Antinociceptive activity of the new triple reuptake inhibitor NS18283 in a mouse model of chemotherapy-induced neuropathic pain

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Conflicts of interest
None declared.

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Abstract

Background: Chronic neuropathic pain can lead to anxiety and depression. Drugs that block reuptake of serotonin, norepinephrine and/or dopamine are widely used to treat depression, and have emerged as useful drugs in the treatment of neuropathic pain. This study compared the acute antinociceptive effects of NS18283, a novel triple monoamine reuptake inhibitor (MRI) with indatraline, venlafaxine and escitalopram in a mouse model of neuropathic pain.

Method: Neuropathic pain-like behaviours were induced in mice by repeated injections of oxaliplatin (OXA), and assessed using the von Frey hair test, the cold plate test and the thermal preference plate test. Anxio/depressive phenotype and antidepressant-like properties of compounds were assessed by the novelty suppressed feeding test and the tail suspension test, respectively.

Results: In vivo microdialysis experiments showed that each MRI increased extracellular serotonin, norepinephrine and/or dopamine levels in the cingulate cortex, in agreement with their in vitro reuptake inhibitory properties. Indatraline (3 mg/kg) reversed the full repertoire of OXA-induced neuropathic hypersensitivity. NS18283 (10 mg/kg) reversed OXA-induced mechano-hypersensitivity and cold allodynia. Venlafaxine (16 mg/kg) and escitalopram (4 mg/kg) only reversed cold allodynia and mechano-hypersensitivity, respectively. All MRIs produced antidepressant-like activity in anxio/depressive phenotype of OXA mice.

Conclusions: Acute administration of drugs that enhance the activity of serotonin, norepinephrine and dopamine neurotransmission within nociceptive pathways may provide a broader spectrum of antinociception than dual or selective reuptake inhibitors in animal models of neuropathic pain. Whether similar observations would occur after repeated administration of such compounds in an attempt to simulate dosing in humans, or be compromised by dopaminergic-mediated adverse effects warrants further investigation.

1. Introduction

Involvement of monoaminergic systems in pain pathophysiology has formed the basis for the use of tricyclic antidepressant drugs to treat neuropathic pain (Sindrup et al., 2005). However, their therapeutic use has been limited by a poor side effect profile (Lader, 1996). More recent dual serotonin (5-HT) and norepinephrine (NE) reuptake inhibitors (SNRIs), such as venlafaxine or duloxetine, appear to be devoid of
many of these side effects and yet still attenuate neuropathic pain in preclinical (Bomholt et al., 2005; Pedersen et al., 2005) and clinical studies (Wei et al., 2009; Attal et al., 2010). Compelling evidence also suggests that dopamine (DA) is involved in the regulation of pain and more particularly in analgesia (Wood, 2008). For example, the enhancement of DA transmission induced by L-DOPA or bupropion produces analgesia in several models of neuropathic pain (Pedersen et al., 2005; Cobacho et al., 2010). Conversely, lesion of DA neurons by the neurotoxin 6-hydroxydopamine in rodents attenuates the analgesic effect of morphine (Deakin and Dostrovsky, 1978).

DA is also involved in locomotor activity, cognition, reward and emotion (Missale et al., 1998), which might participate in the modulation of pain (Hache et al., 2011). In particular, there is growing interest for DA in the field of mood disorders. Accordingly, the selective D2/D3 receptor agonist pramipexole has been shown to be effective in depression as an augmentation strategy for patients refractory to selective serotonin reuptake inhibitors (SSRIs) (Lattanzi et al., 2002; Goldberg et al., 2004). Furthermore, degeneration of DA neurons in Parkinson’s disease patients leads to a 50% prevalence of depression (McDonald et al., 2003). Together, these converging lines of evidence have led to the development of a new generation of antidepressants: the triple reuptake inhibitors (TRIs). These compounds block 5-HT, NE and DA transporters thereby increasing all three monoamine levels in the synapse (Guiard et al., 2009). Enhancement of dopaminergic neurotransmission might expand the favourable antidepressant (Bourin et al., 2009; Ghanbari et al., 2012) and analgesic (Pedersen et al., 2005) profile of SNRIs. In this regard, the TRI bicifadine attenuates nociceptive behaviours in animal models of acute, persistent and chronic pain (Basile et al., 2007). Moreover, the DA D2 receptor antagonist (−)-sulpiride partially attenuated bicifadine-mediated reversal of mechanical hypersensitivity in nerve-injured rats, thereby emphasizing the importance of DA reuptake in its antinociceptive actions.

Within the brain, the anterior cingulate cortex (ACC) receives a dense monoaminergic innervation, the activation of which is reduced in patients with chronic pain (Honda et al., 2007) and is differentially modulated between responders and non-responders to antidepressant therapy (Mulert et al., 2007). In the current study, after initially assessing the in vitro reuptake inhibitory properties of NS18283, we used microdialysis within the ACC of naïve mice to confirm that these selectivity profiles of two structurally unrelated TRIs, NS18283 and indatraline, were also observed in vivo. We then proceeded to compare the antinociceptive properties NS18283 and indatraline, with those of the SNRI venlafaxine and the SSRI escitalopram in a mouse model of chemotherapy-induced neuropathic pain. The antidepressant-like properties of these compounds were also investigated in this model in order to unveil a putative link in the relief of pain and mood disorders.

### 2. Materials and methods

#### 2.1 Animals

Experiments were performed on male C57BL/6j mice (7–8 weeks) (Janvier, Le Genest sur l’Isle, France) housed in groups of 5 per cage under a 12-h light/dark cycle (lights on at 07:00 h) at a constant room temperature of 23 °C. To minimize aggressive behaviours, no females were present in the room during the study. In addition, group-housed male mice were routinely monitored for the possible presence of defeated/injured individuals, so that they could be excluded from the study proper. Food and water were available ad libitum. All experiments were performed during the light period. Pharmacological tests and care of animals were conducted in accordance with the guidelines of the International Association for the Study of Pain (Zimmermann, 1983), the recommendation of the European Community (86/609/EEC) and the French National Committee (87/848) for care and use of laboratory animals, and approved by the local committee of ethics (permission #B 92–373, F. Coudoré).

#### 2.2 Drugs

Oxaliplatin (OXA) (Leancare, Flintshire, UK) was dissolved in Glucose 5% and administered intraperitoneally in a volume of 10 mL/kg. 7-[(exro-8-azabicyclo[3.2.1]octan-3-yl)oxy]-3-methyl-chromen-2-one hydrochloride (NS18283; Neurosearch, Ballerup, Denmark; Supporting Information Fig. S1),
indatraline (Tocris, Bristol, UK; Supporting Information Fig S1), venlafaxine (Biotrend, Zurich, Switzerland), escitalopram (Lundbeck A/S, Valby, Denmark) and morphine (Cooper, Melun, France) were dissolved in saline (0.9% sodium chloride) and administered subcutaneously at doses of 10, 3, 16, 4 and 5 mg/kg, respectively. The doses for escitalopram, venlafaxine and indatraline were chosen based on previous behavioural studies using comparable tests (Tirelli et al., 1998; Yalcin et al., 2009; Zomkowski et al., 2010).

### 2.3 Intracerebral microdialysis

Extracellular levels of 5-HT, NE and DA were measured in the ACC by microdialysis prior to and following drug administration to confirm their monoamine receptor inhibitory profiles under the conditions tested herein. Anaesthetized mice were implanted with probes in the ACC (stereotaxic coordinates in millimetres from bregma according to Hof et al., 2000: A = +1.3, L = +0.2, V = −2.5; A, anterior; L, lateral; V, ventral). Animals were allowed to recover from the surgery overnight. On the next day, approximately 20 h after surgery, the probes were continuously perfused with artificial cerebrospinal fluid (aCSF: NaCl 147 mmol/L, KCl 3.5 mmol/L, CaCl₂ 1.26 mmol/L, MgCl₂ 1.2 mmol/L, NaH₂PO₄ 1.0 mmol/L, NaHCO₃ 25.0 mmol/L; pH 7.4 ± 0.2) at a flow rate of 1.5 μL/min using a CMA/100 pump (CMA/100, Stockholm, Sweden) while animals were awake and freely moving in their cage. Two hours after the start of an aCSF perfusion stabilization period, four fractions were collected every 15 min into small Eppendorf tubes (Eppendorf, Le Pécq, France) to measure the basal values of monoamines (mean ± SEM) calculated for each mouse prior to acute subcutaneous (s.c.) administration of vehicle or test drug. Twelve subsequent 15-min samples were collected (t₁₅₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋Петерс, 2010). This sequence was applied to minimize the impact of stress across tests. A 48-h washout period was used between each assessment in order to facilitate the maximal clearance of injected drugs.

#### 2.4.1 Mechanical behavioural testing (von Frey)

Animals were placed upon an elevated mesh floor surrounded by a clear plastic enclosure (10 × 10 × 10 cm). Mechanical sensitivity was assessed using three von Frey filaments with bending force 0.16, 0.6 and 1.4 g (Bioseb Inc., Vitrolles, France). In ascending order of force, each filament was applied for a duration of 2 s to the mid-plantar area of each hind paw five times, with 3 s between each application. Rapid retraction, shaking and/or licking of the hind paw were considered to represent nociceptive specific behaviours and only one of these responses needed to be displayed to be considered as a positive withdrawal response. Applications were applied to both hind paws, counted and then expressed as an overall percentage response.

#### 2.4.2 Thermal preference plate test

The T2PT was performed as described previously (Hache et al., 2012). Briefly, mice were allowed to freely explore an enclosure in which the floor is composed by two adjoining thermoelectric computer-managed metal plates (Bioseb Inc.). Both plates are enclosed by an opaque Plexiglas box (Bioseb Inc.). Low illumination is used for each compartment. Preliminary experiments determined that ‘naïve’ animals spent a similar amount of time on the two plates when they were set to 30 °C, suggesting that this temperature is perceived as comfortable. Correspondingly, OXA mice also spent a similar time on each plate, confirming that they had similar exploratory behaviour to control mice that received the vehicle (unpublished data). Subsequently, in the present work, the floor of one compartment was maintained at 30 °C and the other plate was set at 20 °C. This latter temperature is well within the threshold range of channel complexes localized within the terminal endings of sensory neurones that are capable of responding to cooling and cold (McKemy et al., 2002). The animals were videotaped from above and the time spent on each plate was used.

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2.4.1.3 Cold plate test

Mice were placed individually on a cold plate at −4.0 ± 0.2 °C with four 30.5-cm Plexiglas walls (Bioseb Inc.) and the reaction time (in seconds) until the first signs of a positive painful response consisting of jumping was recorded. In order to avoid paw damage, mice were immediately removed after the first jump and the cut-off latency was set at 3 min.

2.4.1.4 Novelty suppressed feeding

NSF is a conflict test that elicits competing motivations: the drive to eat and the fear of venturing into the centre of the brightly lit arena to obtain food. The NSF test was performed during a 10-min period as previously described (David et al., 2009). Briefly, 24 h prior to testing, food was removed from the home cage. At the time of testing, a single pellet of food was placed in the centre of the cage on a white enlightened paper to strengthen the anxiogenic context. Latency to begin eating was used as an index of depressive/anxiety-like behaviour (Bodnoff et al., 1988). Food consumption during 5 min in the home cage was immediately measured after the test to control that mice were hungry enough to elicit the conflict.

2.4.1.5 Tail suspension test

The TST is a paradigm commonly used to predict antidepressant-like activity of compounds in mice and was performed as previously described (Cryan et al., 2005). Total duration of immobility during a 6-min session was recorded using an automated apparatus (Bioseb Inc.). A decrease in immobility duration can be interpreted as an antidepressant-like response. However, using this paradigm to describe a depressive-like phenotype remains controversial, especially herein given that the C57BL/6j is a strain that exhibits an elevated baseline for immobility time (Ripoll et al., 2003).

2.5 Data analysis and statistics

Results from data analyses are expressed as mean ± SEM. Data were analysed using StatView 5.0 software (SAS Institute, Cary, NC, USA). For microdialysis experiments, statistical analyses were performed comparing area under the curve (AUC) calculated for the 15- to 180-min period between antidepressants versus vehicle administration. For all experiments, one-way or two-way analyses of variance (ANOVA) with repeated measures were applied to the data as appropriate. Significant main effects and/or interactions were followed by Bonferroni post hoc analysis or Student’s t-tests as appropriate. In the NSF test and the CPT, we used the Kaplan–Meier survival analysis due to the lack of normal distribution of the data. Mantel–Cox log-rank test was used to evaluate differences between experimental groups. The correlation between latency to feed in the NSF and latency of first jump in the CPT was estimated by Pearson’s test; animals reaching the cut-off were excluded from the statistical analysis to avoid any ‘ceiling effect’.

3. Results

3.1 Monoamine reuptake assays

The inhibition of 5-HT, NE and DA uptake mediated by NS18283 was measured in rat synaptosomal preparations (for Material and methods, see Supporting Information Appendix S1) with the following data representing the mean ± SEM of four experiments. NS18283 inhibited uptake of 5-HT most potently with an IC50 = 0.72 ± 0.31 nmol/L. Inhibition of NE uptake by NS18283 was in the order of 10-fold lower (IC50 = 10 ± 5 nmol/L), while inhibition of DA uptake was least affected by NS18283 (IC50 = 150 ± 45 nmol/L). Accordingly, the rank order of potency obtained for NS18283 was 5HT > NE > DA (Supporting Information Table S4).

3.2 Neurochemical effects of monoaminergic antidepressants in the ACC

Using in vivo microdialysis, analyses of AUC (% of baseline values) indicated that escitalopram (4 mg/kg, s.c.) significantly elevated the extracellular 5-HT concentrations in the ACC relative to vehicle [215.7 ± 16.5% vs. 86.19 ± 7.0% of baseline, F(1, 6) = 52.917, p < 0.001]. However, escitalopram did not change NE and DA extracellular concentrations (Fig. 1A and B). Venlafaxine (16 mg/kg, s.c.) significantly increased extracellular concentrations of 5-HT [283.8 ± 34.2 vs. 110.4 ± 3.4% of baseline, F(1, 6) = 25.446, p < 0.01] and NE [259.0 ± 41.2 vs. 61.3 ± 9.4% of baseline, F(1, 6) = 17.513, p < 0.05] (Fig. 1C and D). Indatraline (3 mg/kg, s.c.) significantly increased the extracellular concentrations of 5-HT [194.6 ± 14.4 vs. 104.2 ± 8.03% of baseline, F(1, 6) = 30.114, p < 0.01], NE [262.5 ± 44.4 vs. 94.5 ± 13.9% of baseline, F(1, 6) = 17.070, p < 0.01] and DA [292.7 ± 53.4 vs. 93.7 ± 6.1% of baseline, F(1, 6) = 13.725, p < 0.01] (Fig. 2E and F). Finally, NS18283 (10 mg/kg, s.c.) significantly increased the extracellular concentrations of 5-HT [196.0 ± 36.9 vs. 84.9 ± 11.6% of baseline, F(1, 8) = 8.256, p < 0.05], NE [294.3 ± 44.9 vs. 101.9 ± 22.8% of baseline, F(1, 6) = 14.559, p < 0.01] and DA [230.6 ± 29.4 vs. 124.7 ± 34.0% of baseline, F(1, 8) = 5.547, p < 0.05] (Fig. 1G and H). Importantly, we confirmed functionally that although indatraline and NS18283 increased all three monoamines in the ACC, the other monoaminergic compounds tested herein
increased either 5-HT alone (escitalopram) or both 5-HT and NE (venlafaxine). The ability of indatraline and NS18283 to enhance DA transmission in the ACC was significantly higher than that of escitalopram and venlafaxine (Fig. 1I, statistical comparisons are provided in Supporting Information Table S2). These results demonstrated that the in vivo properties of these monoaminergic agents are in agreement with their pharmacological profiles determined in vitro (for a review, see Hache et al., 2011).

3.3 OXA induces mechano-hypersensitivity, cold allodynia and cold hyperalgesia

In the von Frey hair test, OXA-treated mice had a significantly higher percentage of paw withdrawals to
application of the 1.4 g filament than vehicle-injected mice (54.0 ± 6.5% vs. 20.0 ± 4.7%, \( t(18) = -4.71, p < 0.001 \)), indicative of the presence of mechano-hypersensitivity. Notably, there was no difference in response to the application of 0.16 and 0.6 g filaments (Fig. 2A). Using the T2PT, OXA-treated mice spent significantly less time on the 20 °C plate (15.5 ± 2.1% vs. 37.0 ± 4.6%, \( t(18) = 4.19, p < 0.001 \)) compared with vehicle-treated mice, revealing the presence of cold allodynia (Fig. 2B). Accordingly, we explored whether OXA treatment was able to induce hypersensitivity as assessed in the CPT (Kaplan–Meier survival analysis, Mantel–Cox log-rank test, \( p < 0.01 \); Fig. 2C). Student’s t-test revealed a significant effect of OXA (\( t(18) = 2.88, p < 0.01 \)) on the latency to jump (70.5 ± 22.8 s vs. 153.5 ± 17.6 s, Fig. 2D) confirming the presence of cold hyperalgesia.

### 3.4 Effects of monoamine reuptake inhibitors on nociception

In the von Frey hair test, a one-way ANOVA revealed a significant effect of treatment factor [\( F(5, 64) = 9781, p < 0.001 \)], where the frequency of hind paw withdrawal was significantly lower in escitalopram- (30.8 ± 6.7% of withdrawal paw, \( p < 0.01 \)), indatraline- (26.4 ± 6.2% of withdrawal paw, \( p < 0.001 \)) and NS18283-treated mice (32.5 ± 2.8% of withdrawal paw, \( p < 0.001 \)) compared with vehicle (Fig. 3A). In the T2PT, a one-way ANOVA revealed a significant effect of treatment factor [\( F(5, 64) = 7.72, p < 0.001 \)], where the time spent upon the 20 °C plate was higher in venlafaxine- (36.52 ± 2.9%, \( p < 0.001 \)), indatraline- (37.3 ± 3.9%, \( p < 0.001 \)) and NS18283-treated mice (37.7 ± 3.9%, \( p < 0.001 \)) compared with vehicle (15.5 ± 2.1%; Fig. 4B). In the CPT (Fig. 3C and D), we explored whether escitalopram, venlafaxine, indatraline and NS18283 were able to increase latency of first jump (Fig. 4C: Kaplan–Meier survival analysis, Mantel–Cox log-rank test, \( p < 0.001 \)). One-way ANOVA revealed a significant effect of treatment factor [\( F(5, 64) = 10.514, p < 0.001 \)]. However, only indatraline was able to significantly increase latency of first jump compared with vehicle (170.3 ± 9.7 s vs. 80.3 ± 20.9 s respectively, \( p < 0.001 \), Fig. 3D). In contrast, NS18283, venlafaxine and escitalopram all failed to significantly modify this parameter (100.7 ± 17 s, 101.5 ± 18.1 s and 52.8 ± 12 s, respectively). As expected, the opioid analgesic morphine reversed mechano-hypersensitivity (9.0 ± 2.3% of paw withdrawal, \( p < 0.001 \)), cold allodynia (38.4 ± 4.6% on 20 °C plate, \( p < 0.001 \)) and cold hyperalgesia (180.0 ± 0.0 s, \( p < 0.001 \)). All pairwise comparisons between monoamine reuptake inhibitors by Bonferroni/Dunn analysis are summarized in Supporting Information Table S3.
3.5 Anxio-depressive-like phenotype of OXA mice and antidepressant-like activity of monoamine reuptake inhibitors

The NSF test revealed that OXA mice developed an anxious-depressive-like state (Supporting Information Fig. S2a; Kaplan–Meier survival analysis, Mantel–Cox log-rank test, \( p = 0.09 \)). A Student’s t-test revealed a significant effect of OXA \( [t(18) = -2.79, p < 0.05] \) on the latency to feed (Supporting Information Fig. S2b). Indeed, OXA-treated mice displayed an increased latency to feed compared with control group (429.5 ± 30.2 s vs. 233.2 ± 46.4 s, respectively). No differences in home cage food consumption were observed thereby excluding the possibility of changes on feeding drive between groups \([t(18) = 1.12, p = 0.26]\) (Supporting Information Fig. S2c).

Antidepressant-like properties of escitalopram, venlafaxine, indatraline and NS18283 were subsequently assessed in OXA-treated mice using the TST. Two mice in the indatraline group and one in the NS18283 group were removed because they climbed their tails. A one-way ANOVA revealed a significant effect of treatment \([F(5, 61) = 16.33, p < 0.001]\). A marked decrease in the immobility time was observed for OXA mice treated with either escitalopram (68.6 ± 17.3 s of immobility), venlafaxine (66.3 ± 17.1 s of immobility), indatraline (18.2 ± 14.5 s of immobility) or morphine (mph) 5 mg/kg (n = 10) were evaluated 45 min after their administration in the OXA mice using a battery of nociceptive tests. (A) In the von Frey hair test, values represent the mean + SEM of the percentage of paw withdrawals measured with a 1.4 g filament after administration of each drug. (B) In the thermal preference plate test, values represent the mean + SEM of the time spent on the 20 °C plate. (C) In the cold plate test, results are presented as a Kaplan–Meier survival curve. As indicated, all animals treated with mph reach the cut-off: 1 with esc, 4 with vlfx or NS18283, and 11 with inda. (d) Values represent mean + SEM of latency of first jump in seconds. \(*p < 0.01; \**p < 0.001\): significantly different from vehicle-administered mice.

![Figure 3](https://example.com/fig3.png)

**Figure 3** Antinociceptive effects of administration of various monoamine reuptake inhibitors on oxaliplatin (OXA)-induced neuropathic pain in mice. The analgesic effects of acute s.c. injection of escitalopram (esc) 4 mg/kg (n = 12), venlafaxine (vlfx) 16 mg/kg (n = 12), indatraline (inda) 3 mg/kg (n = 12), NS18283 (ns) 10 mg/kg (n = 12), and morphine (mph) 5 mg/kg (n = 10) were evaluated 45 min after their administration in oxaliplatin-treated mice submitted to a tail suspension test. The antidepressant-like effects of acute s.c. injection of escitalopram (esc) 4 mg/kg (n = 12), venlafaxine (vlfx) 16 mg/kg (n = 12), indatraline (inda) 3 mg/kg (n = 12), NS18283 (ns) 10 mg/kg (n = 12), and morphine (mph) 5 mg/kg (n = 10) were evaluated in the tail suspension test 45 min after their respective administration. Values represent mean + SEM of immobility time in seconds. \(*p < 0.05; \**p < 0.01 and \***p < 0.001\): significantly different from vehicle-administered mice.

![Figure 4](https://example.com/fig4.png)

**Figure 4** Antidepressant-like activity of various monoamine reuptake inhibitors in oxaliplatin-treated mice submitted to the tail suspension test. The antidepressant-like effects of acute s.c. injection of escitalopram (esc) 4 mg/kg (n = 12), venlafaxine (vlfx) 16 mg/kg (n = 12), indatraline (inda) 3 mg/kg (n = 12), NS18283 (ns) 10 mg/kg (n = 12), and morphine (mph) 5 mg/kg (n = 10) were evaluated in the tail suspension test 45 min after their respective administration. Values represent mean + SEM of immobility time in seconds. \(*p < 0.05; \**p < 0.01 and \***p < 0.001\): significantly different from vehicle-administered mice.
NS18283 (83.8 ± 16.0 s of immobility) compared with vehicle (127.9 ± 21.8 s of immobility, Fig. 4). Thus, these data clearly indicate antidepressive-like properties of these drugs in OXA-induced neuropathic pain and anxiodepressive-like phenotype.

4. Discussion

As yet no drugs have been officially approved for the clinical treatment of chemotherapy-induced neuropathic pain. The strongest evidence exists for topical analgesics and SNRIs such as duloxetine and venlafaxine (Pachman et al., 2011; Smith et al., 2013). Opioids are effective but often in a dose range that is poorly tolerated. Thus, we sought to examine the pharmacological sensitivity of OXA-induced neuropathic pain in mice to a diverse range of monoaminergic reuptake inhibitors which are capable of variously increasing extracellular levels of 5-HT, NE and/or DA, and all of which are involved in the modulation of nociceptive transmission within central pain pathways (Hache et al., 2011). Firstly, we assessed their ability to increase extracellular levels of 5-HT, NE and DA within the ACC by performing microdialysis studies in naïve mice using doses carefully chosen from pilot experiments. However, we are aware that facilitatory and inhibitory monoaminergic pathways are modulated in chronic pain to help mediate central sensitization (Millan, 2002), and that by association OXA might modulate extracellular monoamine levels and the neurochemical profiles of the drugs tested herein. We measured from the ACC because considerable evidence implicates this structure in the regulation of cognition, emotion and sensory information (Johansen et al., 2001; Ortega-Legaspi et al., 2011; Shackman et al., 2011). In particular, it is suspected to play a major role in the integration of affective and sensory aspects of neuropathic pain (Descalzi et al., 2012; Widerstrom et al., 2012). Accordingly, the in vivo neurochemical properties of indatraline reported here correlate well with functional studies performed in rat brain synaptosomes, where it inhibits the reuptake of all three monoamines equi potently (IC\textsubscript{50} = 0.48, 0.28 and 0.99 nmol/L for 5-HT transporter (SERT), NE transporter (NET) and DA transporter (DAT), respectively) (Arnt et al., 1985; Bogeso et al., 1985; Hyttel and Larsen, 1985). The ability of NS18283 to increase 5-HT, NE and DA outflows to a similar extent within the ACC was unexpected; however, given that it has a more unbalanced profile (IC\textsubscript{50} = 0.72, 10 and 150 nmol/L for inhibition of 5-HT, NE and DA reuptake in cortex, hippocampus and striatum, respectively). It is conceivable that blockade of 5-HT and NE transporters could have indirect effects on DA outflow given that the ACC dopaminergic system receives dense noradrenergic and serotonergic innervations (Adell and Artigas, 2004; Esposito, 2006). In other brain regions, e.g., 5-HT exerts an inhibitory influence on DA neurotransmission via the 5-HT2C receptor-mediated activation of gamma-aminobutyric acid (GABA) interneurons, decreasing dopaminergic neuronal firing in the ventral tegmental area (VTA) (Di Giovanni et al., 2000; Di Matteo et al., 2000). However, at the nerve terminals such as in the frontal cortex, evidence suggests that activation of 5-HT2A/C receptors may facilitate DA release (Bortolozzi et al., 2005; Pehek et al., 2006; Leggio et al., 2009), raising the possibility that the latter mechanism would have participated to increase cortical extracellular DA levels. Regarding the noradrenergic system, it is difficult to anticipate the effect of a global increase in NE levels. Indeed, α\textsubscript{1}-adrenoceptor activation has been shown to be inhibitory through activation of cortico-VTA pyramidal neurons that project unto GABA neurons in the VTA (Dumont and Williams, 2004; Paladini and Williams, 2004). In contrast, stimulatory effects via direct NE innervation of VTA dopaminergic neurons have also been described (Grenhoff et al., 1995; Steffensen et al., 1998), and as shown by the inhibition of the release of DA in the medial prefrontal cortex or the nucleus accumbens after the local infusion or systemic administration of the α\textsubscript{1}-adrenoceptor antagonist prazosin, respectively (Mathe et al., 1996; Pan et al., 2004). Moreover, it was described that DA reuptake in the frontal cortex or the hippocampus depended primarily on the NET (Moron et al., 2002; Guitard et al., 2008), notably because DAT expression was sparse in these areas (Sesack et al., 1998). Hence, NET inhibition could have also contributed to increased cortical extracellular DA levels through heterologous reuptake. Moreover, the slow time course of NS18283 on extracellular DA levels is not supportive of a direct action on the DAT and suggests the existence of other indirect mechanisms that might have reverberated on its neurochemical activity in the ACC.

Based on the above observations, we started by administering the TRIs indatraline or NS18283 to OXA mice at doses of 3 and 10 mg/kg, respectively, 45 min prior to evaluation of neuropathic hypersensitivity. Accordingly, indatraline reversed all signs of mechanical and cold hypersensitivity in OXA mice with a level of efficacy comparable to that of morphine. Although essentially similar results were obtained with NS18283, it did fail to reverse cold hyperalgesia in the
CPT. Careful consideration of the time course of monoamine levels obtained for these two compounds shows that the increase in extracellular DA levels produced by indatraline was greater than that obtained for NS18283 at this time point. This supported the concept that increasing DA neurotransmission, in addition to 5-HT and NE, provides a broader spectrum of analgesia across a more diverse range of neuropathic signs and symptoms in this animal model of neuropathic pain (Supporting Information Appendix S2). On balance, however, it is important to also consider the present results in relation to the psychostimulant effects of drugs capable of enhancing extracellular DA levels. Accordingly, this might represent a confounding parameter when interpreting drug-induced reductions in behavioural endpoints such as immobility in the TST as reflecting an antidepressant-like response. This said, morphine at the dose tested in the current study stimulates motor activity, yet clearly lacks any antidepressant-like activity in this paradigm.

Depression can occur as a deleterious consequence of chronic stress and chronic painful syndromes (Blackburn-Munro and Blackburn-Munro, 2001; Gambassi, 2009) with a prevalence of up to 30% in patients suffering from neuropathic pain (Gustorff et al., 2008). Surprisingly, only a limited number of preclinical studies have tried to address the relationship between mood disorders and pain, with even fewer having attempted to determine the parallel effects stress and of pharmacological manipulations on sensory and affective behaviours (Munro, 2012). Previously, we have shown that mice exposed to corticosterone for 5 weeks have impaired stress axis function and exhibit an increased latency to feed using the NSF paradigm, strongly suggestive of a robust anxiodepressive-like phenotype (David et al., 2009). Here, OXA treatment produced similar effects on the latency to feed in the NSF. Notably, this correlated with the latency to first jump in the CPT (R = -0.846; p = 0.016; Y = 486.5 – 1.35X; R² = 0.716; Supporting Information Fig. S2d), further supporting a putative link between neuropathic hypersensitivity and anxiodepressive-like phenotype (Hasnie et al., 2007; Suzuki et al., 2007; Matsuzawa-Yanagida et al., 2008; Leite-Almeida et al., 2009; Hu et al., 2010; Norman et al., 2010). The complexity of measuring affective comorbidity in rodent neuropathic models is reflected by another recent study involving neuropathic mice induced by sciatic nerve cuffing (Yalcin et al., 2011). Notably, although these mice developed pronounced mechanical hypersensitivity within days after injury, anxiodepressive-like behaviours detected in NSF were only observed from week 5 onwards. Moreover, depressive-like behaviours measured in the forced swim test were only manifest from 8 weeks after injury, leading the authors to conclude that affective changes of neuropathic pain evolve over time. This is not entirely discordant with our data as we failed to show any impairment in anxious-like behaviour in OXA mice measured in the elevated plus maze (Supporting Information Fig. S3). In contrast, the presence of anxiodepressive-like behaviour was clearly evident in the same OXA mice in the NSF. However, the NSF paradigm is only sensitive to chronic antidepressant treatments (Dulawa and Hen, 2005), unlike commonly used tests of antidepressant response, such as the TST. Therefore, we chose to determine the acute antidepressant-like activity of monoaminergic antidepressants in OXA mice using TST. All of the monoamine reuptake inhibitors tested herein study elicited antidepressant-like activity in OXA-treated mice as shown by a decrease in the time of immobility. The most pronounced effect was obtained with indatraline, complimenting initial data that described the behavioural profile of indatraline as a potential new antidepressant drug (Arnt et al., 1985).

Inevitably, it is tantalizing to speculate that its superior antidepressant efficacy might link directly to enhanced DA-related neurotransmission, and in turn might have been a contributory driver to its superior analgesic profile in OXA mice. A caveat here is that the acute dosing of such compounds in preclinical pain models does not accurately simulate dosing paradigms for antidepressant compounds administered to chronic pain patients. To begin to address this obstacle, we dosed naïve mice with NS18283 (10 mg/kg daily for 28 days) in the hope that putative anxiolytic efficacy would be maintained in the absence of side effects, so that we could adopt a similar dosing strategy in OXA mice. This dosing paradigm was chosen based on acute efficacy data obtained in OXA mice. However, the data were equivocal (Supporting Information Fig. S4), with the hyperlocomotion measured at day 28 in the open field suggesting that under the specific testing conditions employed it could be a predictor of abuse liability in humans (Paterson et al., 2010), and correlate to brain DAT occupancy levels in the region of 70–80% (Desai et al., 2005). Notably, BMS-820836 attains relevant SERT and DAT occupancy in human volunteers, indicating that putative efficacy can be obtained while maintaining an acceptable safety profile (Risinger et al., 2013). Currently, the optimal level of DAT occupancy required to mitigate neuropathic hypersensitivity in animal pain models or pain patients is unknown. Accordingly, a
more detailed knowledge of this parameter should be the driver for choosing a dose of a TRI which will provide stable brain concentrations upon repeated dosing prior to investigating putative analgesic efficacy in preclinical pain models. Even then, the relationship between absolute DAT occupancy and effects on behaviour in rodents per se is complex, with the apparent rate of occupancy playing an important role in behavioural effects including abuse liability (Desai et al., 2005; Volkow et al., 2005). A more pragmatic feature of inducing hyperlocomotion in such models is, as already discussed, that it can potentially confound interpretation of any putative anxiolytic or antidepressant efficacy, despite TRIs having been shown to possess clear antidepressant-like properties in naïve or depressed animals (Guiard et al., 2009).

5. Conclusion

In conclusion, our data highlight that: (1) pain and anxiodepressive-like behaviours in OXA neuropathic mice are interconnected at some level; (2) two structurally unrelated TRIs, indatraline and, to a lesser extent, NS18283, appear to possess superior analgesic profiles in neuropathic mice compared with either escitalopram or venlafaxine; and (3) the enhancement of dopaminergic neurotransmission within the ACC could constitute a key element underpinning the therapeutic activity of these pharmacological agents (Lopez-Avila et al., 2004). Whether this latter facet contributes to pain and mood disorder comorbidity per se remains to be fully elucidated. Moreover, in addition to investigating effects of TRIs on such comorbidities within a single animal model of relevant pathology, where functional changes in the target of interest can impact upon the efficacy obtained, for purposes of translational value to humans we would suggest that safety concerns for this drug class are also considered early on in the drug discovery process.

Author contributions

G.H., B.P.G., T.H.N. and G.Q. did the experiments, analysed the data and wrote the first draft of the manuscript. G.H., B.P.G., A.M.G., D.P., G.M. and F.C. conceived the study, analysed the data and participated in the revision of the final draft. All authors discussed the results and commented on the manuscript.

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References


Triple reuptake inhibitors reduce neuropathic pain

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Figure S1.** Chemical structure of the triple reuptake inhibitors NS18283 (a) and indatraline (b).

**Figure S2.** Anxio-depressive-like phenotype of oxaliplatin-treated mice submitted to the novelty suppressed feeding paradigm. (a) Results are expressed as cumulative survival curve of animals that have not eaten over a 10-min period. (b) Values represent mean ± SEM of latency to feed in seconds. (c) Food intake after testing session. Values represent food intake during 5 min following the testing session in the home cage. p = 0.26. *p < 0.05: significantly different from control mice. (d) Correlation between anxio-depressive-like phenotype and pain in OXA mice. Data from Fig. 2D and Supporting Information Fig. S2b were used to estimate Pearson’s correlation between the latency to feed in the novelty suppressed feeding test and the latency of first jump in the cold plate test. The latency to feed showed a significant correlation (R = 0.846; p = 0.016; Y = 486.5 − 1.35X; R² = 0.716) with the latency of first jump.

**Figure S3.** Assessment of anxiety-like behaviours in oxaliplatin-treated mice exposed to the elevated plus maze. No significant difference between vehicle and oxaliplatin-treated mice was revealed (a, b, c). Assessment of anxiety-like activity after administration of various monoamine reuptake inhibitors in oxaliplatin-treated mice submitted to the elevated plus maze. Compared with vehicle-treated mice, none of the antidepressant tested exhibited an anxiolytic-like effect in these conditions (d, e, f). Values are plotted as mean ± SEM of time spent in open (a, d), closed arms (c, e) and the ratio of entries (b, f).

**Figure S4.** Effects of chronic administration of NS18283 (10 mg/kg daily for 28 days) in naive mice subjected to the open field. Values are plotted as mean ± SEM of entries in the centre (a), time spent in the centre (b), total ambulatory distance (c), and the ratio of distance in the centre to total ambulatory distance (d). **p < 0.01, ***p < 0.001 versus naive mice. Although an anxiolytic-like effect might be suspected from the increased entries into the centre after NS18283 treatment, the parallel increase in total ambulatory distance prevents any clear conclusion from being made in this regard.

**Table S1.** General toxicity of repeated oxaliplatin (OXA) injections in C57BL/6J male mice.

**Table S2.** Statistical pairwise comparison of antidepressant effects upon extracellular concentrations of 5-HT, NE and DA in the anterior cingulate cortex (ACC).

**Table S3.** Statistical pairwise comparison of antidepressant efficacy in relation to attenuating mechanical and thermal hypersensitivity in OXA mice.

**Table S4.** In vitro functional activity of NS18283.