post hoc analysis showed that exposure to the chronic stress protocol impaired the acquisition of operant responding in FR1 (Chronic Stress versus CTR group: $p < 0.05$, session 2; $p < 0.001$, sessions 3, 4 and 5) and FR5 (Chronic Stress versus CTR group: $p < 0.001$, all sessions) (Fig. 3b,c).

After 8 days of lamotrigine treatment, self-administration sessions were resumed under FR5 schedule. Lamotrigine treatment completely restored the competence to acquire sucrose self-administration, thus counteracting the effects of stress (two-way RM ANOVA; group: $F_{2,21} = 9.57$, $p < 0.01$; session: $F_{5,105} = 22.31$, $p < 0.001$;

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interaction: $F_{10,105} = 4.45$, $p < 0.001$). In particular, post hoc analysis showed that the reduced performance in sucrose self-administration induced by stress exposure (Chronic Stress + Vehicle versus Vehicle group, $p < 0.05$, sessions 1; $p < 0.01$, sessions 2 and 4; $p < 0.001$, sessions 3, 5 and 6) was completely rescued by lamotrigine treatment (Chronic Stress + LTG group versus Chronic Stress + Vehicle group: $p < 0.01$, session 5; $p < 0.001$, session 6, Fig. 3d). Moreover, analysis of the BP under the PR schedule confirmed that chronic stress exposure impaired operant responding for sucrose, thus reducing the motivation to operate for a reward and lamotrigine treatment restored the incentive motivation in stressed rats (one-way ANOVA: $F_{2,21} = 18.73$, $p < 0.001$; post hoc test: Chronic Stress + Vehicle versus Vehicle group, $p < 0.01$; Chronic Stress + LTG versus Chronic Stress + Vehicle group, $p < 0.001$, Fig. 3e). In this experiment rats underwent stress exposure for 42 days. Thus, to verify whether lamotrigine administration restored reactivity to aversive stimuli after prolonged exposure to the stress protocol, rats in each group were tested for escape 24 h after the end of self-administration experiments. The number of escapes was different between groups (one-way ANOVA, $F_{2,21} = 67.49$, $p < 0.001$) and post hoc analysis demonstrated a clear-cut escape deficit in the Chronic Stress + Vehicle group, while the Chronic Stress + LTG group showed a performance similar to that of the Vehicle group (Fig. 3f).

4.3. Effects of repeated lamotrigine administration on phosphorylation levels of Thr34-DARPP-32 in the NAcS of rats exposed to the chronic stress protocol

In order to study the dopaminergic response to a natural reinforcer in the NAcS, Thr34-DARPP-32 phosphorylation levels were analyzed by immunoblotting after oral administration of a 10% sucrose solution to rats in the different experimental groups. Levels of total DARPP-32 in the NAcS were similar in the four experimental groups (one-way ANOVA, $F_{3,20} = 0.63$, $p = 0.60$, Fig. 4b). Lamotrigine administration reinstated the DARPP-32 phosphorylation changes in the NAcS in response to acute sucrose consumption disrupted by stress exposure. Analysis by two-way ANOVA of Th34-DARPP-32 phosphorylation levels expressed as percentage of the baseline values of Vehicle group (rats not exposed to sucrose consumption) revealed a significant effect of group ($F_{3,40} = 4.28$, $p < 0.05$), sucrose consumption ($F_{1,40} = 27.06$, $p < 0.001$), and their interaction ($F_{3,40} = 2.99$, $p < 0.05$). Baseline levels of phospho-Thr34 DARPP-32 were similar between groups (Fig. 4c). Chronic stress exposure impaired the increase in phospho-Thr34 DARPP-32 levels in response to sucrose exhibited by control animals (Chronic Stress + Vehicle versus Vehicle group, $p < 0.001$), while lamotrigine administration restored the Thr34-DARPP-32 response to a natural reinforcer (Chronic Stress + Vehicle versus LTG and Chronic Stress + LTG groups, $p < 0.01$, Fig. 4c; original images are shown in the supplementary material).

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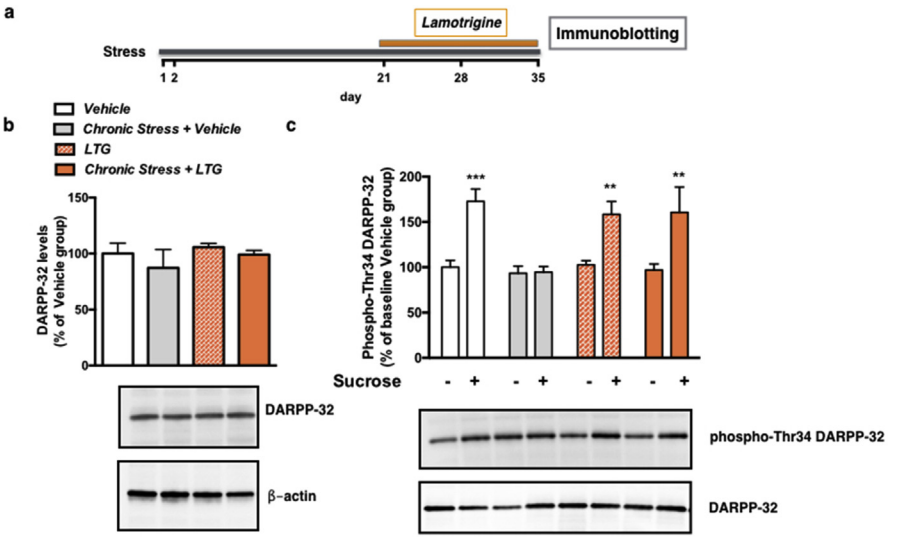

Fig. 4. Lamotrigine reinstated the dopaminergic response to sucrose impaired by stress exposure in the NAcS. As outlined in (a), rats in the Chronic Stress group were exposed to the stress protocol for 3 weeks. On day 21, part of the Control and Chronic Stress rats continued to receive the vehicle (Vehicle and Chronic Stress + Vehicle groups), while the remaining rats were administered lamotrigine (7.5 mg/kg i. p., LTG and Chronic Stress + LTG groups), twice a day. (b) DARPP-32 levels were normalized to their respective β-actin levels and data are expressed as mean ± S.E.M. of percentage modifications compared those of the Vehicle group. (c) Phospho-Thr34 DARPP-32 levels were normalized to the respective DARPP-32 levels and data are expressed as mean ± S.E.M. of percentage modifications compared to those of the baseline (- sucrose) Vehicle group levels. $^{**}p < 0.01$, $^{***}p < 0.001$, versus the Chronic Stress + Vehicle (+ sucrose) group (post hoc Bonferroni's test).

5. Discussion

Repeated lamotrigine administration prevented and relieved the stress-induced hyporeactivity to negative aversive stimuli (that is, escape deficit) and reinstated the physiological response to natural rewarding stimuli (sucrose) in a stress-induced depressive-like phenotype in rats. The pro-motivational effect of repeated lamotrigine administration that we observed in stress-exposed "anhedonic" rats is a novel finding since only the consummatory component of reduced responses to pleasurable stimuli has been previously investigated in animal models [18]. In our experimental conditions appetitive motivational anhedonia is closely linked to reduced dopaminergic responses to sucrose, evaluated in terms of increases in extraneuronal dopamine levels in the NAcS of non food-deprived rats [35]. Indeed, modifications in dopamine D_1 receptor-dependent signaling in the NAcS after sucrose consumption, measured as PKA-dependent phosphorylation of Thr34 DARPP-32, positively correlate with changes reported in extraneuronal dopamine levels [35, 46, 47]. Repeated pharmacological treatments that counteract these blunted dopaminergic responses to a natural reinforcer reestablish the motivation to operate for a reward, as indicated by PR and BP values in sucrose self-administration [35, 37, 38]. The present results show that repeated lamotrigine treatment was able to reinstate the PKA-

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dopamine D_1 receptor-DARPP-32 signaling response elicited by a natural reinforcer in the NAcS of non-food-deprived rats. Confirming our working hypothesis, the effect on dopaminergic transmission was accompanied by the reinstated behavioral response to a positive stimulus, in agreement with our previous studies [35, 37, 38, 39]. The role of the dopamine D_1 receptor pathway in the NAc in hedonic/anhedonic behaviors has also been demonstrated in a rat model of bipolar disorder [48]. Moreover, the effects of repeated lamotrigine administration that we report may be interpreted in the frame of data indicating modulation by this drug of VTA dopaminergic neurons activity and resulting dopaminergic responses in the NAcS [27].

Consistent with previous observations [35], chronic unavoidable stress exposure impaired the reactivity to noxious stimuli and motivation to operate for natural reinforcer. Also, it disrupted the dopaminergic response in the NAcS elicited by the intake of a natural reward in non-food-deprived rats. In rats, the stress-induced escape deficit and the impaired acquisition of an operant behavior maintained by salient rewarding stimuli represent a good translational model that mimic the increased negative affect and the decreased positive affect [34, 35, 49] that have been described in depressed patients [50, 51]. These two deficits induced by chronic unavoidable stress exposure are differently affected by classical antidepressants. In the model used, the escape deficit is completely reverted by a long-term treatment (~3 weeks) with antidepressant drugs, such as imipramine, fluoxetine, reboxetine, mirtazapine [34, 37, 52, 53], while motivational anhedonia is only partially reverted by imipramine and unaffected by fluoxetine treatment [37]. On the other hand, the deficit in appetitive motivation is completely counteracted by lithium, clozapine, aripiprazole, and fenofibrate long-term administration [35, 37, 38, 39]. It is interesting to note that lamotrigine, lithium, and aripiprazole that in our experimental model restored the responses to a natural reward are among the drugs that have been approved by FDA for maintenance treatment of bipolar disorder.

In this study, a 14-day lamotrigine treatment was effective in the prevention of escape deficit development elicited by exposure to acute inescapable stress, and a 21-day lamotrigine treatment rescued the condition of escape deficit maintained by the long-term stress protocol, similar to what antidepressant drugs do on this test [34, 52, 53]. Our findings are consistent with previously published studies that demonstrate antidepressant-like effects of repeated lamotrigine administration on reactivity to aversive stimuli [16, 17, 18], or positive stimuli, e.g., sucrose in the sucrose preference test [18]. Differently from previous findings [16, 18], in our experimental conditions lamotrigine was effective on the stress-induced deficits at the dose of 7.5 mg/kg/twice a day, while the dose of 15 mg/kg/twice a day decreased the escape response, an effect likely related to the slight impairment observed in locomotor activity.

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Several lines of evidence involve alterations in reward signaling in depression and anhedonia [54, 55] and dopamine signaling in the NAc has been identified as playing an important role in the regulation of effort-related and activational aspects of motivation [56]. Burst firing of VTA dopaminergic neurons and the subsequent phasic dopamine release in the NAc encodes the occurrence and the valence of salient stimuli [57, 58], playing an important role in motivated behavior [59]. Moreover, chemogenetic activation of VTA dopaminergic neurons results in an increased responding for sucrose in a PR schedule of reinforcement, that is, it enhances incentive motivation [60]. VTA dopaminergic neurons that project to the NAcS exhibit a large hyperpolarization-activated inward current, I_h, that modulates their electrophysiological properties and firing rate [61]. This current that modulates VTA firing activity seems to be crucial for the formation of cue-reward association and motivated behaviors [27, 61]. Lamotrigine is an enhancer of I_h currents [26] and its antidepressant effects may be related to the influence on VTA dopaminergic neurons firing activity. In a model of chronic social defeat stress, lamotrigine, repeatedly infused in the VTA of the susceptible subpopulation of defeated mice, reverts social avoidance and sucrose preference deficit. In susceptible mice VTA dopaminergic neurons projecting to the NAc show enhanced firing rates that are normalized following lamotrigine-induced sustained I_h current potentiation [27]. These data suggest that the anti-anhedonic-like effects of repeated lamotrigine administration could correlate with its ability to normalize the firing rate of VTA dopaminergic neurons modified by stress exposure. Actually, the results on the PKA-dopamine D_1 receptor-DARPP-32 response to sucrose in the NAcS are consistent with the hypothesis that lamotrigine modulation of VTA dopaminergic neurons activity and the resulting dopaminergic responses in the NAc underlies the effects on motivational anhedonia and depressive-like behaviors.

The results obtained in our experimental model of depressive-like symptoms seem to correlate with the alleged clinical efficacy of lamotrigine in bipolar depression. The pro-motivational activity of lamotrigine that we report may have a counterpart in the proposed efficacy of this drug as augmentation therapy for patients with treatment resistant depression [62, 63, 64], although a clear impact on the anhedonic domain has not been clearly assessed and demonstrated in clinical studies.

In our experimental conditions, lamotrigine effects differ from those observed after administration of aripiprazole, a second generation antipsychotic also used as add-on therapy in the treatment of bipolar disorder and treatment-resistant depression. Aripiprazole differently modulates the responses to aversive and rewarding stimuli, since it restores the motivation to operate for, and the dopaminergic response to, positive stimuli (sucrose), without affecting stress-induced decreased reactivity to aversive stimuli, confirming that motivations to operate for a reward or to avoid negative stimuli could be dissociated [39]. Thus, experimental models of depressive symptoms suggest that different drugs used for the treatment of mood disorders

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preferentially affect some of these domains and show that the pro-motivational effects of lithium and lamotrigine develop faster than those of classical antidepressants [35, 37, 39]. These results on the antidepressant-like and pro-motivational activity of lamotrigine in an animal model are endowed with translational value as they could suggest a clinical use of this drug to target the symptom domain of motivational anhedonia in unipolar or bipolar depression.

5.1. Limitations

The pro-motivational effects of lamotrigine observed in our model may not fully transpose to the patients, since anhedonia is a multidimensional construct and the motivational aspects although relevant, are not the only components.

6. Conclusions

This study demonstrates a clear pro-motivational effect of repeated lamotrigine administration, accompanied by a restored dopaminergic response in the NAcS, possibly consequent to the modulation of VTA dopaminergic neurons firing rate. These data are suggestive of a correlation with the results of clinical studies where early improvement in positive affect has been shown to be predictive of antidepressant treatment response [51, 65]. Thus, studies of drugs activity on different behavioral symptoms of mood disorders modeled in animals may support clinical choices of pharmacological therapies oriented to treat prevalent symptom domains.

Declarations

Author contribution statement

Carla Gambarana, Maria Graziella De Montis: Conceived and designed the experiments; Wrote the paper.

Simona Scheggi: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Alessandro Cuomo: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

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Additional information

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