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Research paper

# Duloxetine and 8-OH-DPAT, but not fluoxetine, reduce depression-like behaviour in an animal model of chronic neuropathic pain

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## HIGHLIGHTS

- Antidepressant duloxetine but not fluoxetine reverses depression-like behaviour derived from persistent neuropathic pain.
- The selective 5-$HT_{1A}$ agonist 8-OH-DPAT can attenuate neuropathic pain induced depression-like behaviour.
- Noradrenergic and 5-$HT_{1A}$ receptors are promising targets to treat chronic neuropathic pain induced depression.

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## ABSTRACT

The current study assessed whether antidepressant and/or antinociceptive drugs, duloxetine, fluoxetine as well as (±)-8-hydroxy-2-[di-*n*-propylamino] tetralin (8-OH-DPAT), are able to reverse depression-like behaviour in animals with chronic neuropathic pain. Chronic constriction injury (CCI) of the sciatic nerve in rats was selected as neuropathic pain model. Mechanical hypersensitivity and depression-like behaviour were evaluated 4 weeks after surgery by "electronic algometer" and forced swimming test (FST), which measured the time of immobility, and active behaviours climbing and swimming. The selective noradrenergic and serotonergic uptake blocker duloxetine (20 mg/kg) and the selective 5-$HT_{1A}$ agonist 8-OH-DPAT (0.5 mg/kg) significantly reversed both mechanical hypersensitivity and depression-like behaviour in CCI animals. Duloxetine significantly reversed depression-like behaviour in CCI rats by increasing the time of climbing and swimming, while 8-OH-DPAT attenuated depression-like behaviour mainly by increasing the time of swimming. However, the selective serotonergic uptake blocker fluoxetine (20 mg/kg) failed to attenuate mechanical hypersensitivity and depression-like behaviour, possibly due to confounding pro-nociceptive actions at 5-$HT_3$ receptors. These data suggest to target noradrenergic and 5-$HT_{1A}$ receptors for treatment of chronic pain and its comorbidity depression.



## 1. Introduction

Several clinical trials have shown that members of the new selective serotonin (5-HT) and noradrenalin (NA) reuptake inhibitor class of antidepressants (SNRIs), such as venlafaxine and duloxetine, tend to have a better efficacy against neuropathic pain than noradrenergic reuptake inhibitors (NRIs), such as desipramine and maprotiline, which in turn are twofold more effective than selective serotonin inhibitors (SRIs), like fluoxetine and citalopram [1–3]. This may be due to pronociceptive actions of serotonin on 5-$HT_3$ receptors [4], but preclinical evidence on the relationship among the NA and/or 5-HT reuptake inhibitors, 5-HT receptor agonists, depression and chronic pain is scanty. Therefore, the present study was undertaken to compare the effect of duloxetine and fluoxetine on rats with neuropathic pain related depression because it has been reported that both drugs display antidepressant activity in humans and rats without nerve injuries [5,6]. Moreover, we wondered whether an agonist acting on the antinociceptive serotonin 5-$HT_{1A}$ receptor [7] can produce antidepressive efficacy on depression-like behaviour arising from persistent neuropathic

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pain. Therefore, the selective 5-$HT_{1A}$ agonist (±)-8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) was examined as well [8].

A higher mechanical hypersensitivity than in other models of peripheral neuropathic pain has been reported in chronic constriction injury (CCI) animals [9]. Furthermore, these animals display depression-like behaviour without locomotion deficits [10,11]. Therefore, to study the association between peripheral nerve injury and depression-like behaviour, the CCI model has been selected in the present study. Forced swimming test (FST) has been used in the present study due to its ease of use and reliability [12,6].

In FST, a high baseline level of depression-like behaviour is useful in order to demonstrate antidepressant activity without encountering floor effects. In pain research, a low baseline level of depression-like behaviour is essential in order to demonstrate the induction of depression-like behaviour by chronic pain without encountering ceiling effects. Therefore, in the present study the water depth was increased from traditional depths of 15–18 to 30 cm to reduce the time of immobility in control rats [12,6].

To test whether the NA and/or 5-HT descending pain control systems contribute to the pain-like and depression-like behaviour of CCI animals, we have measured in the FST the time of climbing and swimming which are mediated by the NA and 5-HT system, respectively [12,6].

The aim of this study was to better understand the relationship of the 5-HT and NA neuronal systems with chronic neuropathic pain induced depression, by investigating the efficacy of NA and 5-HT associated antidepressants and/or antinociceptive drugs on this disorder.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats (HsdCpb: Wu), weighing 210–230 g at the beginning of the experiment were housed in standard light cycle (6:00 a.m.–6:00 p.m.) and temperature controlled environment (23 ± 1 °C), with food and water ad libitum. Gender differences were not the topic of this study, but are known to exist for both chronic pain and depression [13]. Therefore, the restriction to male rats is a limitation of this study. The behavioural tests were performed during the light phase (9:00 a.m.–4:00 p.m.). Housing, handling and testing of the animals were conducted according to the Guidelines on Ethical Standards for investigation of Experimental Pain in Animals [14]. The experimental protocols were reviewed and approved by the Animal Experimentation Ethics Committee of the Government Presidium of Tübingen, Germany (permission VVH07-016).

### 2.2. Animal surgery

Under pentobarbital sodium (60 mg/kg intraperitoneally), chronic constriction injury was performed based on the original description by Bennett and Xie [15]. Sham operation was performed by exposing the sciatic nerve except for nerve ligation. Animals were inspected every two days and tested 28 days after surgery.

### 2.3. Measurement of mechanical hypersensitivity

Mechanical hypersensitivity was measured by using an automated algometer (Somedic, Hörby, Sweden). The animals were placed in a plexiglas cage (16 × 24 × 14 cm) with a grid bottom and adapted for at least 30 min. Mechanical stimuli were generated by touching the plantar region of the left and right hind paw of the rat with a continuously increasing pressure (5 g/s, 0.3 mm diameter cylindrical probe). The paw withdrawal thresholds represent the mean of three independent measurements. The observer was blinded to pharmacological treatments [16].

### 2.4. Depression like behaviour: forced swimming test

The rats were placed individually into Plexiglas cylinders (height 40 cm, diameter 18 cm) filled with 30 cm, 25 ± 1 °C water, which is close to the thermally neutral range and does not induce avoidance behaviour even in thermal hypersensitivity. Two different sessions were performed: a 15-min pre-medication-test followed by a 5-min post-medication-test 24 h later. Test sessions were videotaped. The time of immobility is determined when no additional activity is observed other than the movements necessary to keep the rat's head above the water. The time of climbing is measured when the rat was making upward movements vigorously with its forepaw in and out of the water. Swimming is considered when the rat is showing active swimming movement, e.g., moving around in the cylinder.

### 2.5. Statistics and drugs

The experiments were performed in a randomized manner between 9:00 a.m. and 04:00 p.m. The dosages of 0.5 mg/kg for 8-OH-DPAT, 20 mg/kg for fluoxetine as well as duloxetine were selected as it was reported that these doses produced antidepressive effect [17–20]. All drugs were administered intraperitoneally 23, 5 and 1 h before behavioural tests as this subchronic treatment is effective to evaluate/predict antidepressant efficacy in FST [21,18,22]. Moreover, in humans, antinociceptive actions of antidepressants can even be detected after a single administration [23].

The effects of duloxetine, fluoxetine and 8-OH-DPAT in sham operated and CCI animals were analyzed by performing a parametric ANOVA two-way analysis of variance, including the factors surgery (sham and CCI), treatment (duloxetine, fluoxetine or 8-OH-DPAT) and interaction, and followed by adjusted Bonferroni's multiple comparison test.

A $P$ value of $<0.05$ was considered to be significant. Analysis of the data was performed using GraphPad Prism 5.01 for Windows. Data are given as means ± SEM.

Duloxetine, fluoxetine and (±)-8-OH-DPAT hydrobromide were purchased from Sigma–Aldrich (Sigma, Germany). All compounds were dissolved in saline (0.9% NaCl).

### 2.6. Dropouts

Animals that did not develop an enhanced response to mechanical stimuli (behavioural hyperalgesia) following nerve injury or showed self mutilation at the paw were excluded from both the depression-like behaviour and the pain behaviour studies. Lack of behavioural hyperalgesia is defined as a decrease of PWTs less than 10% of the average value of the control group. Dropouts were not replaced.

## 3. Results

### 3.1. Effect of duloxetine, fluoxetine and 8-OH-DPAT on mechanical hypersensitivity

Duloxetine significantly reversed mechanical hypersensitivity (Fig. 1A). ANOVA two-way comparison revealed a statistically significant main effect for the treatment ($P < 0.01$), for the surgery ($P < 0.001$) and for interaction ($P < 0.05$). Paw withdrawal thresholds (PWTs) of CCI animals were significantly decreased when compared to sham-operated animals (sham + saline 31 ± 2 g, CCI + saline 14 ± 1 g; $P < 0.001$). Duloxetine significantly reversed mechanical hypersensitivity from 14 ± 1 g in saline-treated CCI animals to