JPET Fast Forward. Published on April 12, 2007 as DOI:10.1124/jpet.107.120915

IN VIVO EFFECT OF VENLAFAXINE ON LOCUS COERULEUS NEURONS: ROLE OF OPIOID, α_2-ADRENERGIC AND 5-HT_{1A} RECEPTORS

Esther Berrocoso and Juan Antonio Mico*

Pharmacology and Neuroscience Research Group, Department of Neuroscience (Pharmacology and Psychiatry), School of Medicine, University of Cádiz, Plaza Falla 9, 11003 Cádiz, Spain.

Copyright 2007 by the American Society for Pharmacology and Experimental Therapeutics.

RUNNING TITLE PAGE

Running title: *In vivo* venlafaxine effects on locus coeruleus neurons

Corresponding author:

Juan A. Mico, Pharmacology and Neuroscience Research Group, Department of Neuroscience (Pharmacology and Psychiatry), School of Medicine, University of Cádiz, Plaza Fragela 9, 11003 Cádiz, Spain.

Phone: +34 956015247; Fax: +34 956015225; e-mail: juanantonio.mico@uca.es

Number of text pages: 32

Number of figures: 7

Number of references: 42

Number of words in the *Abstract*: 233

Number of words in the *Introduction*: 781

Number of words in the *Discussion*: 1453

Abbreviations: 5-HT, serotonin or 5-hydroxytryptamine; NA, noradrenaline; dual 5-HT/NA re-uptake inhibitor, SNRI; locus coeruleus, LC; monoamine oxidase inhibitor MAOIs; ED_{50}, 50% effective dose; ANOVA, analysis of variance.

Section assignment: Neuropharmacology

Abstract

The locus coeruleus (LC) is involved in several neural pathways responsible for some somatic and emotional processes, such as pain and depression; its activity is regulated by several receptors, such as opioid, α_2-adrenergic and 5-HT_{1A} receptors. The present study investigates the in vivo effects of venlafaxine, an antidepressant with analgesic properties, on locus coeruleus neurons, and its modulation by opioid, α_2-adrenergic and 5-HT_{1A} receptors. The results show that acute administration of venlafaxine produced a dose-dependent, complete inhibition of LC activity. This inhibitory effect was not reversed by the opioid receptor antagonist naloxone, but subsequent administration of idazoxan, an α_2-adrenoceptor antagonist, did reverse it. The pre-administration of the 5-HT_{1A} receptor agonist, 8-OH-DPAT (1 and 40 μg/kg), significantly enhanced the venlafaxine inhibitory effect, decreasing the ED_{50} by 56% and 44% respectively. A 14-day treatment with venlafaxine (40 mg/kg/day) induced a suppression of the firing activity of LC neurons. In these treated animals, venlafaxine produced a similar inhibitory effect to in non-treated animals. This inhibitory effect was not reversed by naloxone, but was reversed by idazoxan. In addition, the pre-administration of 8-OH-DPAT (40 μg/kg) significantly enhanced the venlafaxine effect, decreasing the ED_{50} by 60 %. These results suggest that the effect of venlafaxine on LC neurons is modulated by α_2-adrenergic and 5-HT_{1A} receptors, and not by opioid receptors. These data could contribute to the further understanding of the antidepressant and analgesic mechanism of action of venlafaxine.

Introduction

Venlafaxine is a dual 5-HT/NA (serotonin/noradrenaline) re-uptake inhibitor (SNRI) antidepressant that blocks the synaptosomal uptake of 5-HT at low doses and NA at higher doses (Muth et al., 1986; Dawson et al., 1999). In addition to its antidepressant effect, venlafaxine also has analgesic properties (Saarto and Wiffen, 2005). The fact that antidepressants have antidepressant and analgesic properties suggests that monoamines, and primarily 5HT and NA, are implicated in depression as well as in pain. These pathologies have a high comorbidity, implying a possible interrelated or common neurochemical mechanism and/or neuroanatomical substrate.

One of the most important nuclei which is clearly implicated in depression and pain, and by extension involved in the mechanism of action of antidepressants (Svensson and Usdin, 1978), is the locus coeruleus (LC). LC electrical activity is regulated by α_2-adrenoceptors and opioid μ-receptors and the activation of these receptors leads to a progressive reduction in firing activity (Egan et al., 1983). In addition, the LC is a strategically situated nucleus, located where the noradrenergic and the serotonergic systems establish a close functional relationship. The LC receives dense 5-HT projections coming from dorsal raphe and pericoerulear 5-HT neurons (Segal, 1979). Interestingly, it has been suggested that LC neurons are subject to the indirect influence of 5-HT_{1A} receptors (Szabo and Blier, 2001c). Therefore, taking into account all these data, it seems clear that LC electrical activity is regulated by a great variety of receptors that contribute to regulating the functional state of LC neurons in physiological and pathological conditions, such as depression or pain; by extension, it may have crucial implications in the antidepressant and analgesic effects of antidepressants.

Several findings have demonstrated that venlafaxine and other antidepressants regulate the electrophysiological properties of the LC. In fact, previous electrophysiological studies have shown that venlafaxine inhibited the firing of LC neurons through an α_2-adrenoceptor mechanism (Beique et al., 1999). Regarding opioid receptors, it is not clear if venlafaxine has any effect on them in LC neurons. The analgesic mechanism of action of antidepressants has been attributed mainly to the enhancement of monoaminergic neurotransmission. However, other mechanisms, such as the activation of the endogenous opioid system, have been proposed (Mico et al., 2006), even though antidepressants have no affinity for opioid receptors. Most data come from studies of tricyclic antidepressants, which have been used as analgesics for a long time. Evidence of the opioid system's involvement in the antidepressant effect is that the antinociceptive effect of some tricyclics is inhibited by naloxone (Valverde et al., 1994). Furthermore, tricyclics are able to increase the levels of enkephalins in some CNS regions (Hamon et al., 1987) and displace radiolabelled opioid receptor ligands and, after repeated administration, to modify receptor density (Isenberg and Cicero, 1984; Hamon et al., 1987). However, no data are available for the new SNRIs (venlafaxine, duloxetine and milnacipran), which are emerging as analgesic antidepressants with fewer side effects than tricyclics. With venlafaxine, controversy exists about the possible role the opioid system plays in its antinociceptive effect (Schreiber et al., 1999; Marchand et al., 2003). However, interestingly, we have previously shown that the antidepressant-like effect of venlafaxine is blocked by naloxone in the forced-swimming test (Berrocoso et al., 2004).

Regarding the role of 5-HT_{1A} receptors, it has been shown previously that the blockade of 5-HT_{1A} receptors potentiates the inhibitory effect of venlafaxine on wind-up activity in mononeuropathic rats (Marchand et al., 2004). Furthermore, we have found that the activation of 5-HT_{1A} receptors enhances the antidepressant-like effect of venlafaxine and blocks its antinociceptive effect (unpublished data).

Finally, some studies with tricyclic antidepressants have reported that chronic treatment with these drugs resulted in tolerance to the effects they had on spontaneous and/or sensory-evoked LC discharge due to a desensitization of α_2-adrenergic receptors (Svensson and Usdin, 1978; Valentino et al., 1990). Thus, the functional desensitization of α_2-adrenergic receptors in the LC has been claimed to be necessary for the onset of the clinical antidepressant effect. However, other data argue that this does not occur after chronic treatment with MAOIs (Blier and de Montigny, 1985), reboxetine (Szabo and Blier, 2001a) and even with other tricyclics (Lacroix et al., 1991). As for venlafaxine, no data are available. Similarly, no data exist regarding opioid and 5-HT_{1A} receptors in LC neurons after long-term venlafaxine treatment.

Therefore, considering that LC activity is subject to the action of these receptors, the first aim of our study will be to explore the role of α_2-adrenergic, opioid, and 5-HT_{1A} receptors in the acute effect in vivo of venlafaxine on LC neurons. This will be achieved using electrophysiological techniques after both acute and long-term treatment and will help us to further understand both the antidepressant and analgesic effects of venlafaxine, and the relationship between them.

Methods

Animal treatments and surgical procedure

The experiments were performed using adult male Sprague-Dawley rats weighing 220 - 300g or 180 – 200g, for acute or long-term experiments respectively. The animals were housed under standard laboratory conditions (22ºC, 12h light/dark cycles, lights on at 8am, food and water *ad libitum*). Every effort was made to minimize animal suffering and to use the minimum possible number of animals. The experimental protocols were reviewed and approved by the Committee for Animal Experimentation at the University of Cádiz and complied with the International Association for the Study of Pain ethical guidelines (Zimmermann, 1983). Animal care and use procedures conformed to European Ethical Standards (86/609-EEC) and Spanish Law (RD 1201/2005) for the care and use of laboratory animals. Animals were housed in groups of 4 and a 7-day acclimatization period was allowed before the experiments.

Rats were anaesthetized with chloral hydrate (400 mg/kg i.p.); subsequently a cannula was inserted into the trachea and the right jugular vein was cannulated for systemic (i.v.) injections of anaesthetic and drugs. Supplemental doses of anaesthetic were given to maintain constant anaesthesia and to prevent any nociceptive reaction. Body temperature was maintained at 37°C with a heating pad. The rat was placed in a stereotaxic frame with its head at a 15° angle to the horizontal plane (nose down). To approach the LC, the skull was exposed, and a hole (approximately 3mm diameter) was drilled for the insertion of the recording electrode at 1.1mm lateral to the midline and 3.7mm posterior to the lamboid fontanel over the cerebellum. The dura over the cerebellum was carefully removed.

For sustained treatment regimes, rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) for subcutaneous implantation of osmotic minipumps (ALZET®). The skin was shaved and sterilized with an antiseptic solution (Betadine®). An incision of about 2cm was made between the scapulae and the filled pump was inserted, stitched and supported in place with three clips. Doses of venlafaxine (mg/kg per day) were calculated estimating the weight of the rat at the middle of treatment time by assuming that the rat would gain 50g/week, and this value was used to prepare the solution. Experiments were carried out 24 hours after minipump removal.

To study the acute effect of venlafaxine on LC neurons *in vivo*, dose-effect curves were performed for venlafaxine, which was injected at 2 – 3 min intervals, in doubling doses, until maximal effect was reached. This experimental group will be referred to as the "control group". A subsequent injection of naloxone (5 mg/kg, i.v.), an opioid receptor antagonist, was administered. If the admistration of naloxone (5 mg/kg, i.v.) did not reverse the inhibitory effect of venlafaxine, idazoxan (1 mg/kg, i.v.), an α_2-adrenoceptor antagonist, was subsequently administered in order to achieve this reversal. Secondly, the role of 5-HT_{1A} receptors in the effect displayed by venlafaxine was evaluated by pre-treating animals with the selective agonist of 5-HT_{1A} receptors, 8-OH-DPAT (1 and 40 μg/kg, i.v.), 3 – 6 min before dose-response curves for venlafaxine were obtained. Thirdly, the possible tolerance effect to venlafaxine after long-term treatment (40 mg/kg/day, 14 days) and its modulation by opioid, α_2-adrenergic and 5-HT_{1A} receptors was explored. Initially, the effect of 14 days of venlafaxine treatment on basal firing activity was recorded. To do this, three to seven consecutive LC cells were randomly recorded (3-5 minutes each cell) and the mean firing rate value for each treated group

was compared with that obtained in the control group. Subsequently, in one experimental group, to explore a possible tolerance effect to venlafaxine treatment, the acute effect of venlafaxine was studied after sustained treatment. Dose-effect curves were performed for this drug until maximal effect was reached. Following the procedure described above, a subsequent injection of naloxone (5 mg/kg, i.v.) was administered. If the inhibitory effect of venlafaxine was not reversed after the administration of naloxone, idazoxan (1 mg/kg, i.v.) was subsequently administered in order to reverse it. In another experimental group, the modulatory effect of the activation of 5-HT_{1A} receptors on the venlafaxine inhibitory effect was explored in long-term treated animals. In this case, animals were pre-treated with 8-OH-DPAT, 4 – 6 min before dose-response curves for venlafaxine were obtained, following the same protocol as described before.

The doses used for these compounds were chosen on the basis of previous successful experiments carried out in our laboratory and data available in the literature. Naloxone was injected at 5 mg/kg, a dose which blocks the effect of opioid compounds on LC neurons (Ruiz-Durantez et al., 2003). Idazoxan was injected at 1 mg/kg, a dose previously used to reversed the inhibitory effect of a NA reuptake blocker (Szabo and Blier, 2001a). 8-OH-DPAT was tested at low and high doses (1 and 40 μg/kg) following previous studies into the role of 5-HT_{1A} receptors in LC firing activity (Ruiz-Ortega and Ugedo, 1997; Szabo and Blier, 2001a; Berrocoso et al., 2006). For long-term administration, venlafaxine was infused in a dose of 40 mg/kg/day for 14 days (via osmotic minipumps) to ensure the dual effect on the reuptake inhibition of both 5-HT and NA, following previous studies (Beique et al., 2000b; Beique et al., 2000a).

Extracellular recordings of LC neurons in vivo

Single-unit extracellular recordings of LC neurons were performed as described previously (Ugedo et al., 1998). The recording electrode was an Omegadot single-barrel glass micropipette filled with a 2% solution of Pontamine Sky Blue in 0.5% sodium acetate and broken back to a tip diameter of 1 - 2.5μm. The extracellular signal from the electrode was amplified, discriminated and monitored on an oscilloscope and with an audio monitor too. Discriminated spikes were fed into a PC and processed using computer software (CED micro 1401 interface and Spike2 software, Cambridge Electronic Design, U.K.). LC neurons were encountered 5.5 - 6.0mm below the dural surface, just ventral to a zone of relative silence (corresponding to the IV^{th} ventricle), and medial to neurons of the mesencephalic nucleus of the V^{th} cranial nerve (which could be activated by depression of the mandible). LC neurons were identified by standard criteria that included: long duration action potential (> 2ms); spontaneous firing at a regular rhythm; a slow firing rate; and characteristic spikes with a long-lasting positive-negative waveform (Aghajanian et al., 1977). The basal firing rate was recorded at least 2 min prior to any drug administration. Only one noradrenergic LC cell was pharmacologically studied in each animal.

At the end of the experiment, a 5μA cathodal current was passed through the recording electrode to leave a blue spot at the recording site. The brain was fixed and removed, cut into sections of 50μm and stained with neutral red. The site of recording was examined microscopically. Only measurements from cells within the LC were included in this study.

Analysis of data and Statistical analyses

Changes in firing rate are expressed as percentages of the baseline firing rate (defined as 0%). Dose-concentration-effect curves were analyzed for the best non-linear fit to a logistic three-parameter equation (Parker and Waud, 1971): $E = E_{max}\ [A]^n / (ED_{50}^n + [A]^n)$, where [*A*] is the i.v. dose of venlafaxine and *E* is the effect on the firing rate induced by *A*; E_{max} is the maximal percentage change at "infinite" dose (100 %); ED_{50} is the effective dose for eliciting 50 % of E_{max}; *n* is the slope factor of the dose-response curve. Experimental data were analyzed by using the computer program Graphd Prism (v. 3.0; GraphPad Software, Inc.). In the experiments which evaluated the role of 5-HT_{1A} receptors in the inhibitory effect displayed by venlafaxine, the baseline firing rate after the injection of 8-OH-DPAT was taken as 0%. Statistical significance was assessed by means of a two-way repeated measures analysis of variance (ANOVA; with venlafaxine doses and 8-OH-DPAT pre-treatment or venlafaxine long-term treatment as main factors) followed by the Bonferroni test. For comparisons between more than two groups, one-way repeated measures or a one-way ANOVA followed by the Newman-Keuls test were chosen. For comparisons between two groups and for the analysis of the firing rate before and after drug administration, unpaired and paired Student's *t*-tests were used respectively. The level of significance was considered as $p < 0.05$. Data are reported as mean ± S.E.M.

Drugs

The following drugs were used: venlafaxine (1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol hydrochloride, courtesy of Wyeth Research, USA); 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide), idazoxan (2-(1,4-benzodioxan-2-yl)-2-imidazoline hydrochloride) and naloxone ((5α)-4,5-epoxy-3,14-dihydro-17-(2-propenyl)morphinan-6-one hydrochloride) provided by Sigma Chemicals

Co, Spain). All drugs solutions were dissolved in saline (0.9% NaCl) and those used for i.v. administration were prepared immediately before each trial.

Results

Effect of acute administration of venlafaxine on the firing activity of locus coeruleus neurons and its interaction with opioid and α_2-adrenergic receptors

Administration of venlafaxine, as expected, depressed the spontaneous activity of LC neurons in a dose-dependent manner ($F_{(6,41)}$ = 3.96, p < 0.01, one-way repeated measures ANOVA; n = 7; Fig. 1). Complete inhibition was achieved in all cells tested and the mean ED_{50} value estimated from the dose-effect curves was 1.8 ± 0.2 mg/kg (n = 7). After complete inhibition, the injection of naloxone (5 mg/kg, i.v.) failed to reverse venlafaxine inhibition (expressed relative to baseline values (0%), venlafaxine + vehicle = -100.0 ± 0.0 % (n = 7) *versus* venlafaxine + naloxone = -100.0 ± 0.0 % (n = 7), p > 0.05, unpaired Student's *t*-test; Fig 1). However, subsequent idazoxan (1 mg/kg, i.v.) administration produced a complete reversion of the firing activity (venlafaxine + naloxone = -100.0 ± 0.0 % (n = 7) *versus* venlafaxine + naloxone + idazoxan = +5.3 ± 13.8 % (n = 7), p < 0.0001, unpaired Student`s *t*-test; Fig 1 and Fig. 3).

The administration of naloxone alone (5 mg/kg, i.v.) did not modify the basal firing rate (+9.8 ± 6.3 % (n = 5), p > 0.05, paired Student's *t*-test; Fig. 3) as we have previously shown (see Methods section for rationale behind the selection of antagonist compounds) (Illes and Norenberg, 1990; Berrocoso et al., 2006). Subsequently, an injection of idazoxan at 1 mg/kg, enhanced the firing activity (+50.6 ± 6.8 % (n = 5), p < 0.01, paired Student's *t*-test) in a similar way to the administration of idazoxan alone (+42.8 ± 8.3 % (n = 5), p < 0.01, paired Student's *t*-test; Fig. 3) in agreement with previous studies (Freedman and Aghajanian, 1984; Szabo and Blier, 2001b).

Effect of the pre-treatment of 8-OH-DPAT, a 5-HT$_{1A}$ receptor agonist, on the acute inhibitory effect of venlafaxine on locus coeruleus neurons

Next, we examined the involvement of the 5-HT$_{1A}$ receptor in the acute effect of venlafaxine on LC neurons. The well-established agonist of the 5-HT$_{1A}$ receptor, 8-OH-DPAT, was intravenously administrated at doses of 1 and 40 µg/kg. After the drug application, there was a slight increase in the firing rate (8-OH-DPAT (1 µg/kg) = +14.0 ± 10.6 % (n = 5) or 8-OH-DPAT (40 µg/kg) = +15.2 ± 7.6 % (n = 10), $p > 0.05$, paired Student's *t*-test respectively). Venlafaxine dose-response curves were performed when the cell was stabilized. Pre-treatment with 8-OH-DPAT at both 1 and 40 µg/kg (n = 5 and n = 9 respectively), significantly enhanced the venlafaxine-induced inhibition of LC neurons (8-OH-DPAT factor: $F_{(2,90)} = 3.49$, $p > 0.05$; venlafaxine doses factor: $F_{(5,90)} = 156.9$, $p < 0.0001$; interaction factor: $F_{(10,90)} = 5.57$, $p < 0.0001$; two-way repeated measures ANOVA), so the dose-response curves showed a shift to the left and the ED$_{50}$ value was reduced to 0.8 ± 0.3 mg/kg and 1.0 ± 0.3 mg/kg respectively, compared to the control group ($F_{(2,18)} = 4,43$; $p < 0.05$, one-way ANOVA followed by Newman-Keuls test; Fig. 2A, Fig. 2B and Fig. 5). As previously found, this inhibitory effect was rapidly and completely reversed by idazoxan (1 mg/kg, i.v.) in 8-OH-DPAT pre-treated animals (40 µg/kg, i.v.) (8-OH-DPAT + venlafaxine = -100.0 ± 0.0 % (n = 9) *versus* 8-OH-DPAT + venlafaxine + idazoxan = +21.9 ± 8.0 % (n = 5), $p < 0.0001$, unpaired Student *t*-test; Fig. 2B and Fig. 3).

Effect of acute administration of venlafaxine on the firing activity of locus coeruleus neurons in long-term venlafaxine treated rats. Interaction with opioid, α_2-adrenergic and 5-HT$_{1A}$ receptors

Rats were treated with venlafaxine (40 mg/kg/day, via osmotic minipumps for 14 days) in order to study: first, the effect of the acute administration of venlafaxine in long-term venlafaxine treated rats and second, the role of 5-HT_{1A} receptors in the acute inhibitory effect of venlafaxine in these animals. After long-term venlafaxine treatment (40 mg/kg/day, 14 days), the mean basal firing rates of LC neurons in the treated group significantly decreased with respect to the non-treated group (basal firing rate of the non-treated group (2.2 ± 0.1 Hz) compared to the basal firing rate of the treated group (1.1 ± 0.1 Hz), $p < 0.0001$, paired Student's *t*-test, n = 58).

First, we studied the effect of the acute administration of venlafaxine in long-term venlafaxine treated rats. In this experimental group, the acute administration of venlafaxine depressed the spontaneous activity of LC neurons in a dose-dependent manner. This effect was not different to the effect displayed by acute administration of venlafaxine in non-treated animals (control group) (long-term treatment factor: $F_{(1,55)} = 0.35$ $p > 0.05$; venlafaxine doses factor: $F_{(5,55)} = 121.2$, $p < 0.0001$; interaction factor: $F_{(5,55)} = 2.38$, $p > 0.05$; two-way repeated measures ANOVA). Complete inhibition was achieved in all cells tested and the mean ED_{50} value (2.0 ± 0.5 mg/kg, n = 6) did not vary from the control group ($p > 0.05$, unpaired Student's *t*-test). In addition, this inhibitory effect was not reversed by naloxone administration (5 mg/kg, i.v.) (venlafaxine + vehicle = -100.0 ± 0.0 % (n =6) *versus* venlafaxine + naloxone = -99.2 ± 0.8 % (n = 6), $p < 0.05$, unpaired Student's *t*-test). Subsequent idazoxan administration (1 mg/kg, i.v.) produced a reversal in the firing activity that exceeded the initial basal value (venlafaxine + naloxone = -99.2 ± 0.8 % (n = 6) *versus* venlafaxine + naloxone + idazoxan = +57.7 ± 14.3 % (n = 6), $p < 0.001$, unpaired Student's *t*-test; Fig. 3).

Second, another group of rats were treated with venlafaxine (40 mg/kg/day) for 14 days in order to study the role of 5-HT_{1A} receptors in the acute inhibitory effect of venlafaxine in these rats. 8-OH-DAPT (40 µg/kg, i.v.) application slightly increased the firing rate (+7.8 ± 9.0 %, $p > 0.05$, paired Student's *t*-test, n = 5). Venlafaxine dose-response curves were performed when the cell was stabilized, similar to the above procedure (Fig. 4B). As is shown in Figure 4A, pre-treatment with 8-OH-DPAT significantly enhanced the venlafaxine effect compared with the effect of long-term venlafaxine treated animals (n = 4 and n = 6 respectively; 8-OH-DPAT factor: $F_{(1,40)} = 5.33$, $p < 0.05$; venlafaxine doses factor: $F_{(5,40)} = 58.07$, $p < 0.0001$; interaction factor: $F_{(5,40)} = 1.44$, $p > 0.05$; two-way repeated measures ANOVA). Consequently, the ED_{50} value (0.8 ± 0.1 mg/kg) was reduced in comparison with the long-term venlafaxine treated animals and was similar to that displayed by venlafaxine in 8-OH-DPAT (40 µg/kg, i.v.) pre-treated animals that were not submitted to long-term treatment ($F_{(2,18)} = 3.87$; $p < 0.05$, one-way ANOVA followed by Newman-Keuls test; Fig. 5).

Discussion

The results of the present study indicate that the SNRI venlafaxine depressed the spontaneous activity of LC neurons in acute and long-term venlafaxine treatment in vivo. These effects seem to be mediated mainly by α_2-adrenoceptors and not by opioid receptors. Furthermore, the results indicate that the activation of 5-HT_{1A} receptors significantly enhanced venlafaxine inhibition.

The decrease in the spontaneous activity of LC neurons induced by venlafaxine is dose-dependent and is in agreement with other previous studies (Beique et al., 1999; Millan et al., 2001). This suppressant effect was not reversed by the opioid antagonist naloxone. Nevertheless, it was reversed by an antagonist of α_2-adrenoceptors, suggesting that the enhancement of NA concentrations in the LC, via venlafaxine blockade of NA transporters, produced an over-activation of α_2-adrenoceptors and suppression of NA neurons, in a similar way to other NA reuptake blocker antidepressants, such as desipramine (Egan et al., 1983). These data imply that NA contributes to the antidepressant and analgesic effect of venlafaxine. Indeed, it has been shown that its antidepressant and antinociceptive effects were blocked by an inhibitor of NA synthesis and a noradrenergic neurotoxin respectively (Redrobe et al., 1998; Marchand et al., 2003). In addition, parachlorophenylalanine, an inhibitor of 5-HT synthesis, blocked both effects (Redrobe et al., 1998; Marchand et al., 2003), suggesting that the noradrenergic and serotonergic systems participate in the antidepressant and analgesic effect of venlafaxine.

After long-term venlafaxine administration, animals exhibited a firing activity of LC neurons that was around half that of non-treated animals. It has been suggested that after chronic antidepressant administration the firing rate is reversed due to a functional desensitization of somatodendritic α_2-autoreceptors and a consequent increase in the central availability of NA. This event has been related to the onset of the antidepressant effect (Linner et al., 1999). However, there are some studies that fail to confirm this hypothesis (Blier and de Montigny, 1985; Lacroix et al., 1991; Szabo and Blier, 2001a). Furthermore, venlafaxine plasma concentrations have not been tested, so it can be not excluded that venlafaxine or its metabolites were still in the brain, in spite of the fact that the osmotic minipumps were removed 24 h before the test.

Furthermore, the current study shows that after long-term venlafaxine treatment its inhibitory effect on LC firing rate was similar in 14-day treated and non-treated animals. Thus, our data disagree with the studies that suggest that after long-term antidepressant treatment, acute antidepressant administration inhibits the firing rate of LC neurons to a lesser extent, i.e. tolerance occurred to the acute administration (Svensson and Usdin, 1978; Valentino et al., 1990). This fact has been related to the desensitization of α_2-adrenoceptors. Therefore, our results indirectly suggest that chronic treatment with venlafaxine did not modify venlafaxine action in the LC neurons and we argue against the desensitization of LC α_2-adrenoceptors being a critical point for the onset of action of the clinical antidepressant effect. Looking at the analgesic effect of venlafaxine these results are very interesting if we consider that its onset of action as an analgesic in painful conditions is shorter than its onset time as an antidepressant (Saarto and Wiffen, 2005). So, in summary, it seems likely that desensitization of α_2-adrenoceptors in the

LC is not necessary for a venlafaxine induced antidepressant effect or analgesia either because after 14 days of antidepressant treatment, both the antidepressant and the analgesic effect is present both clinically and pre-clinically. In addition, our study shows that brain noradrenergic activity can be markedly augmented by systemic administration of an antagonist of α_2-adrenoceptors, even after chronic treatment. This data support the idea that α_2-adrenoceptors are functioning normally and suggest that the blockade of α_2-adrenoceptors at LC level could be a pharmacological strategy for increasing NA release in terminal areas (Dawson et al., 1999; Linner et al., 1999) resulting in a greater antidepressant or analgesic effect.

Antidepressants that inhibit the reuptake of NA and 5-HT are common drugs for the treatment of chronic pain. Specifically, tricyclic antidepressants are the first-line drugs for the treatment of neuropathic pain, although their efficacy is limited due to their side effects. Other non-tryciclic antidepressants (SNRIs: venlafaxine, duloxetine and milnacipran) that inhibit NA and 5-HT uptake but do not act upon the α-adrenergic, muscarinic and histaminergic receptors (responsible for the main side effects of tricyclics) have recently appeared as antidepressants which act upon mood and pain but with fewer side effects. Several studies, mainly investigating tricyclics' analgesic effect, have shown that apart from the monoaminergic mechanism, tricyclic analgesia implies an activation of opioid system (for review see Mico et al., 2006) even though tricyclics do not bind to opioid receptors. SNRIs do not bind to opioid receptors either but it is not yet known if the action of SNRIs involves an interaction with the opioid system, similar to tricyclics. Regarding the antinociceptive effect of venlafaxine and its involvement with opioid receptors, only a few pre-clinical studies have been performed to study this

subject, with apparently opposing results. Schreiber (Schreiber et al., 1999) showed that venlafaxine-induced antinociception was mediated through opioid and adrenergic systems in healthy mice submitted to an acute model of pain. However, Marchand and collaborators (Marchand et al., 2003) showed that naloxone failed to block the antihyperalgesic effect of venlafaxine in mononeuropathic and diabetic rats. Our study showed that naloxone did not modify venlafaxine inhibition after either acute or chronic venlafaxine administration. There is a previous study suggesting that naloxone has no effect on venlafaxine inhibition in LC neurons in acute treatment (Haskins et al., 1985). Therefore, considering that LC neurons have an abundant population of μ-opioid receptors (Williams and North, 1984), our study suggests that the inhibitory effect of venlafaxine on LC neurons is not related to the opioid system in healthy rats under acute or long-term venlafaxine treatment. There are no data available for other SNRIs (duloxetine and milnacipran), but our results suggest that the opioid system does not contribute to the effect of SNRIs, contrary to the existing knowledge for tricyclic antidepressants.

This study also shows that the activation of 5-HT_{1A} receptors significantly enhances the venlafaxine-induced inhibition of LC neurons. There are several lines of evidence supporting the notion that 5-HT_{1A} receptors modulate LC neuron activity (Ruiz-Ortega and Ugedo, 1997; Szabo and Blier, 2001c). In fact, the pre-administration of 8-OH-DPAT enhanced the inhibitory effect of the agonist of α_2-adrenoceptors, clonidine, in LC neurons (Ruiz-Ortega and Ugedo, 1997). The exact location of the 5-HT_{1A} receptors that modulate LC activity it is not known, but it has been suggested that they are not the

5-HT_{1A} receptors controlling dorsal raphe neuron firing activity, although it appears that an intact 5-HT system is necessary for the modulator effect of 5-HT_{1A} on LC neurons (Szabo and Blier, 2001c). In addition, it has been suggested that 5-HT_{1A} receptors could be located on glutamatergic projections in the LC (Millan et al., 2000; Szabo and Blier, 2001c).

In another series of studies, we have shown that in LC neurons in vivo the inhibitory effect of the analgesic tramadol, a weak μ-opioid agonist and dual 5-HT/NE reuptake inhibitor, is augmented by the pre-administration of 8-OH-DPAT (Berrocoso et al., 2006). These data suggest that the activation of 5-HT_{1A} receptors modulates the response of the LC to venlafaxine and tramadol. This fact could be important when looking into nociception. The LC is highly implicated in the endogenous descending inhibitory system. Several data have demonstrated that electrical or chemical stimulation of LC neurons produces antinociception (Jones and Gebhart, 1988) and significantly increases the level of NE metabolites in the spinal cord (Crawley et al., 1979). Thus, considering that 8-OH-DPAT attenuates venlafaxine or tramadol-induced analgesia in different pain models (Rojas-Corrales et al., 2000) (unpublished data), it could be suggested that the association of 8-OH-DPAT with venlafaxine or tramadol may lead to a cooperative role in decreasing NA neurotransmission. That is, the combination of a dual 5-HT/NA reuptake inhibitor and the activation of 5-HT_{1A} receptors enhances the suppression of LC activity, obstructing the descending noradrenergic pathway and consequently blocking the analgesic effect of venlafaxine. Furthermore, our study shows that the enhancing effect of 8-OH-DPAT on venlafaxine inhibition is maintained after long-term venlafaxine treatment in LC neurons. Long-term studies of microdialysis with venlafaxine showed no desensitization of $5HT_{1A}$

postsynaptic receptors in the frontal cortex or hippocampus, although desensitization has been reported in the hypothalamus (Gur et al., 1999).

In summary, the present results indicate that venlafaxine inhibits LC firing through a mechanism independent of opioid receptors and dependent on α_2-adrenoceptors. In addition, the activation of 5-HT_{1A} receptors significantly enhances the inhibitory effect of venlafaxine in LC neurons in non-treated and long-term venlafaxine treated animals. This data could contribute to understanding the effect of venlafaxine in LC neurons and elucidating its mechanism of action in depression and analgesia.

References

Aghajanian GK, Cedarbaum JM and Wang RY (1977) Evidence for norepinephrine-mediated collateral inhibition of locus coeruleus neurons. *Brain Res* 136:570-577.

Beique J, de Montigny C, Blier P and Debonnel G (2000a) Effects of sustained administration of the serotonin and norepinephrine reuptake inhibitor venlafaxine: I. in vivo electrophysiological studies in the rat. *Neuropharmacology* 39:1800-1812.

Beique J, de Montigny C, Blier P and Debonnel G (2000b) Effects of sustained administration of the serotonin and norepinephrine reuptake inhibitor venlafaxine: II. In vitro studies in the rat. *Neuropharmacology* 39:1813-1822.

Beique JC, de Montigny C, Blier P and Debonnel G (1999) Venlafaxine: discrepancy between in vivo 5-HT and NE reuptake blockade and affinity for reuptake sites. *Synapse* 32:198-211.

Berrocoso E, Mico JA and Ugedo L (2006) In vivo effect of tramadol on locus coeruleus neurons is mediated by alpha2-adrenoceptors and modulated by serotonin. *Neuropharmacology* 51:146-153.

Berrocoso E, Rojas-Corrales MO and Mico JA (2004) Non-selective opioid receptor antagonism of the antidepressant-like effect of venlafaxine in the forced swimming test in mice. *Neurosci Lett* 363:25-28.

Blier P and de Montigny C (1985) Serotoninergic but not noradrenergic neurons in rat central nervous system adapt to long-term treatment with monoamine oxidase inhibitors. *Neuroscience* 16:949-955.

Crawley JN, Roth RH and Maas JW (1979) Locus coeruleus stimulation increases noradrenergic metabolite levels in rat spinal cord. *Brain Res* 166:180-184.

Dawson LA, Nguyen HQ and Geiger A (1999) Effects of venlafaxine on extracellular concentrations of 5-HT and noradrenaline in the rat frontal cortex: augmentation via 5-HT1A receptor antagonism. *Neuropharmacology* 38:1153-1163.

Egan TM, Henderson G, North RA and Williams JT (1983) Noradrenaline-mediated synaptic inhibition in rat locus coeruleus neurones. *J Physiol* 345:477-488.

Freedman JE and Aghajanian GK (1984) Idazoxan (RX 781094) selectively antagonizes alpha 2-adrenoceptors on rat central neurons. *Eur J Pharmacol* 105:265-272.

Gur E, Dremencov E, Lerer B and Newman ME (1999) Venlafaxine: acute and chronic effects on 5-hydroxytryptamine levels in rat brain in vivo. *Eur J Pharmacol* 372:17-24.

Hamon M, Gozlan H, Bourgoin S, Benoliel JJ, Mauborgne A, Taquet H, Cesselin F and Mico JA (1987) Opioid receptors and neuropeptides in the CNS in rats treated chronically with amoxapine or amitriptyline. *Neuropharmacology* 26:531-539.

Haskins JT, Moyer JA, Muth EA and Sigg EB (1985) DMI, Wy-45,030, Wy-45,881 and ciramadol inhibit locus coeruleus neuronal activity. *Eur J Pharmacol* 115:139-146.

Illes P and Norenberg W (1990) Blockade of alpha 2-adrenoceptors increases opioid mu-receptor-mediated inhibition of the firing rate of rat locus coeruleus neurones. *Naunyn Schmiedebergs Arch Pharmacol* 342:490-496.

Isenberg KE and Cicero TJ (1984) Possible involvement of opiate receptors in the pharmacological profiles of antidepressant compounds. *Eur J Pharmacol* 103:57-63.

Jones SL and Gebhart GF (1988) Inhibition of spinal nociceptive transmission from the midbrain, pons and medulla in the rat: activation of descending inhibition by morphine, glutamate and electrical stimulation. *Brain Res* 460:281-296.

Lacroix D, Blier P, Curet O and de Montigny C (1991) Effects of long-term desipramine administration on noradrenergic neurotransmission: electrophysiological studies in the rat brain. *J Pharmacol Exp Ther* 257:1081-1090.

Linner L, Arborelius L, Nomikos GG, Bertilsson L and Svensson TH (1999) Locus coeruleus neuronal activity and noradrenaline availability in the frontal cortex of rats chronically treated with imipramine: effect of alpha 2-adrenoceptor blockade. *Biol Psychiatry* 46:766-774.

Marchand F, Alloui A, Chapuy E, Jourdan D, Pelissier T, Ardid D, Hernandez A and Eschalier A (2003) Evidence for a monoamine mediated, opioid-independent, antihyperalgesic effect of venlafaxine, a non-tricyclic antidepressant, in a neurogenic pain model in rats. *Pain* 103:229-235.

Marchand F, Pelissier T, Eschalier A, Ardid D, Alloui A, Soto-Moyano R, Mondaca M, Laurido C, Constandil L and Hernandez A (2004) Blockade of supraspinal 5-HT1A receptors potentiates the inhibitory effect of venlafaxine on wind-up activity in mononeuropathic rats. *Brain Res* 1008:288-292.

Mico JA, Ardid D, Berrocoso E and Eschalier A (2006) Antidepressants and pain. *Trends Pharmacol Sci* 27:348-354.

Millan MJ, Gobert A, Lejeune F, Newman-Tancredi A, Rivet JM, Auclair A and Peglion JL (2001) S33005, a novel ligand at both serotonin and norepinephrine transporters: I. Receptor binding, electrophysiological, and neurochemical

profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. *J Pharmacol Exp Ther* 298:565-580.

Millan MJ, Lejeune F and Gobert A (2000) Reciprocal autoreceptor and heteroreceptor control of serotonergic, dopaminergic and noradrenergic transmission in the frontal cortex: relevance to the actions of antidepressant agents. *J Psychopharmacol* 14:114-138.

Muth EA, Haskins JT, Moyer JA, Husbands GE, Nielsen ST and Sigg EB (1986) Antidepressant biochemical profile of the novel bicyclic compound Wy-45,030, an ethyl cyclohexanol derivative. *Biochem Pharmacol* 35:4493-4497.

Parker RB and Waud DR (1971) Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. I. Agonists. *J Pharmacol Exp Ther* 177:1-12.

Redrobe JP, Bourin M, Colombel MC and Baker GB (1998) Dose-dependent noradrenergic and serotonergic properties of venlafaxine in animal models indicative of antidepressant activity. *Psychopharmacology (Berl)* 138:1-8.

Rojas-Corrales MO, Ortega-Alvaro A, Gibert-Rahola J, Roca-Vinardell A and Mico JA (2000) Pindolol, a beta-adrenoceptor blocker/5-hydroxytryptamine(1A/1B) antagonist, enhances the analgesic effect of tramadol. *Pain* 88:119-124.

Ruiz-Durantez E, Torrecilla M, Pineda J and Ugedo L (2003) Attenuation of acute and chronic effects of morphine by the imidazoline receptor ligand 2-(2-benzofuranyl)-2-imidazoline in rat locus coeruleus neurons. *Br J Pharmacol* 138:494-500.

Ruiz-Ortega JA and Ugedo L (1997) Activation of 5-HT1A receptors potentiates the clonidine inhibitory effect in the locus coeruleus. *Eur J Pharmacol* 333:159-162.

Saarto T and Wiffen PJ (2005) Antidepressants for neuropathic pain. *Cochrane Database Syst Rev*:CD005454.

Schreiber S, Backer MM and Pick CG (1999) The antinociceptive effect of venlafaxine in mice is mediated through opioid and adrenergic mechanisms. *Neurosci Lett* 273:85-88.

Segal M (1979) Serotonergic innervation of the locus coeruleus from the dorsal raphe and its action on responses to noxious stimuli. *J Physiol* 286:401-415.

Svensson TH and Usdin T (1978) Feedback inhibition of brain noradrenaline neurons by tricyclic antidepressants: alpha-receptor mediation. *Science* 202:1089-1091.

Szabo ST and Blier P (2001a) Effect of the selective noradrenergic reuptake inhibitor reboxetine on the firing activity of noradrenaline and serotonin neurons. *Eur J Neurosci* 13:2077-2087.

Szabo ST and Blier P (2001b) Functional and pharmacological characterization of the modulatory role of serotonin on the firing activity of locus coeruleus norepinephrine neurons. *Brain Res* 922:9-20.

Szabo ST and Blier P (2001c) Serotonin (1A) receptor ligands act on norepinephrine neuron firing through excitatory amino acid and GABA(A) receptors: a microiontophoretic study in the rat locus coeruleus. *Synapse* 42:203-212.

Ugedo L, Pineda J, Ruiz-Ortega JA and Martin-Ruiz R (1998) Stimulation of locus coeruleus neurons by non-I1/I2-type imidazoline receptors: an in vivo and in vitro electrophysiological study. *Br J Pharmacol* 125:1685-1694.

Valentino RJ, Curtis AL, Parris DG and Wehby RG (1990) Antidepressant actions on brain noradrenergic neurons. *J Pharmacol Exp Ther* 253:833-840.

Valverde O, Mico JA, Maldonado R, Mellado M and Gibert-Rahola J (1994) Participation of opioid and monoaminergic mechanisms on the antinociceptive

effect induced by tricyclic antidepressants in two behavioural pain tests in mice. *Prog Neuropsychopharmacol Biol Psychiatry* 18:1073-1092.

Williams JT and North RA (1984) Opiate-receptor interactions on single locus coeruleus neurones. *Mol Pharmacol* 26:489-497.

Zimmermann M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 16:109-110.

FOOTNOTES

Sources of financial support: This study has been supported by "Fondo de Investigación Sanitaria" (PI031430) and "Plan Andaluz de Investigación" (CTS - 510).

Address correspondence to: Prof. Juan A. Micó, Pharmacology and Neuroscience Research Group, Department of Neuroscience (Pharmacology and Psychiatry), School of Medicine, University of Cádiz, Plaza Fragela 9, 11003 Cádiz, Spain. Phone: +34 956015247; Fax: +34 956015225; e-mail: juanantonio.mico@uca.es

Legends for Figures

Fig. 1

Effect of intravenous administration of venlafaxine on spontaneous activity of locus coeruleus neurons and its involvement with opioid and α_2-adrenergic receptors. Symbols represent mean ± S.E.M. (n = 7). Note that the dose-dependent inhibitory effect of venlafaxine was not reversed by the opioid receptor antagonist naloxone (5 mg/kg, i.v.), but it was reversed by the subsequent administration of the antagonist of α_2-adrenoceptors idazoxan (1 mg/kg, i.v.). The horizontal dashed line represents baseline unit activity.

Fig. 2

(A) Dose-effect curves illustrating the inhibitory effect of venlafaxine on locus coeruleus neuron firing rate after the administration of 8-OH-DPAT. Symbols represent mean ± S.E.M. of the percentage of reduction from basal firing rate in the control (○, n = 7) and experimental groups, 3 - 6 min after the intravenous administration of 8-OH-DPAT at 1 μg/kg (□, n = 5) and 40 μg/kg (■, n = 9). The horizontal axis represents the cumulative doses of venlafaxine administered i.v. at 2 - 3 min intervals. Note that the pre-treatment with 8-OH-DPAT, an agonist of 5-HT_{1A} receptors, shifted the dose-response curve to the left and the ED_{50} value decreased by 56% and 44% respectively with respect to the control group. (B) Effect of cumulative doses of venlafaxine after the administration of 8-OH-DPAT (40 μg/kg, i.v., DPAT) on spontaneous activity. Symbols represent mean ± S.E.M. (n = 9). Note that the subsequent injection of idazoxan (1 mg/kg, i.v.) completely reversed the venlafaxine effect. The horizontal dashed line represents baseline unit activity.

Fig. 3

Percentage of change from basal firing rate of the selective antagonist of α_2-adrenoceptors, idazoxan, on locus coeruleus neurons. Mean ± S.E.M. of the influence of idazoxan (1 mg/kg, i.v.) (IDX) subsequent to the i.v. administration of vehicle (NaCl 0.9%, Veh); naloxone (5 mg/kg) (NLX); venlafaxine (19.2 mg/kg) + naloxone (5 mg/kg) (represented as VLX+NLX); 8-OH-DPAT (40 μg/kg) + venlafaxine (9.6 mg/kg) (represented as DPAT+VLX); venlafaxine (19.2 mg/kg) + naloxone (5 mg/kg) in long-term venlafaxine treatment group (40 mg/kg/day X 14 days) (represented as VLX+NLX [VLX,14 days]). Note that idazoxan and naloxone + idazoxan enhanced the firing activity in a similar way. In addition, idazoxan completely reversed venlafaxine inhibition in non-treated, 8-OH-DPAT pre-treated and long-term venlafaxine treated groups. *$p<0.05$ (one-way ANOVA followed by Newman-Keuls test). n.s. = not significant.

Fig. 4

(A) Dose-effect curves illustrating the inhibitory effect of venlafaxine on locus coeruleus neuron firing rate after the administration of 8-OH-DPAT in long-term treated animals (venlafaxine, 40 mg/kg/day, 14 days via osmotic minipumps). Symbols represent mean ± S.E.M. of the percentage of reduction from basal firing rate in the control (◊, n = 6) and experimental groups, 3 - 6 min after the intravenous administration of 8-OH-DPAT at 40 μg/kg (♦, n = 4). The horizontal axis represents the cumulative doses of venlafaxine administered i.v. at 2 - 3 min intervals. Note that the pre-treatment with 8-OH-DPAT, an agonist of 5-HT_{1A} receptors, shifted the dose-response curve to the left and the ED_{50} value decreased by 60% with respect to the

control group. (B) Effect of cumulative doses of venlafaxine on spontaneous activity after the administration of 8-OH-DPAT (40 μg/kg, i.v., DPAT) in long-term treated animals. Symbols represent mean ± S.E.M. (n = 4). The horizontal dashed line represents baseline unit activity.

Fig. 5

Bar histograms showing the mean ED_{50} value, estimated from the inhibition induced by venlafaxine after the administration of 8-OH-DPAT in control and long-term treated animals (VLX [14 days] = venlafaxine, 40 mg/kg/day, 14 days via osmotic minipumps). Columns represent the mean ED_{50} value (± S.E.M.) that was calculated for each dose-response curve using the Parker and Waud equation. Values between brackets represent the doses of 8-OH-DPAT (DPAT) in μg/kg. *$p<0.05$ (one-way ANOVA followed by Newman-Keuls test) compared to respective venlafaxine group (control group and long-term treated group); n.s. = not significant.

Figure 1

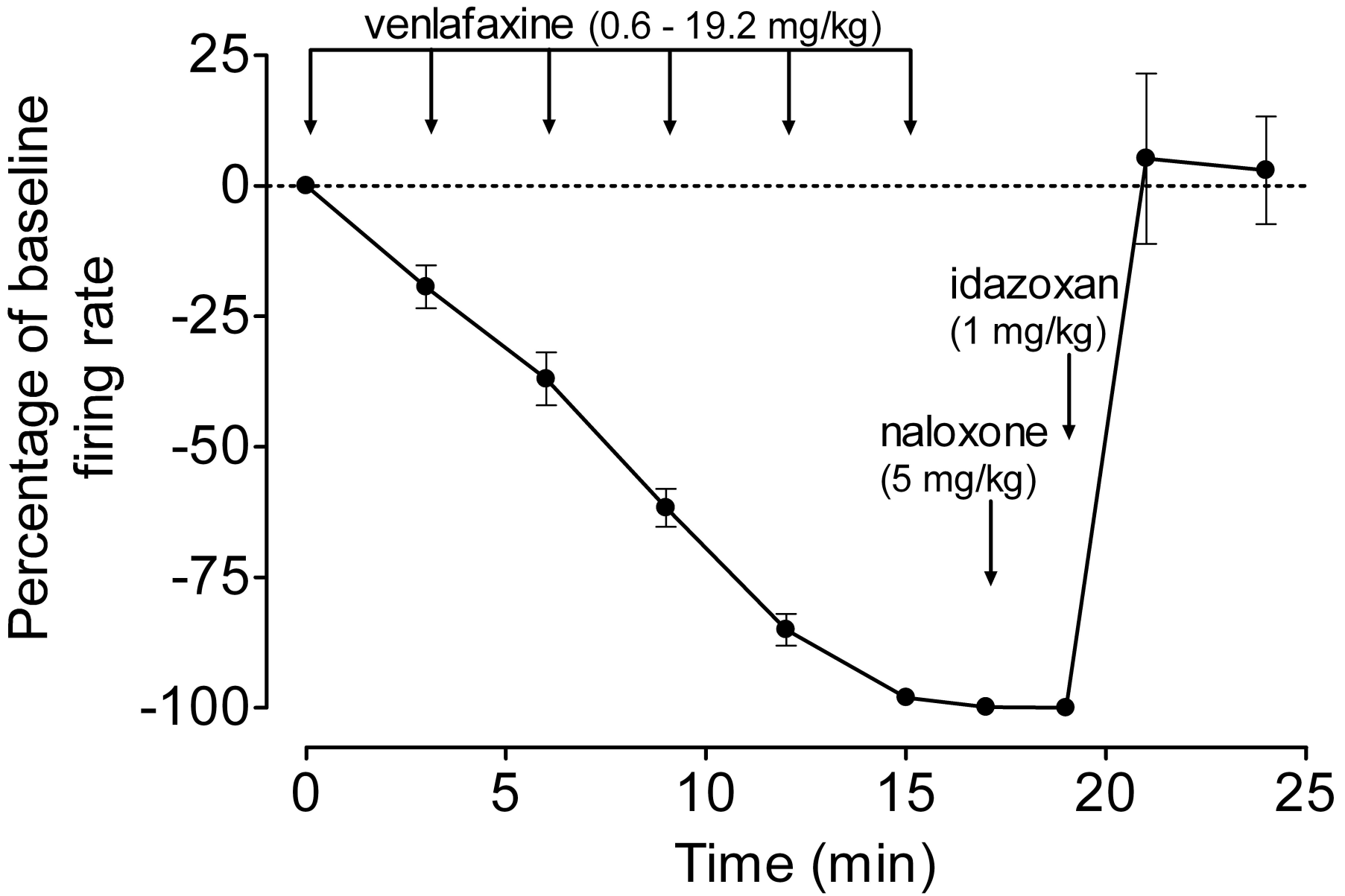

venlafaxine (0.6 - 19.2 mg/kg)
idazoxan
(1 mg/kg)
naloxone
(5 mg/kg)
Percentage of baseline
firing rate
25
0
-25
-50
-75
-100
0
5
10
15
20
25
Time (min)

Figure 2A

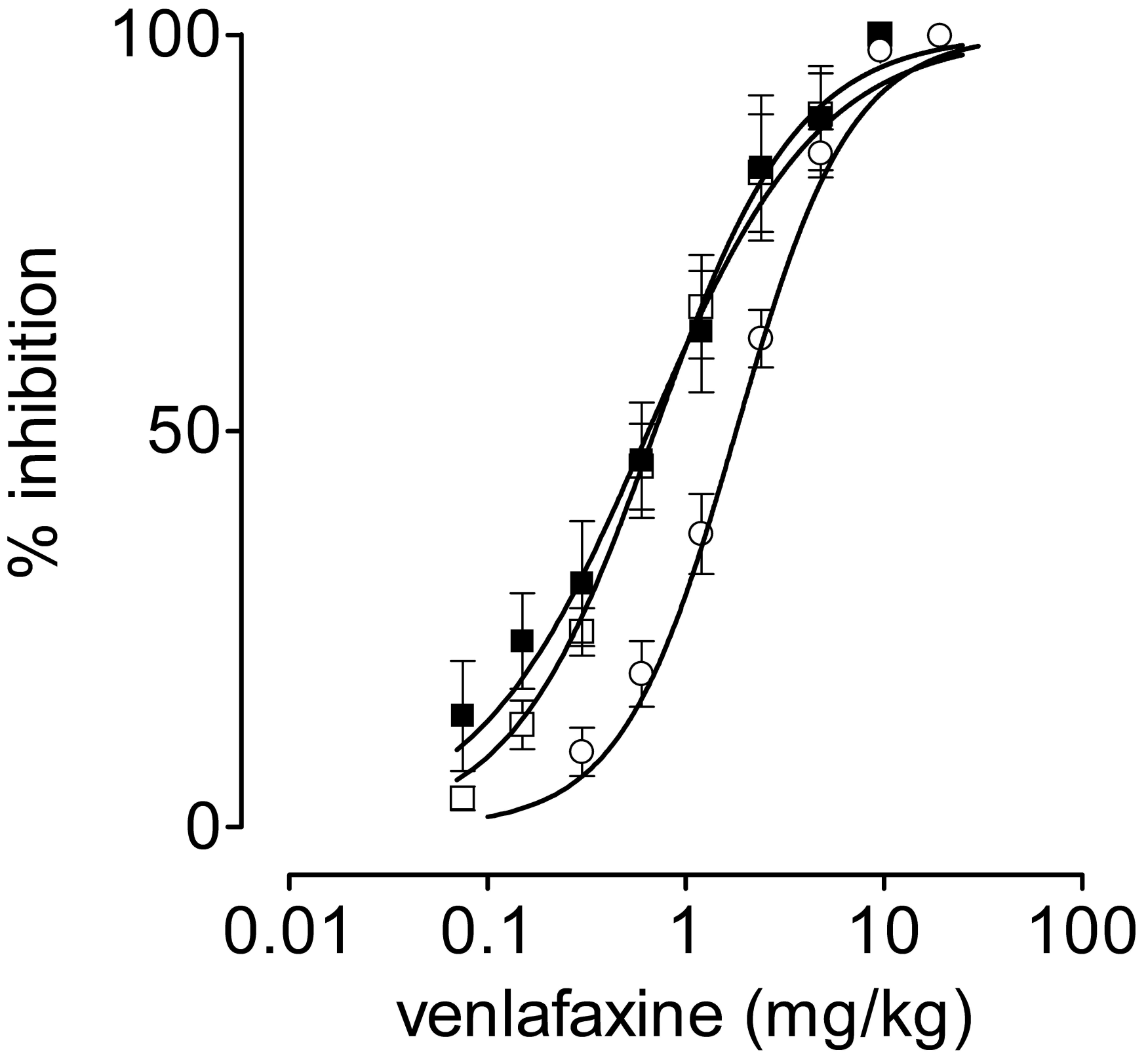


Figure 2B

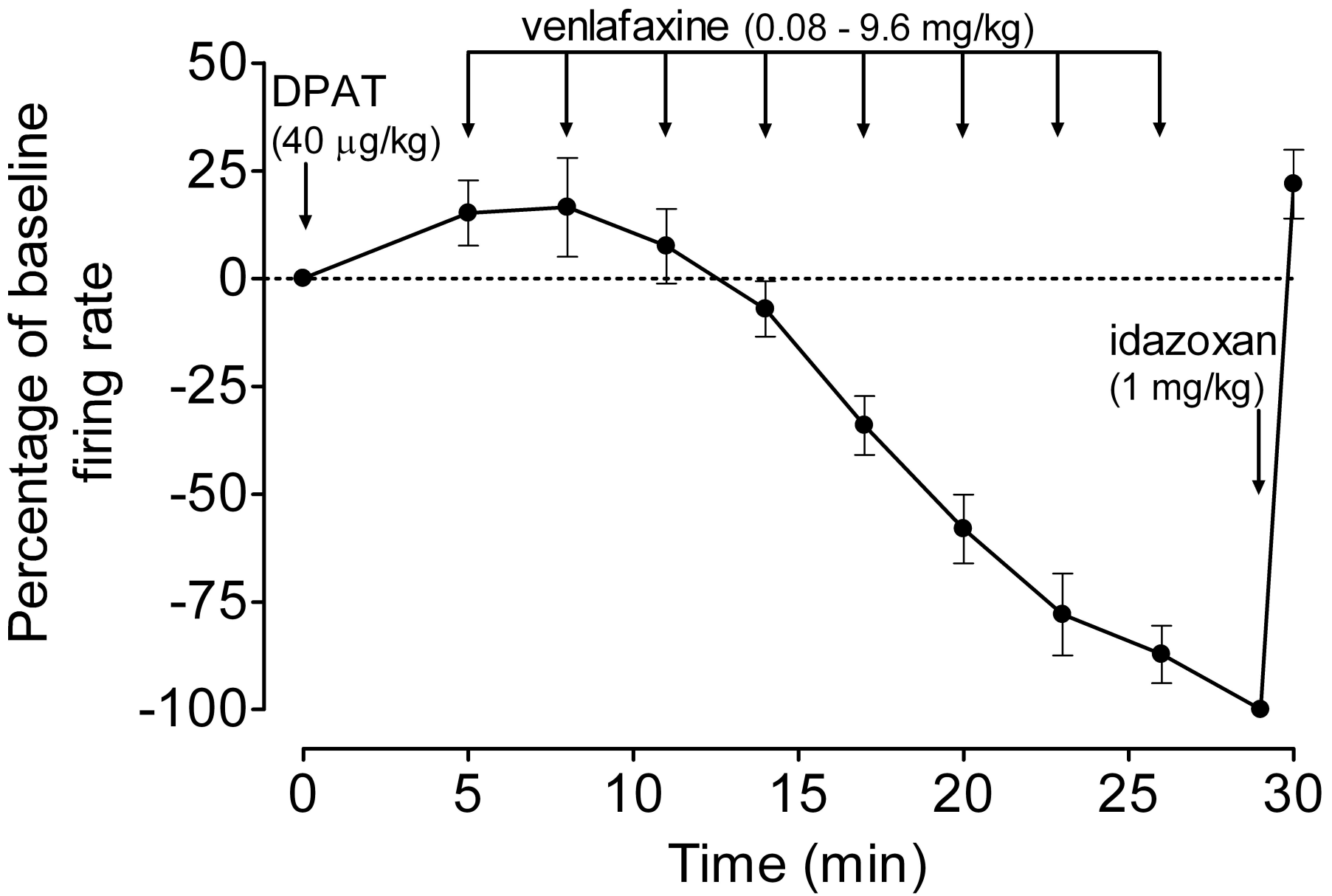

venlafaxine (0.08 - 9.6 mg/kg)
DPAT
(40 μg/kg)
idazoxan
(1 mg/kg)
Percentage of baseline
firing rate
50
25
0
-25
-50
-75
-100
0
5
10
15
20
25
30
Time (min)

Figure 3

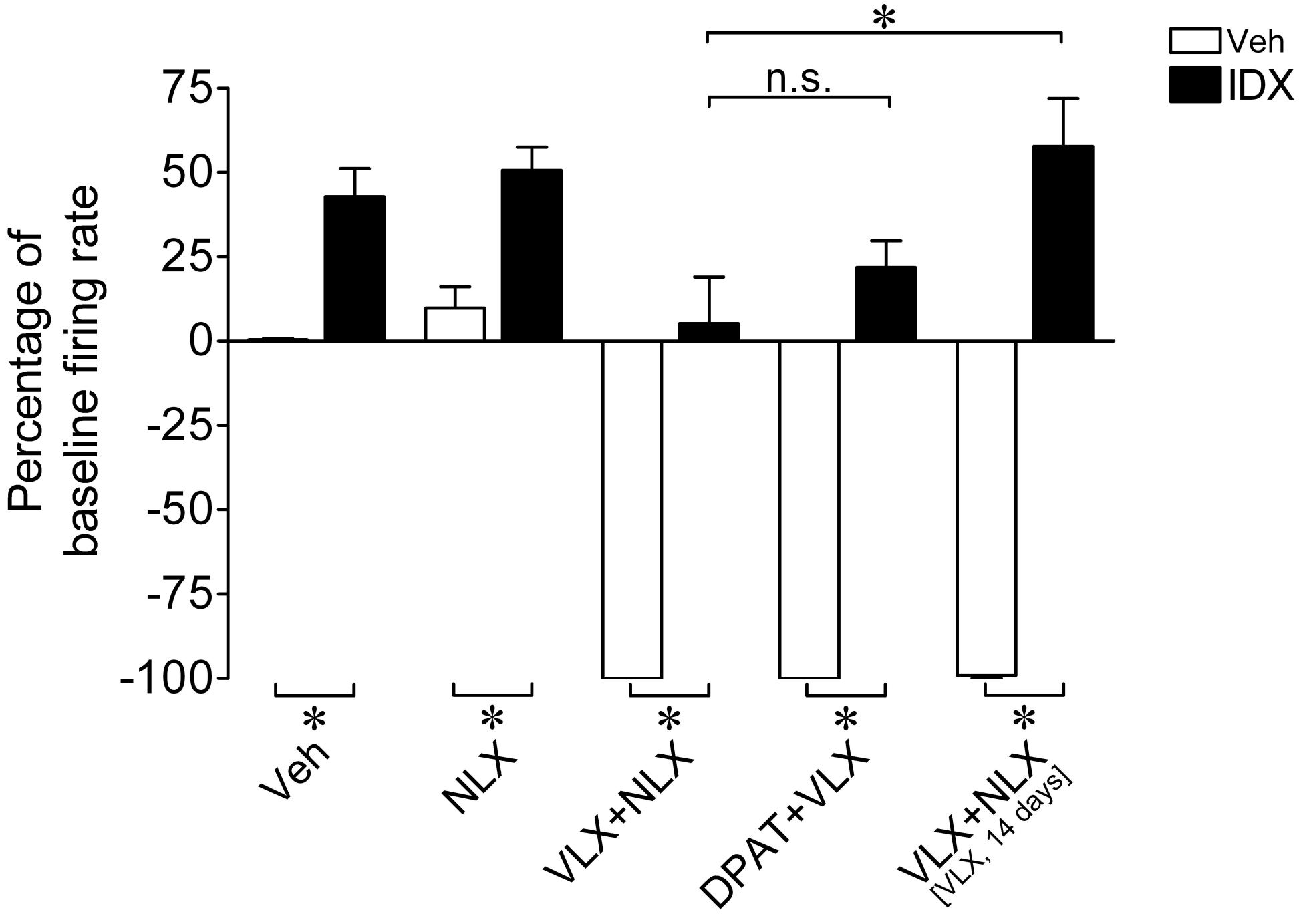

*
n.s.
Veh
IDX
75
50
25
0
-25
-50
-75
-100
Percentage of baseline firing rate
Veh *
NLX *
VLX+NLX *
DPAT+VLX *
VLX+NLX [VLX, 14 days] *

Figure 4A

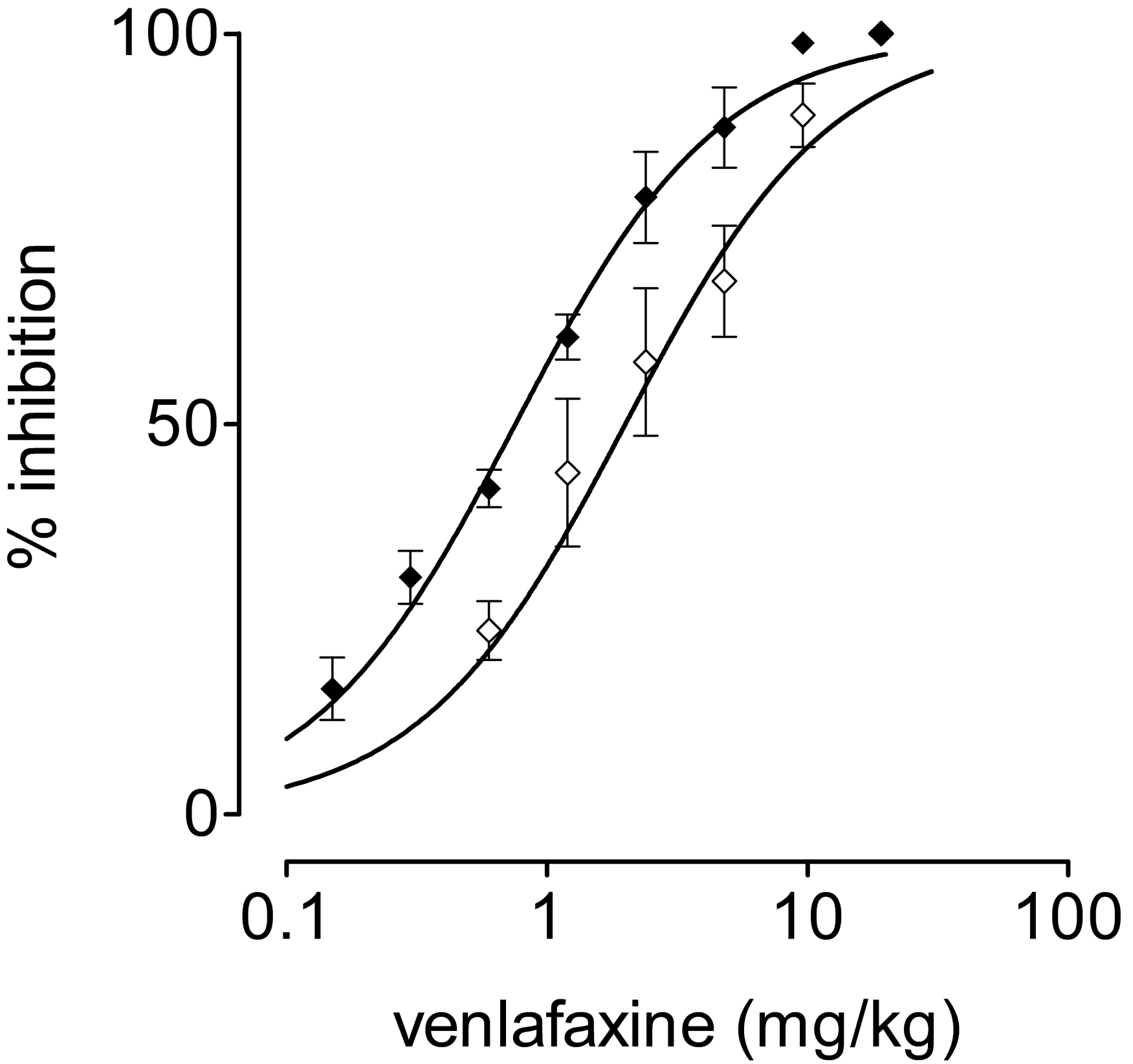


Figure 4B

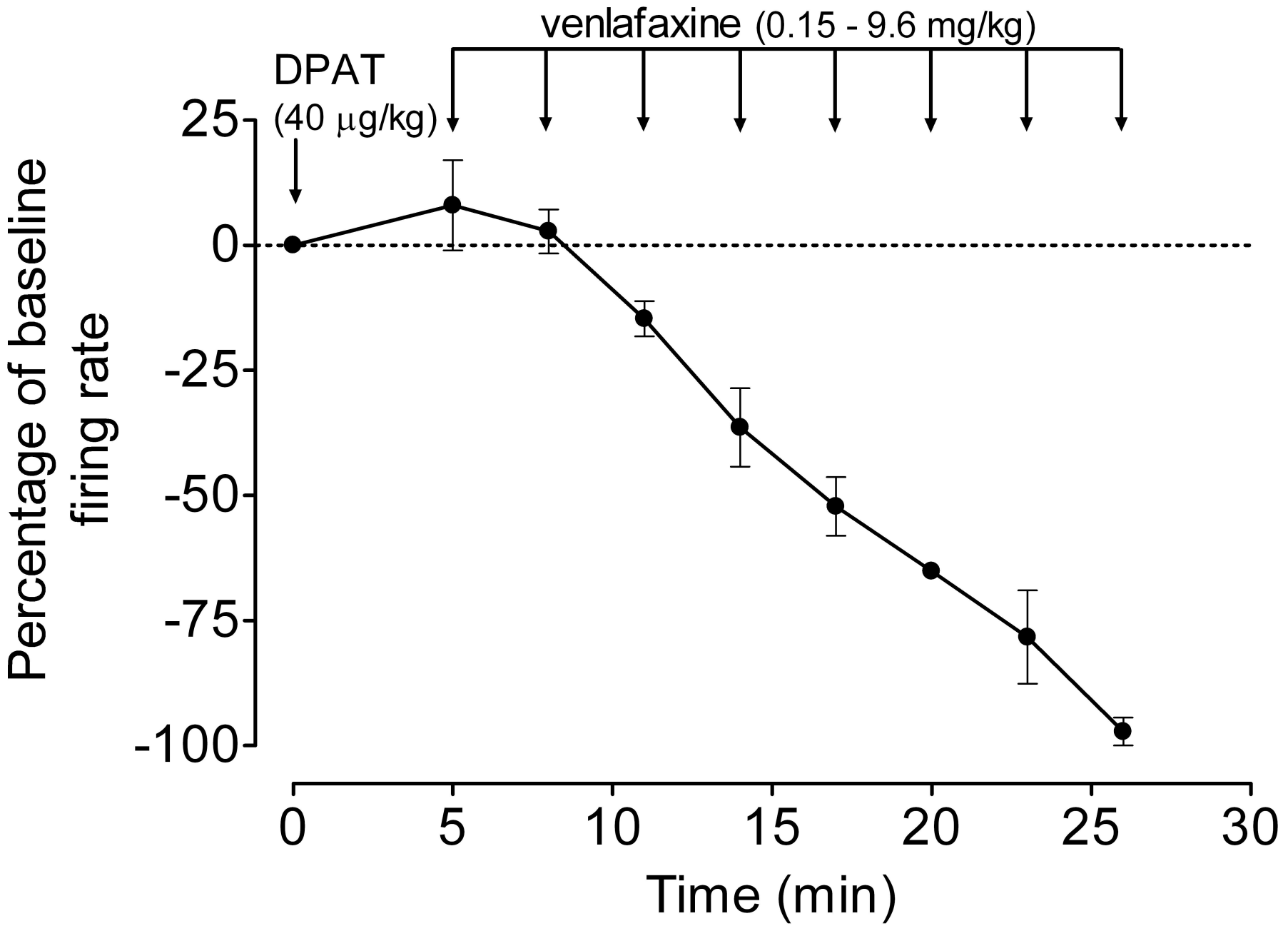
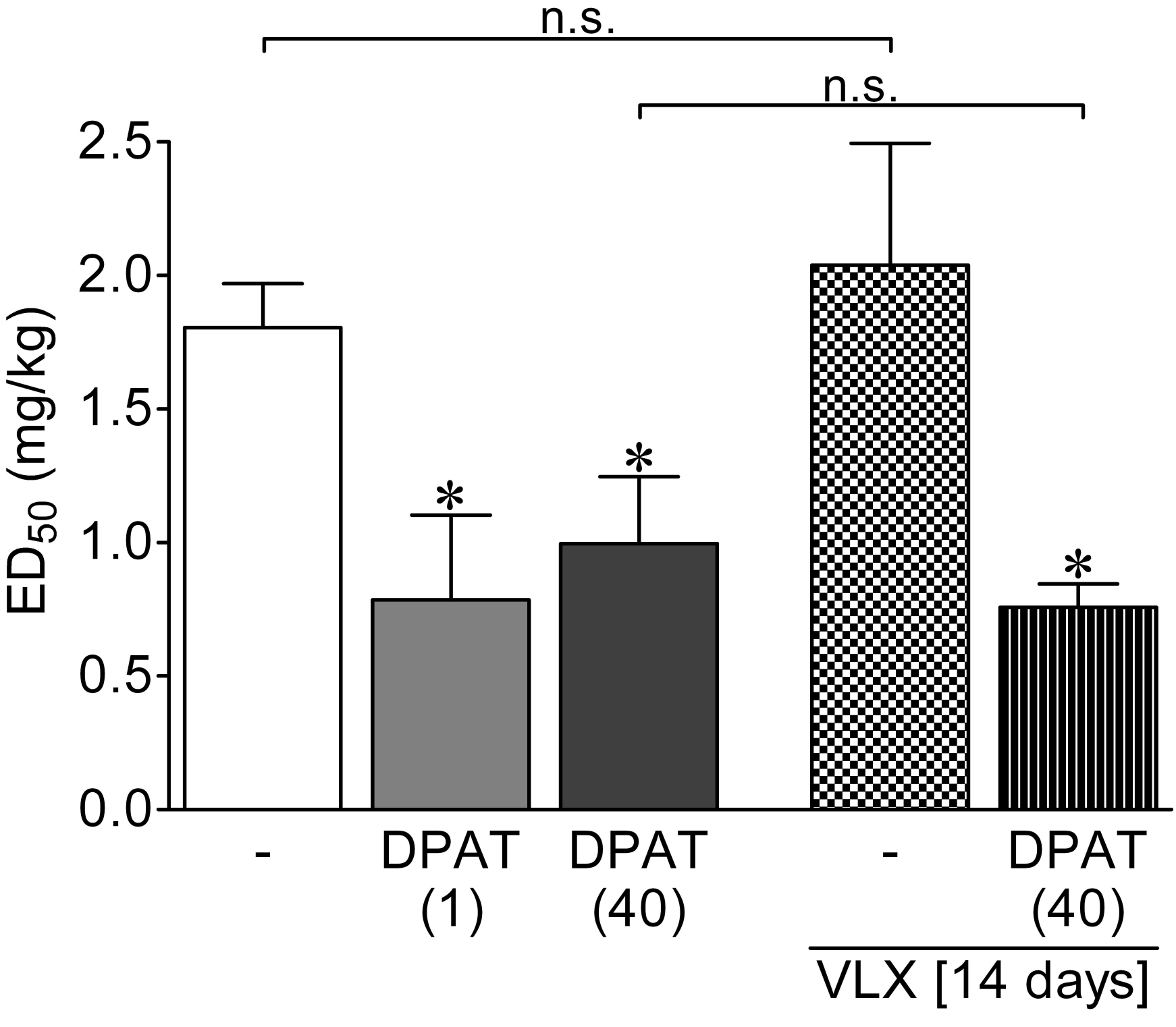

venlafaxine (0.15 - 9.6 mg/kg)
DPAT
(40 μg/kg)
25
0
-25
-50
-75
-100
Percentage of baseline
firing rate
0
5
10
15
20
25
30
Time (min)

Figure 5


n.s.
n.s.
2.5
2.0
1.5
1.0
0.5
0.0
ED50 (mg/kg)
*
*
*
-
DPAT (1)
DPAT (40)
-
DPAT (40)
VLX [14 days]