Antinociceptive effects of fluoxetine in a mouse model of anxiety/depression
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Pain was reported by 60–90% of patients with depression, and chronic pain states are often linked to depression. Animal models of pain/depression are generally lacking for the identification of centrally active drugs. In the present study, pain sensitivity was assessed in a mouse model of anxiety/depression on the basis of chronic corticosterone (CORT) administration through the drinking water (CORT model). We measured thermal hyperalgesia as shown by a decrease in the latency to hind paw licking in the hot plate test and cold allodynia reflected by a decrease in the time spent on the plate set at 20°C in the thermal preference plate test. Subsequently, we determined the effect of chronic administration of the selective serotonin reuptake inhibitor fluoxetine (an antidepressant known to reverse anxiety/depressive-like state in CORT-treated mice) on pain relief. Fluoxetine administration reduced both heat hyperalgesia and cold allodynia, thus unveiling a putative link between mood and nociception in the CORT model.

Introduction
Antidepressants including selective serotonin reuptake inhibitors (SSRIs) are able to prevent or reverse hyperalgesia in various animal models of pain [1]. Accordingly, acute or chronic administration of the SSRI fluoxetine produces antinociceptive effects in a thermal pain test in rodents [2,3]. Remarkably, intracerebroventricular injection of the serotonin (5-HT)-selective neurotoxin 5,6-Dihydrotryptamine, which induces a marked depletion in brain tissue levels of 5-HT, suppressed the antinociceptive effects of fluoxetine [3]. These results strongly suggest that SSRI-induced antinociception involves central serotonergic pathways. However, other studies have failed to demonstrate the antinociceptive effects of SSRIs in chronic pain models [4,5]. Although the reasons for such an inconsistency remain unknown, it is possible that animal models do not cover all dimensions of pain. Chronic pain is a common comorbidity factor accompanying depression: pain complaints are reported by 60–90% of depressed patients [6]. Although animal research in the field of pain has mainly focused on the mechanisms of nociception, pain perception in humans involves integration of sensory and emotional components [7]. These considerations indicate that pain and depressive symptoms need to be examined in parallel in a single animal model rather than by independent studies. However, animal models of comorbid pain/depression are generally lacking for the identification of centrally active drugs.

This hypothesis is consistent with previous clinical studies reporting the analgesic efficacy of fluoxetine in depressed patients suffering from pain disorders. Together, these results suggest that the CORT model, with pain/anxiety/depressive-like state, is a good candidate for translational research. NeuroReport 23:525–529 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins

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Materials and methods
Animals
C57BL/6 male mice were purchased from Elevage Janvier (Le Genest sur l’Isle, France) and were 8–10 weeks of age. They were kept in a temperature-controlled room (22 ± 2°C) on a 12-h light–dark cycle. Food and water were freely available. Experimental procedures were performed according to the recommendation of the European Community (86/609/EEC) and the French National Committee (87/848) for care and use of laboratory animals (permission #B 92–373, F. Coudore). Nociceptive tests were carried out according to the ethical guidelines of the International Association for the Study of Pain [9].

Induction and validation of the anxious/depressive-like state
The anxious/depressive-like state was induced in mice through CORT treatment (Sigma-Aldrich, St Louis, Missouri, USA; 6–7 mg/kg/day in drinking water for 8
weeks) [8]. The phenotype was analyzed using the following paradigms: open field (OF), the four-plate test (FPT), the novelty suppressed feeding (NSF) test, and coat state examination.

The OF test was performed as described previously [10] to compare anxiety-like responses between vehicle-treated and CORT-treated mice. In brief, motor activity was quantified in four Plexiglas boxes 43 × 43 cm² (MED Associates, Georgia, Vermont, USA). Activity chambers were computer interfaced for data sampling. The computer defined grid lines dividing the center and surrounding regions, with the center square consisting of four lines, 11 cm from the wall of the cage.

The FPT is an anxiety test based on spontaneous responses. Animals were exposed to a novel environment and the exploration was suppressed by the delivery of a mild electric foot shock contingent (0.6 mA; 0.5 s) to quadrant crossing. Animals can only escape from this aversive situation by remaining motionless (passive avoidance) [11].

The NSF is a conflict test that elicits competing motivations between the drive to eat and the fear of venturing into the center of the brightly lit arena [8]. A longer latency to feed reveals an anxio/depressive state.

The coat state measure is a reliable and well-validated index of a depressed-like state in mice [10]. The total score results from the sum of the score of five different body parts of the mouse: head, neck, dorsal/ventral coat, tail, fore/hindpaws. For each body part, a score of 0 was given for a well-groomed coat and 1 for an unkempt coat [12].

Behavioral assessment of nociception

Thermal hyperalgesia was tested with the hot plate test (HPT) [9]. Mice were placed on a metal plate maintained at 55°C. The latency to the first hind paw licking response was taken as an index of the heat nociceptive threshold. The cutoff was set at 1 min to avoid damage to the paw.

Cold sensitivity was assessed using two paradigms, the cold plate test and the thermal preference plate test (T2PT) (Bioseb, Vitrolles, France). Mice were placed on a metal plate maintained at 2°C. The latency to the first jump was taken as an index of the cold nociceptive threshold. The cutoff was set at 3 min to avoid paw damage. Cold allodynia was assessed by T2PT, where mice were allowed to explore an enclosure in which the floor was composed of two computer-managed metal plates. One plate was set at a comfortably tolerated temperature, that is 30°C, and the other at the test temperature. The time spent on each plate was measured.

Drug and reagents

CORT (Sigma-Aldrich) was dissolved in a vehicle (0.45% hydroxy-l-β-cyclodextrin; Sigma-Aldrich). Fluoxetine hydrochloride was purchased from Anawa Trading (Wangen, Zurich, Switzerland). CORT (6–7 mg/kg/day) was delivered in opaque bottles to protect it from light, available ad libitum in the drinking water. Control mice received β-cyclodextrin. Fluoxetine (18 mg/kg/day) was added to drinking water after 4 weeks of CORT treatment. The dose and duration used were chosen on the basis of their ability to reverse an anxiodepressive phenotype in this model [8].

Statistical analysis

All data are shown as mean ± SEM. Statistical analyses were performed using Stat-View 5.0 software (Abacus Concepts Inc., Berkeley, California, USA). Student’s t-test was used for comparison between two groups of data. A two-way analysis of variance (ANOVA) followed by a Bonferroni’s post-hoc test was performed for T2PT (Fig. 2b) and a one-way ANOVA with repeated measures was used to determine the effect of fluoxetine in the HPT (Fig. 3a). The accepted level of significance was defined as a P value less than 0.05.

Results

Validation of the anxio/depressive phenotype

Using the OF test, the chronic exposure to exogenous CORT produced a marked decrease in the time spent in the center of the arena (248 ± 23 vs. 158 ± 15 s in CORT-treated and vehicle-treated mice, respectively [t(26) = 3.27, P < 0.01]; Fig. 1a). This behavior indicates an anxiogenic-like phenotype.

The conditioned fear induced by the FPT was first validated using a pharmacological approach with morphine (10 mg/kg, intraperitonially). As the mice received an electric shock, it was possible that some modifications of (a) pain perception, (b) transfer, and/or (c) central integration of the nociceptive message were involved in the modified responses to the electrical stimuli. To disambiguate drug-induced anti-punishment effects obtained in FPT from alteration of pain sensitivity, we confirmed that morphine did not increase the number of shocks received in this test as observed with classic anxiolytic treatment with diazepam (1 mg/kg, intraperitonially; data not shown). The chronic CORT treatment led to a significant decrease in the punished passages between plates during a 1-min-session test (3.6 ± 0.45 vs. 2.1 ± 0.29 in CORT-treated and vehicle-treated mice, respectively [t(38) = 3.81, P < 0.001]; Fig. 1b). This confirms the anxiety-like phenotype unveiled in the OF paradigm.

In the NSF test, chronic CORT treatment increased the latency to feed (105 ± 14 vs. 377 ± 55 s in CORT-treated and vehicle-administered mice, respectively [t(38) = 5.20, P < 0.001]; Fig. 1c) during the 10-min period. This result suggests that CORT-treated mice displayed a depressive/anxiety-like behavior.

Finally, the coat state measure showed that chronic CORT treatment induced a deterioration of the coat
A 4-week chronic corticosterone (CORT) treatment (6–7 mg/kg/day) induced an anxiety/depressive-like phenotype. The treatment induces a highly anxious state reflected by (a) a reduced total time spent in the center in the open field, (b) a decreased number of punished passages in the four-plate test, (c) an increased latency to feed in the NSF paradigm that assesses anxiety/depressive-like behaviors, and (d) alterations in coat state, an index of self-neglect in CORT-treated mice. Data represent mean ± SEM. **P<0.01 and ***P<0.001: significantly different from vehicle (Veh)-treated mice.

Assessment of the antinociceptive effects of the chronic fluoxetine treatment
In the HPT (55 ± 0.5°C), 4 weeks of fluoxetine treatment (18 mg/kg/day) produced an increase in hind paw licking latencies in CORT mice (23.8 ± 5.1 vs. 11.6 ± 1.2 s; \( t_{(35)} = 2.19, \ P < 0.001 \); Fig. 3a). Using the T2PT, 4 weeks of fluoxetine treatment resulted in an increase in the time spent on the plate stored at 20°C for CORT-treated mice compared with vehicle-treated mice (49.3 ± 22.3 vs. –16.2 ± 20.1 s; \( t_{(22)} = 2.81, \ P < 0.01 \); Fig. 3b). Together, these results emphasized a significant analgesic effect of fluoxetine on both heat hyperalgesia and cold allodynia.

Discussion
The present study reveals for the first time important modifications in nociceptive endpoints, such as thermal hyperalgesia and cold allodynia, in a mouse model of anxiety/depression based on chronic administration of CORT to the drinking water. Signs and symptoms of nociceptive hyper-sensitivity in mouse models of anxiety/depression are poorly described in the literature. Although Sacharczuk et al. [13] reported thermal hyperalgesia in mice with high opioid system activity subjected to chronic mild stress, further investigations are required to confirm the nociceptive state of depressed animals such as mice subjected to a learned helplessness protocol or olfactory bulbectomy. A recent study, however, reported a possible relation between a brief and reversible increase in stress-induced CORT concentrations and appearance of abnormal pain sensitivity in mice.
Nevertheless, these observations were made in a fibromyalgia-like animal model, which does not display depressive phenotype [14]. In marked contrast, social defeated mice [15] displayed an opioid-dependent analgesia [16]. Interestingly, our results are consistent with studies performed in other species. For example, in rats, an acute local CORT administration within the amygdala resulted in both long-term anxiety and increased sensitivity to painful stimuli [17], suggesting that stress and anxiety can enhance nociceptive processing by descending supraspinal pathways originating from within the central nucleus of the amygdala.

One of the most remarkable results obtained herein is the observation that a chronic treatment with the SSRI fluoxetine, which normalizes anxiety-depressive-like phenotype in CORT-treated mice [8], also attenuated both thermal hyperalgesia and cold allodynia in this model. Moreover, the lack of effects of fluoxetine on nociceptive thresholds in control mice emphasizes the importance of the affective dimension of pain and suggests that the altered pain perception in CORT-treated mice is likely related to the anxiety/depressive state. Some SSRIs have shown efficacy in animal models of pain [1] and greater efficacy compared with placebo in chronic pain management [18]. However, they are not recommended for the treatment of chronic pain [19]. Indeed, in humans, various neuropathic pain conditions can respond to dual-acting agents, but not to SSRIs [20].

The possibility that fluoxetine may be active on pain, specifically in the context of mood disorders, is consistent with previous clinical studies reporting the analgesic effects.
efficacy of fluoxetine in pain disorders in patients with depression [21]. In the latter study, results were analyzed by separating depressive and nondepressive groups. A significant difference was observed between fluoxetine and placebo in patients with persistent somatoform pain disorder and depression, whereas no difference was found in the nondepressive group. SSRIs may thus exert their analgesic effect by modulating the affective aspect of pain in addition to a putative influence on sensory mechanisms. Interestingly, it has been shown recently that sustained administration of fluoxetine produced a greater enhancement of serotoninergic neurotransmission in CORT-treated compared with vehicle-treated mice [22]. The latter results provide cellular explanations for the antidepressant and likely analgesic effects produced by SSRIs under pathological conditions.

Altered functioning of descending inhibitory and/or facilitatory pathways in patients with depression seems to be one of the major physiopathological hypotheses contributing to chronic pain. The involvement of monoamines, including serotonin and noradrenaline, in these pathways [23] is in agreement with the prescription of monoaminergic antidepressants such as tricyclics, SSRIs, and the dual serotonin and norepinephrine reuptake inhibitor duloxetine or venlafaxine to treat chronic pain [24]. Further investigations in the CORT model should concentrate on the implication of brain monoaminergic regions involved in the initiation of the descending controls of pain as a main target mediating antidepressant-like effects. Interestingly, the amygdala and the cingulate cortex are two connected brain regions modulating both sensory and emotional components of pain [25]. This neural connectivity could mediate the relay of emotion-related information that may be recruited and/or affected in diseases in which mood is altered.

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Conflicts of interest

There are no conflicts of interest.

References