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Efficacy of duloxetine, a potent and balanced serotonin-norepinephrine reuptake inhibitor in persistent pain models in rats.

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List of Nonstandard Abbreviations:

5HT=5– hydroxytryptamine or serotonin

α-MMT =α-methyl-m-tyrosine

p-CA = parachloramphetamine

NE= norepinephrine

NRI=norepinephrine reuptake inhibitor

SNRI= serotonin norepinephrine reuptake inhibitor

SSRI= selective serotonin reuptake inhibitor

Section Guide

Behavioral Pharmacology or Neuropharmacology.

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Abstract

Serotonin (5-HT) and norepinephrine (NE) are implicated in modulating descending inhibitory pain pathways in the central nervous system. Duloxetine is a selective and potent dual 5-HT and NE reuptake inhibitor (SNRI). The ability of duloxetine to antagonize 5-HT depletion in parachloramphetamine (p-CA)-treated rats was comparable to paroxetine, a selective serotonin reuptake inhibitor (SSRI), while its ability to antagonize NE depletion in α-methyl-m-tyrosine (α-MMT)-treated rats was similar to norepinephrine reuptake inhibitors (NRI), thionisoxetine or desipramine. In this paradigm, duloxetine was also more potent than other SNRIs, including venlafaxine or milnacipran and amitriptyline. Low doses of the SSRI, paroxetine or the NRI, thionisoxetine, alone did not have an effect on late phase paw-licking pain behavior in the formalin model of persistent pain; however, when combined, significantly attenuated this pain behavior. Duloxetine (3-15 mg/kg, intraperitoneal) significantly attenuated late phase paw-licking behavior in a dose-dependent manner in the formalin model and was more potent than venlafaxine, milnacipran and amitriptyline. These effects of duloxetine were evident at doses that did not cause neurologic deficits in the rotorod test. Duloxetine (5-30 mg/kg, oral) was also more potent and efficacious than venlafaxine and milnacipran in reversing mechanical allodynia behavior in the L5/L6 spinal nerve-ligation model of neuropathic pain. Duloxetine (3-30 mg/kg, oral) was minimally efficacious in the tail-flick model of acute nociceptive pain. These data

suggest that inhibition of both 5-HT and NE uptake may account for attenuation of persistent pain mechanisms. Thus, duloxetine may have utility in treatment of human persistent and neuropathic pain states.

Introduction

Persistent pain results from changes in sensitivity within both ascending and descending pain pathways in the brain and the spinal cord (Wall, 1999; Hunt and Mantyh, 2001). While a number of neurotransmitters likely modulate the ascending and descending pain pathways, serotonin (5-HT) and norepinephrine (NE) have been implicated as mediators of endogenous analgesic mechanisms in the descending pain pathways (Yaksh, 1985; Jones, 1991; Fields et al., 1991; Clark and Proudfit, 1993; Willis and Westlund, 1997; Fields and Basbaum, 1999). Descending input from the cortex, hypothalamus and amygdala and pretectal nucleus is provided to the midbrain periaqueductal gray, the rostroventral medulla (RVM), and the dorsolateral pontomesencephalic tegmentum (DLPT). Both RVM and DLPT project to the spinal dorsal horn. The descending pain pathways form an endogenous pain-modulating circuit consisting of both a descending inhibitory and facilitatory component (Zhuo and Gebhart, 1997; Ren et al., 2000; Ren and Dubner, 2002; Millan, 2002).

Neuropathic pain is a type of persistent pain that arises from functional changes occurring in the pain sensory system following peripheral nerve injury. Sustained or prolonged stimulation of nociceptive afferents (afferent barrage) due to tissue damage or peripheral nerve injury has been implicated in the initiation and maintenance of central neuroplastic changes (Woolf and Mannion, 1999; Ren and Dubner, 2002) culminating in central neuronal hyperexcitability, possibly due to reduced inhibition of

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nociceptive neurons by neurotransmitters, such as 5-HT and NE in both spinal and supraspinal structures (Ren et al., 2000; Ren and Dubner, 2002; Millan, 2002). The resultant state of central sensitization can produce an ongoing condition of spontaneous, persistent pain as well as an increased sensitivity to painful stimuli (hyperalgesia) or to normally non-painful mechanical or thermal stimuli (allodynia) (Woolf and Mannion, 1999). While, the precise mechanisms involved in the pathogenesis of persistent pain states are not fully understood, there is a growing recognition that disinhibition and imbalance of 5-HT and NE in endogenous pain inhibitory pathways could contribute to persistent pain mechanisms (Ren et al., 2000).

Tricyclic antidepressants (TCAs) that have 5-HT and/or NE reuptake inhibitor properties, such as amitriptyline and desipramine, are used in the clinical management of persistent pain conditions (Sindrup and Jensen, 1999; Collins et al., 2000; Lynch, 2001) but are limited by side effects including sedation, hypotension, anticholinergic effects and cardiovascular abnormalities likely due to the affinity of these drugs for cholinergic, adrenergic, and other receptors. In addition, drugs such as amitriptyline have also been suggested to have Na^+ channel blocking properties that could also be contributing to their clinical efficacy.

Duloxetine hydrochloride is a potent and selective 5-HT and NE reuptake inhibitor (SNRI) (Wong et al., 1993) that lacks significant affinity for muscarinic, histamine1, α_1-adrenergic, dopamine, 5-HT1A, 5-HT1B, 5-HT1D, 5-HT2A, 5-HT2C, opioid receptors

and ion channels including Na+ channels (Bymaster et al., 2001). Duloxetine has also been shown in several *in vivo* studies to be a balanced inhibitor of 5-HT and NE reuptake (Wong et al., 1993; Wong and Bymaster, 2002). Recently, duloxetine has been shown in humans to be safe and effective in the treatment of depression (Goldstein et al., 2002; Detke et al., 2002; Nemeroff et al., 2002). Because of the proposed role of 5-HT and NE as key mediators of descending pain pathways, duloxetine was evaluated in animal models of persistent and neuropathic pain and in a model of acute nociceptive pain.

The formalin model of persistent pain involves moderate, continuous pain generated by injured tissue following subcutaneous injection of formalin into the hindpaw of the rat, consisting of an early and late phase of paw-licking behavior where the late phase is considered to be an index of persistent pain mechanisms (Shibata et al., 1989; Wheeler-Aceto et al., 1990; Tjolsen et al., 1992). Tight ligation of lumbar L5/L6 spinal nerves (Kim and Chung. 1992) results in signs of neuropathic pain behavior including mechanical allodynia behavior and is a well-accepted model of neuropathic pain mechanisms (Kim and Chung, 1992). The tail-flick model, in contrast, measures an acute nociceptive response to radiant heat and does not involve a tissue or nerve insult.

Duloxetine, was evaluated in the formalin model of persistent pain and the L5/L6 spinal nerve ligation model of neuropathic pain at doses of drug that caused reuptake

blockade of 5-HT and NE *in vivo* and compared with other reuptake inhibitors, including venlafaxine and milnacipran.

In addition, the effects of duloxetine, venlafaxine, and milnacipran on α-MMT–induced depletion of NE and p-CA-induced depletion of 5-HT, *in vivo,* were also compared with those of paroxetine, thionisoxetine, amitriptyline, and desipramine.

Methods

Animals: Rats were maintained at constant temperature and light (12h light/12h dark) for 4-7 days prior to the studies. All testing was conducted in the light cycle and the testing room temperature was maintained at 21-23 ^{O}C. Animals had free access to food and water at all times prior to the day of the experiment. All experiment protocols were approved by the Eli Lilly and Company Institutional Animal Care and Use Committee.

Drugs and injections: Duloxetine hydrochloride, [+]-N-methyl-3-(1-napthalenyloxy)3-(2-thienyl) propanamine, paroxetine (Paxil®), and thionisoxetine were synthesized at Eli Lilly and Co. (Indianapolis, IN) for research purposes. Amitriptyline hydrochloride and desipramine hydrochloride were purchased from RBI, Inc. (Natick, MA). Gabapentin (Neurontin®) and Venlafaxine (Effexor®) (Lang et al., 1996) were purchased from Bergen Brunswig Drug Co. (Louisville, KY) for research purposes. Drugs or vehicle were administered by intraperitoneal (i.p.) injection or by oral gavage (p.o.) in a volume of 1 to 5 ml/kg. Vehicle for all test compounds was double-distilled water. A pretreatment time of 30 minutes was chosen for i.p. administration and 60 minutes for oral administration.

Blockade of transporters *in vivo*: Male, SD rats (180-230 g, Harlan Labs, IN). Inhibition of the transporters *in vivo* was determined for 5-HT transporters by blockade of p-chloramphetamine (p-CA,10 mg/kg)-induced depletion of serotonin in rat brain and

for NE transporters by α-methyl-m-tyrosine (α -MMT, 6.25 mg/kg)-induced depletion of norepinephrine in rat cerebral cortex as previously described (Koch et al., 2003). Monoamine concentrations were determined by HPLC-EC techniques (Koch et al., 2003).

Formalin Model: The formalin test was performed in custom made Plexiglas® boxes 25x25x20 cm (length x width x height) in size according to (Simmons et al., 1998) based on (Shibata et al., 1989) and (Wheeler-Aceto et al., 1990). Early (0-5 minutes) and late (15-40 minutes) phases of paw-licking behavior were quantitated visually by a blinded observer using an automated behavioral timer, as previously described (Simmons et al., 1998). A mirror placed at the back of the box allowed the unhindered observation of the formalin injected paw. Male Sprague Dawley Charles River rats (Portage, MI) weighing 200-230 grams (g) were acclimatized individually in the cubicles at least 30 minutes prior to the experiment. Formalin (50 µl of a 5% solution in saline) was injected subcutaneously into the dorsal lateral surface of the right hind paw with a 27-gauge needle. Observation started immediately after the formalin injection. Formalin-induced pain was quantified by recording in 5-minute intervals the number of seconds each licking event lasted. These recordings were made for 50 minutes after the formalin injection, using an automated behavioral timer connected to an IBM PC. Scoring in the formalin test was performed according to (Coderre et al., 1993; Abbott et al., 1995) and Simmons et al. (Simmons et al., 1998). The sum of time spent licking in seconds from

time 0 to 5 minutes was considered the early phase while the late phase was taken as the sum of seconds spent licking from 15 to 40 minutes. Drugs were administered intraperitoneally 30 minutes prior to formalin.

L5/L6 Nerve Ligation (Chung model): Male Sprague Dawley rats (Harlan, Indianapolis, IN), weighing 150-200 g at the time of surgery, were used for these experiments. Surgery was performed as previously described (Kim and Chung, 1992). Briefly, neuropathic injury was produced by tightly ligating the left L5 and L6 spinal nerves under gas anesthesia with a mixture of isoflurane (3% for induction and 2% for maintenance) and O_2. Following surgery, development of neuropathic pain was evaluated daily by measuring mechanical sensitivity of the injured paw to von Frey filaments with incremental bending forces (0.5 – 15 g) as described by (Chaplan et al., 1994). Animals were considered to be neuropathic when they exhibited mechanical allodynia, i.e. paw flinch behavior response to the application of a bending force of less than 2 g for 2 days. Test drug or vehicle was administered i.p. and mechanical threshold for paw flinching was measured at 0.5, 1, 2, 3, 4 and 6 hours after dosing. Measurement of the mechanical threshold for paw flinching was also done prior to surgery (preoperative control). Data are expressed as the threshold force required to elicit a response (g) and are means $\pm$ S.E.M. (standard error of the mean).

Rotorod test: The ability of the tested compounds to induce ataxia was examined using an automated accelerating rotorod (Omnitech Electronics Inc., Columbus, OH)

connected to an IBM PC computer (Simmons et al., 1998). For training and testing purposes, the rotorod was set up to accelerate to 17 r.p.m. in 5 seconds and maintain that speed for 40 seconds. Male Sprague-Dawley rats (Charles River, Portage, MI) weighing 200-230 g were given 3 training trials to learn to maintain posture on the rotorod prior to the day of drug testing. The following day, rotorod testing was conducted both at 1 and at 2hours following administration of drug. Rats that maintained posture and did not fall off the rotorod were given a maximum score of 40 seconds.

Tail-flick latency test: Adult male Sprague Dawley rats weighing 200-230 g (Harlan, Indianapolis, IN) were used for these experiments. The tail-flick measurement was made using the Ugo Basile Tail-Flick Unit (Ugo Basile, 21025, Comerio (VA), Italy) based on a modification of a method described originally by D'Amour and Smith, (1941). The tail-flick unit consisted of an infrared heat source (I.R. source, 50W bulb) of adjustable intensity that was set at 40 units (determined to elicit tail flick latency of 2-4 seconds as baseline in naive animals). The I.R. source was focused to the base of the tail. The latency time (in seconds) required by the rat to reach the thermal threshold for pain and flick its tail was recorded. Each rat was given one test to determine baseline latency to tail flick with a cutoff of 10 seconds set to avoid tissue damage. Animals were then given drug or vehicle and tested at varying time points after administration. Data were calculated as % MPE (maximum possible effect) and expressed as means ±

S.E.M. % MPE was calculated using the following formula: % MPE = [(Test latency – Baseline latency) / (Cut off latency (10 sec) – baseline latency)] x 100.

Data analysis for all tests: Data were analyzed by ANOVA and Dunnett's t-test and Tukey's test using JMPv3.2 (SAS Institute Inc., Cary, NC) statistical software. A significance of $p<.05$ was considered to be statistically different from vehicle group. All data are presented as means ± S.E.M.

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Results

1a. Antagonism of p-CA-induced induced depletion of brain 5-HT concentrations by uptake inhibitors (Figure 1a)

Duloxetine was compared with a number of compounds known to be inhibitors of 5-HT reuptake. The potency of compounds to inhibit the p-CA-induced depletion of whole rat brain 5-HT is shown in Figure 1a. Data are expressed as percent control, where each individual experiment was plotted against its own control group. (Overall Mean control 5-HT = 2.76 $\pm$ 0.34 nmol/g; Mean p-CA-treated 5-HT = 1.48 $\pm$ 0.27 nmol/g, N = 6 experiments, 3-5% variability between each experiment). Compounds were administered i.p. 1 hour prior to 10 mg/kg, i.p. p-CA, which depleted brain 5-HT between 37% and 56%, 2 hours after p-CA administration Paroxetine was the most potent blocker of the p-CA -induced depletion of brain 5-HT, the dose required to block 5-HT depletion by 50% (ED_{50} dose) was 1.2 mg/kg, i.p. followed by duloxetine (ED_{50} = 2.3 mg/kg, i.p.), venlafaxine (ED_{50} = 5.9 mg/kg, i.p.), thionisoxetine (ED_{50} = 14.1 mg/kg, i.p.), amitriptyline (ED_{50} = 22.7 mg/kg, i.p.), desipramine (ED_{50} = 23.0 mg/kg, i.p.) and milnacipran (ED_{50} = 24.6 mg/kg, i.p.). More importantly, the efficacy of duloxetine was comparable to paroxetine in blocking p-CA-induced depletion of brain 5-HT, suggesting that in vivo, duloxetine was a potent and efficacious 5-HT reuptake inhibitor.

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1b. Antagonism of alpha-MMT-induced depletion of brain NE concentrations by uptake inhibitors (Figure 1b)

Figure 1b compares the potency of duloxetine with the ability of several reuptake inhibitors to inhibit the α-MMT-induced depletion of rat cortical norepinephrine concentrations. Data are expressed as percent control, where each individual experiment was plotted against its own control group (Overall Mean control norepinephrine = 2.17 ± 0.20nmol/g; Mean α-MMT norepinephrine = 1.02 ± 0.16 nmol/g, N = 6 experiments, 3-5% variability between each experiment). Compounds were administered i.p. 1 hour prior to 6.25 mg/kg, sc α-MMT, which depleted cortical norepinephrine concentrations between 41% and 63%, 4 hours after administration average α-MMT norepinephrine = 1.02 ± 0.16 nmol/g, N = 6 experiments). Desipramine was the most potent blocker of the α-MMT-induced depletion of cortical norepinephrine concentrations (ED_{50} = 2.5 mg/kg, i.p.), followed by thionisoxetine (ED_{50} = 3.8 mg/kg, i.p.), duloxetine (ED_{50} = 14.9 mg/kg, i.p.), and milnacipran (ED_{50} = 43.5 mg/kg, i.p.). Amitriptyline and paroxetine had ED_{50}'s > 30 mg/kg, i.p. (the highest doses tested), and venlafaxine had an ED_{50} > 100 mg/kg, i.p.). The efficacy of duloxetine was comparable to that of selective NRIs, desipramine and thionisoxetine, in blocking α-MMT-induced depletion of cortical norepinephrine concentrations, confirming that in vivo, duloxetine was also a potent and efficacious NRI.

2. Effects of a combination of thionisoxetine and paroxetine on formalin-induced late phase paw-licking pain behavior (Figure 2)

The selective NRI, thionisoxetine alone did not have an effect on late phase paw-licking pain behavior in the formalin model at the low doses of 0.3, 1 and 3 mg/kg, administered intraperitoneally (Liu et al., 2002) 30 minutes prior to formalin.

However, when these doses of thionisoxetine were combined with a 1 mg/kg, i.p. dose of paroxetine, a selective SSRI, the 1 and 3 mg/kg dose of thionisoxetine showed a statistically significant attenuation of formalin-induced paw-licking behavior when administered 30 minutes prior to formalin. When analyzed by a two way analysis of variance test, the parallel shift of the thionisoxetine dose response in the presence of paroxetine was shown statistically to be additive. These data suggested that while inhibition of 5-HT or NE reuptake alone had a minimal effect in attenuating formalin-induced late phase paw-licking pain, the combination of both actions produced a significant reduction in pain behavior.

Total paw-licking time in the late phase is expressed as percent control. Overall total paw-licking time in the late phase was 377 ± 22 seconds.

3. Effects of duloxetine, venlafaxine, milnacipran, amitriptyline and gabapentin on formalin-induced late phase paw-licking pain behavior (Figure 3a, 3b and 3c)

Duloxetine (3, 10, 15 mg/kg, i.p.) significantly attenuated formalin-induced late phase paw-licking behavior in a dose-dependent manner. Two other selective dual reuptake inhibitors, venlafaxine (10 and 30 mg/kg, i.p.) and milnacipran (10, 30 mg/kg, i.p.) also attenuated late phase paw-licking behavior in this model. However, duloxetine was more potent than either venlafaxine or milnacipran in attenuating pain behavior in this model of persistent pain (Figure 3a). Amitriptyline was efficacious in attenuating late phase paw-licking behavior at 10 and 30 mg/kg, i.p. (Figure 3b).

Gabapentin (10, 30 and 50 mg/kg, i.p.), an anti-epileptic drug that is widely used in the treatment of various neuropathic pain states, attenuated formalin-induced late phase paw-licking behavior, but the effects beyond 30 mg/kg showed a plateau effect. Duloxetine was more potent and efficacious in this model (Figure 3c).

Total paw-licking time in the late phase is expressed as percent control. Each compound was plotted against its own vehicle control. Overall total paw-licking time in the late phase across compounds was 359.16 ± 30.98 seconds, and variability across experiments was 5-9%.

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4. Reversal of mechanical allodynia behavior by duloxetine in L5/L6 spinal nerve ligated rats and comparison with venlafaxine and milnacipran (Figures 4a, b, c)

Duloxetine was tested for its ability to reverse mechanical allodynia behavior in L5/L6 spinal nerve ligated rats. The effects of orally administered duloxetine (10, 20 and 30 mg/kg, p.o.) were evaluated over several time-points. Duloxetine (Figure 4a) dose-dependently reversed mechanical allodynia behavior in this model of neuropathic pain. The effects of the highest (30 mg/kg) dose of duloxetine were shown to occur at 1, 2, 3 and 4 hours after administration and last up to 6 hours, with the peak effects occurring 3 hours after oral administration, while the effects of the 20 mg/kg dose of duloxetine were highest at 2 and 3 hours after administration.

Venlafaxine (100 and 300 mg/kg, p.o, Figure 4b) and milnacipran (200 and 300 mg/kg, p.o., Figure 4c) also significantly reversed mechanical allodynia behavior in the L5/L6 spinal nerve ligated rats. The effects of 300 mg/kg venlafaxine were evident from 2-6 hours after dosing, while the effects of 300 mg/kg milnacipran were evident from 1-6 hours after dosing. However, in comparison to duloxetine, both dual reuptake inhibitors were less potent and less efficacious in this model of neuropathic pain.

5. Effects of duloxetine in the tail-flick test of acute nociceptive pain:

Duloxetine (3, 10, 30 mg/kg, p.o.) produced minimal effects in attenuating the tail-flick latency in the tail-flick test of acute nociceptive pain, with statistically significant, but

modest effects occurring only at the 20 mg/kg and 30 mg/kg doses at 4 hours after administration (Figure 5).

6. Effects of duloxetine, venlafaxine, milnacipran and amitriptyline in the rotorod test of sedation/ataxia and neuromuscular function (Figure 6a, b, c, d)

Duloxetine (Figure 6a), venlafaxine, (Figure 6b) and milnacipran (Figure 6c) did not show performance deficits in the rototod test of sedation/ataxia and neuromuscular function at any of the doses tested after i.p. administration. These doses showed efficacy in the formalin model. Higher doses of milnacipran showed visible signs of ataxic behavior and were not used in pain studies. While amitriptyline did not have significant effects at doses below 50 mg/kg (Figure 6d) it caused performance deficits at the 50 mg/kg dose in this test.

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Discussion

In vivo neurochemical studies on synaptosomal uptake inhibition and microdialysis studies have previously shown that duloxetine is a potent and selective 5-HT and NE reuptake inhibitor in brain (Wong et al., 1993; Bymaster et al., 2001; Wong and Bymaster, 2002; Koch et al., 2003). Koch et al (Koch et al., 2003) showed that while duloxetine, venlafaxine and milnacipran exhibited dual 5-HT and NE reuptake inhibition properties *in vivo*, duloxetine was more potent. In the present study, in rats that were depleted of serotonin with p-CA, duloxetine was as efficacious as paroxetine, an SSRI, in blocking p-CA-induced depletion of 5-HT content, whereas in rats depleted of norepinephrine with α-MMT, duloxetine was as efficacious as the selective NRI, thionisoxetine or desipramine, in increasing NE content. These data reiterate that functionally, duloxetine is a relatively balanced dual reuptake inhibitor of 5-HT and NE *in vivo*, consistent with other published studies on duloxetine's ability to change extracellular levels of 5-HT and NE in the brain (Wong and Bymaster, 2002).

The present study has further demonstrated the efficacy of duloxetine in two preclinical models of persistent pain, the formalin model and the L5/L6 spinal nerve ligation model at doses consistent with uptake inhibition in vivo, and a rank order of potency of duloxetine>venlafaxine$\geq$ milnacipran, similar to that observed in the neurochemical studies [Figures 1a and b and (Koch et al., 2003)]. Thus, we postulate

that the efficacy of duloxetine in these pain models is mediated via enhanced 5-HT and NE transmission resulting from potent *in vivo* blockade of 5-HT and NE reuptake sites.

in the formalin model, the selective NRI, thionisoxetine, or the SSRI, paroxetine alone did not have an effect on late phase paw-licking pain behavior in the formalin model when tested at doses that show functional activity as a NRI or SSRI in the neurotransmitter depletion studies described above (Figure 1a and b). However, when an inactive dose of paroxetine was combined with inactive doses of thionisoxetine, a statistically significant attenuation of late phase formalin-induced paw-licking behavior was observed. These data suggested that while inhibition of 5-HT or NE reuptake alone had a minimal effect in attenuating formalin-induced late phase paw-licking pain, the combination of both actions produced a significant reduction in pain behavior. Thus the effects of paroxetine and thionisoxetine together was found to be additive. Thus, increasing both 5-HT and NE via reuptake inhibition may be more beneficial in the attenuation of persistent pain mechanisms. However, the possibility that a pharmacokinetic interaction between paroxetine and thionisoxetine may have led to enhanced activity when combined cannot be ruled out.

Duloxetine was efficacious in reversing late phase paw-licking behavior, an index of persistent pain, in the formalin model in rats following intraperitoneal administration. The effects were dose-dependent. Furthermore, duloxetine displayed higher potency than both venlafaxine and milnacipran in reversing late phase paw-licking behavior in

the formalin test. In the effective dose range duloxetine was comparable to or more potent than amitriptyline and gabapentin in this model.

Importantly, the selective dual reuptake inhibitors, duloxetine as well as venlafaxine and milnacipran, did not show neurological deficits as measured by the rotorod test at doses that attenuated pain behavior in the formalin model, whereas amitriptyline caused significant deficits in performance in the rotorod test at the highest dose tested, likely due to being less selective than duloxetine, venlafaxine and milnacipran.

In the lumbar L5/L6 spinal nerve ligation model of neuropathic pain, duloxetine (10-30 mg/kg) significantly reversed mechanical allodynia behavior by 3 hours after oral administration. In this neuropathic pain model, duloxetine was more potent than venlafaxine and milnacipran.

Of particular interest in these studies is the reduced efficacy of duloxetine in the tail flick test of acute nociceptive pain at oral doses that showed good efficacy in the L5/L6 spinal nerve ligation model. Several factors may account for this discrepancy: 1) In the tail flick test, there is no tissue or nerve damage involved, unlike in the formalin or neuropathic pain models; 2) The tail-flick nociceptive pain response involves mainly a spinally mediated reflex, whereas injury or nerve ligation triggers more complex supraspinal neurotransmitter mediation and modulation. These data would thus suggest that duloxetine is more likely to be effective when there is persistent activation of pain pathways, unlike opiate agents, such as morphine which are known to be more

efficacious in acute nociceptive pain models. The present data support the efficacy of duloxetine in persistent rather than acute pain states.

Recent studies suggest that persistent inflammation or tissue or nerve injury results in hyperexcitability of dorsal horn neurons within the spinal cord, also called central sensitization (Dubner and Ruda, 1992; Coderre and Katz, 1997; Mannion and Woolf, 2000). Central sensitization is characterized by altered responsiveness of dorsal horn neurons, expansion of receptive fields and plasticity of neuronal connections within the pain transmitting pathways leading to increased neuronal activity at supraspinal sites and to dysfunction of the endogenous spinal and supraspinal pain inhibitory mechanisms (Coderre and Katz, 1997; Urban and Gebhart, 1999; Mannion and Woolf, 2000; Ren et al., 2000). An imbalance of the excitatory and inhibitory mechanisms within both the ascending and descending pain inhibitory pathways could ultimately lead to persistent pain (Urban and Gebhart, 1999; Woolf and Mannion, 1999; Ren et al., 2000; Hunt and Mantyh, 2001; Ren and Dubner, 2002). Thus, restoring this balance, for example with 5-HT and NE reuptake inhibitors could be beneficial in persistent pain conditions in man.

Selective serotonin reuptake inhibitors (SSRIs) have not proven to be as effective against neuropathic pain as anticipated (Sindrup and Jensen, 1999). In a study examining pain reduction among patients with neuropathic pain, fluoxetine was less effective than tricyclic antidepressants (TCAs) amitriptyline and desipramine and faired

no better than placebo (Max et al., 1992). Paroxetine (Kennedy et al., 2000) has found some utility in the treatment of chronic, daily headaches (Jackson, 1998). In general, the SSRIs are less effective in the treatment of diabetic neuropathy, compared to TCAs (Sindrup and Jensen, 1999). Venlafaxine may have analgesic effects (Lang et al., 1996) since it inhibits the reuptake of both serotonin and norepinephrine. Its side effect profile is similar to the other SSRIs and can include agitation, insomnia, or somnolence, gastrointestinal distress and inhibition of sexual functioning, consistent with the possibility that at lower doses, venlafaxine appears to be more of an SSRI and requires higher doses to show dual SNRI properties in man as is evident in one reported study of venlafaxine in diabetic neuropathic pain (Davis and Smith, 1999). Several meta-analyses of randomized double blind controlled studies of reuptake inhibitors including SSRIs and TCAs, in neuropathic pain conditions (Sindrup and Jensen, 1999; Collins et al., 2000; Lynch, 2001), have further suggested that enhancing both 5-HT and NE transmission as opposed to either one alone could result in a better therapeutic outcome. The TCAs have been used for the treatment and management of neuropathic pain for some 25 years (Max et al., 1992; Sindrup and Jensen, 1999; Collins et al., 2000; Lynch, 2001). The mechanism of action in relieving of neuropathic pain by the TCAs is thought to be due to the inhibition of reuptake of serotonin and norepinephrine or just norepinephrine within the central nervous system, however, other possible mechanisms of action include alpha–adrenergic blockade, sodium channel effects and

NMDA receptor antagonism. Nevertheless, the side effect profile of the TCAs including sedation, hypotension, anticholinergic and cardiovascular liabilities has limited their usage in the treatment and management of neuropathic and other persistent pain states. Thus, the more selective dual uptake inhibitors may offer a safer alternative.

In summary, the present results with duloxetine, a selective and potent SNRI that enhances 5-HT and NE neurotransmission in the central nervous system in a relatively balanced way, further supports the suggestion that 5-HT and NE, play a significant role in attenuating persistent pain mechanisms, presumably via descending modulatory pain pathways. The efficacy of duloxetine in models of persistent pain and neuropathic pain, suggests that in addition to its reported antidepressant activity, duloxetine may exhibit efficacy in the treatment of neuropathic pain and other persistent pain conditions in humans. In addition, persistent pain is a frequently cited feature of depression among primary care patients in a wide range of cultural settings that can be refractory to multiple treatment modalities where duloxetine may have utility.

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Figure Legends

Figure 1. Effects of uptake inhibitors on 5-HT or NE depletion. **(a)** Antagonism of p-CA-induced depletion (10 mg/kg, i.p.) of rat brain 5-HT concentrations by uptake inhibitors administered 1 hour before p-CA. Tissue samples collected 2 hours after p-CA. The data are expressed as percent antagonism of p-CA induced depletion of rat brain 5-HT concentrations. Mean ± S.E., N=5/group. *p<.05 compared to the p-CA group. **(b)** Antagonism of α-MMT -induced depletion of rat cerebral cortical concentrations of NE by uptake inhibitors administered 1 hour prior to α-MMT (6.25 mg/kg, s.c.). Tissue samples collected 4 hours after α-MMT. Data expressed as percent control. Mean ± S.E., N=5/group. * p<.05 compared to the α-MMT group. Data for duloxetine, venlafaxine and milnacipran are replotted from Koch et al., 2003 for comparative purposes.

Figure 2. Effects on formalin-induced paw-licking behavior with selective norepinephrine reuptake inhibitor, thionisoxetine (0.3, 1 and 3 mg/kg) and selective serotonin reuptake inhibitor, paroxetine (1 mg/kg) alone or thionisoxetine (0.3, 1 and 3 mg/kg) in the presence of paroxetine (1 mg/kg) administered intraperitoneally, 30 minutes prior to formalin injected into the paw. In combination experiments, paroxetine and thionisoxetine were administered on two different sides, one minute apart. While thiotomoxetine or paroxetine alone did not attenuate paw-licking behavior, when combined, showed a significant attenuation of formalin-induced paw-licking behavior.

Mean ± S.E., N=6-8. Data expressed as inhibition of total time spent licking the injured paw as percent control. Late phase = 15-40 minutes after administration of formalin into the paw.

Figure 3. Effects of **(a)** duloxetine, venlafaxine and milnacipran, **(b)** duloxetine and amitriptyline and **(c)** duloxetine and gabapentin on formalin-induced late phase paw-licking behavior administered 30 minutes prior to formalin, by intraperitoneal (Liu et al., 2002) administration. Mean ± S.E., N=6-9. Data expressed as inhibition of total time spent licking the injured paw as percent control. Late phase = 15-40 minutes after administration of formalin into the paw.

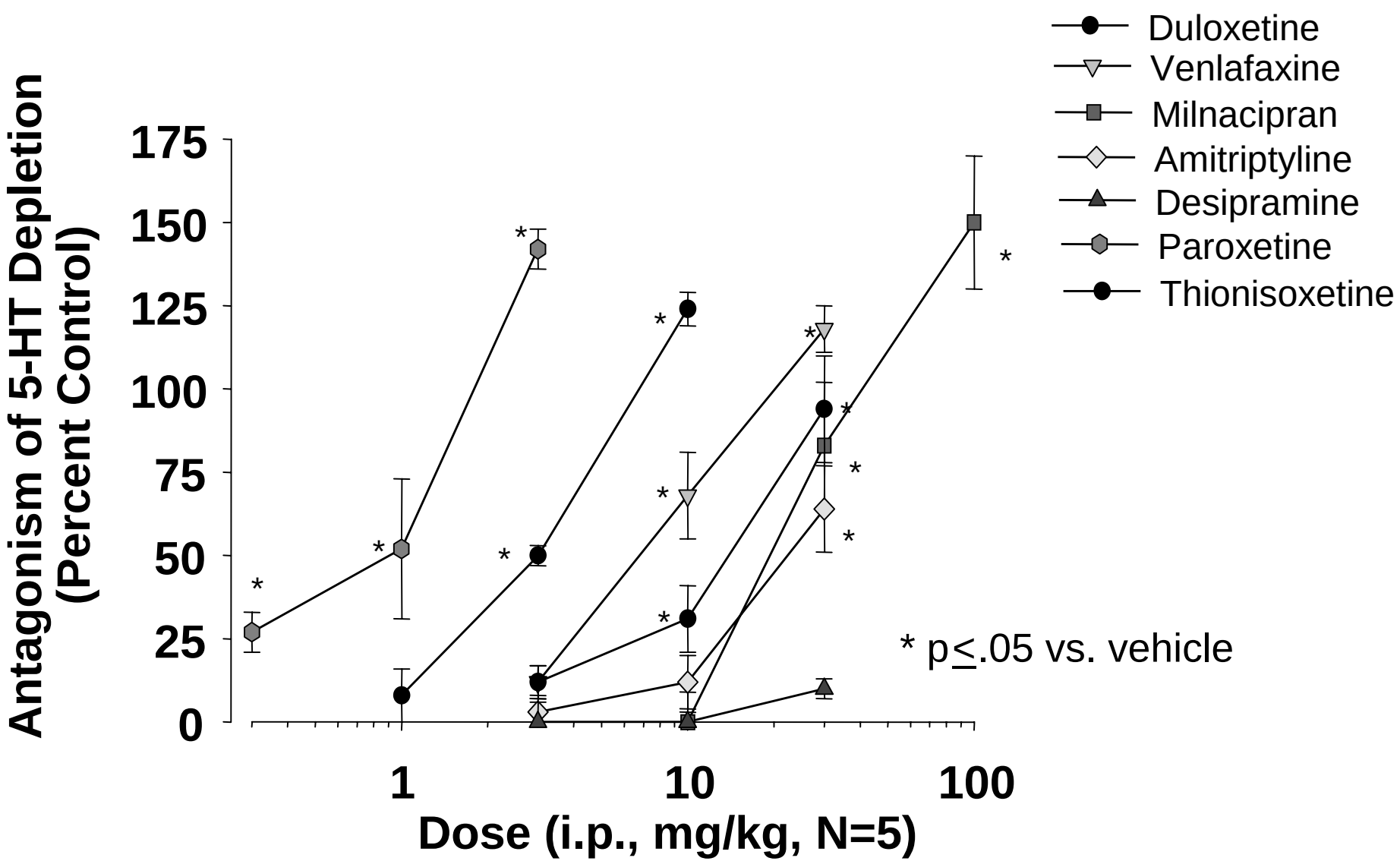
Figure 4. Effects of duloxetine, venlafaxine, and milnacipran on mechanical allodynia behavior over time in L5/L6 spinal nerve-ligated rats as measured by graded von Frey filaments. **(a)** Duloxetine (10, 20,30 mg/kg, p.o., N=6-15) showed dose-dependent reversal of mechanical allodynia behavior in this model. Effects were evident for 4 hours after administration. **(b)** Venlafaxine (p.o. N=8) reversed mechanical allodynia at 1 and 2 hours at the 100 mg/kg dose, and from 2-6 hours at the 300 mg/kg dose. **(c)** Milnacipran (p.o., N=10-12) significantly reversed mechanical allodynia at the 300 mg/kg dose beginning at 1 hour after administration. The effects were evident till the 6 hour time point measured. The 200 mg/kg dose showed effects only at the 4 hour dose. p.o.). Mean ± S.E. * $p<.05$ compared to vehicle, # $p<.1$ compared to vehicle. Pre =

presurgery baseline; base = post-surgery baseline; Data expressed as response (g.) = gram force applied to ipsilateral hindpaw.

Figure 5. Effects of duloxetine on tail flick latency in the tail-flick test of acute nociceptive pain after oral (p.o.) administration. Duloxetine caused a small but significant increase in tail flick latency to radiant heat only at the 20 and 30 mg/kg doses, 4 hours after administration of drug. Mean ± S.E. *p<.05 compared to vehicle. N=6-10. # p<.10 compared to vehicle. Data expressed as % MPE = Maximum Possible Effect [postdose latency (secs) – predose latency (secs)/cut off latency (10 secs) – predose latency X 100].

Figure 6. Effects on performance in the rotorod test of sedation/ataxia. Measurements were made at different time points after intraperitoneal administration in pre-trained rats **(a)** duloxetine, N=8; **(b)** venlafaxine, N=9; **(c)** milnacipran, N=9; **(d)** amitriptyline, N=8; no deficits in performance were noted at doses tested, except at the highest dose tested (50 mg/kg) in amitriptyline treated rats. *p<.05 compared to vehicle.

Figure 1a



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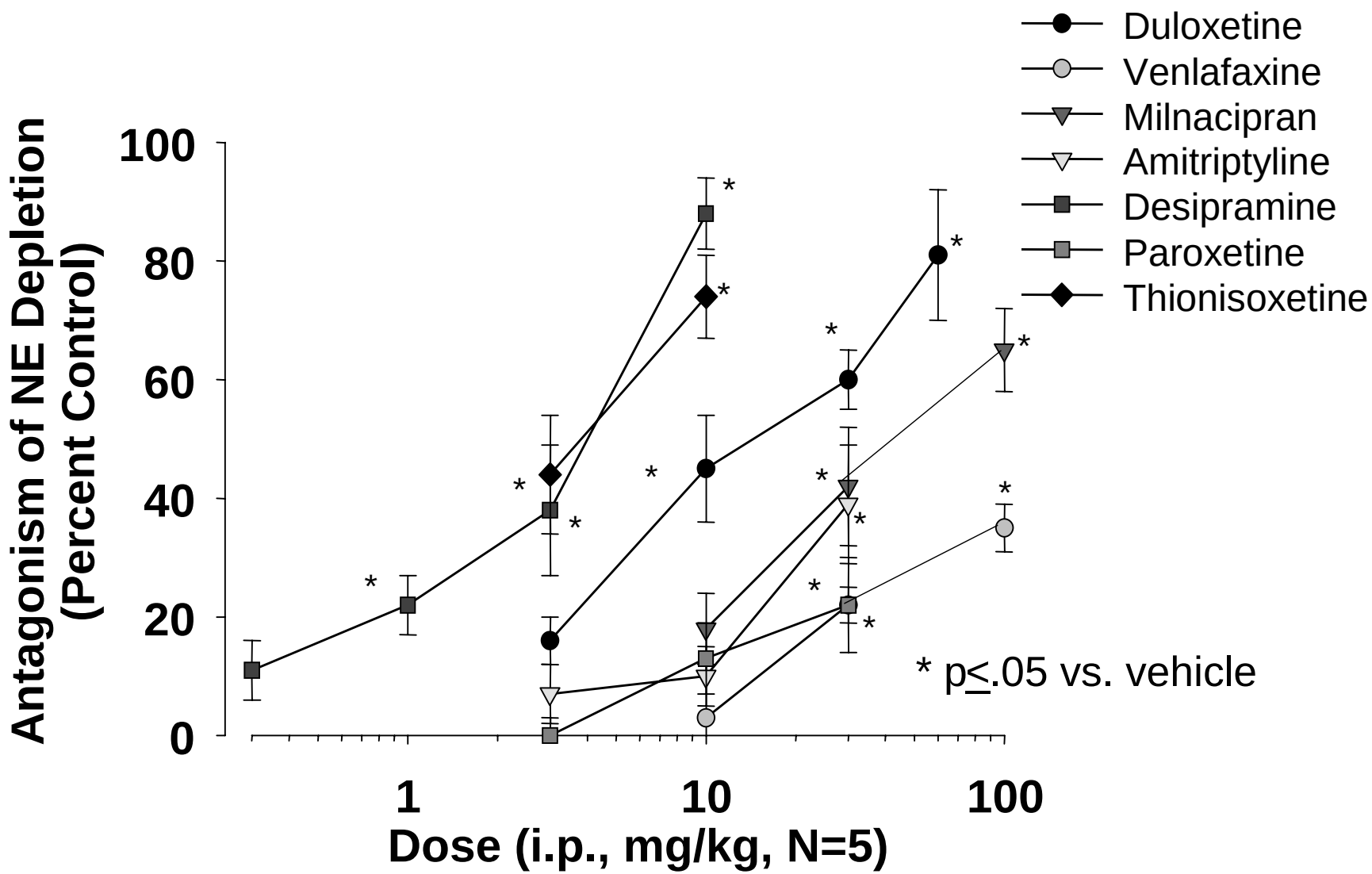

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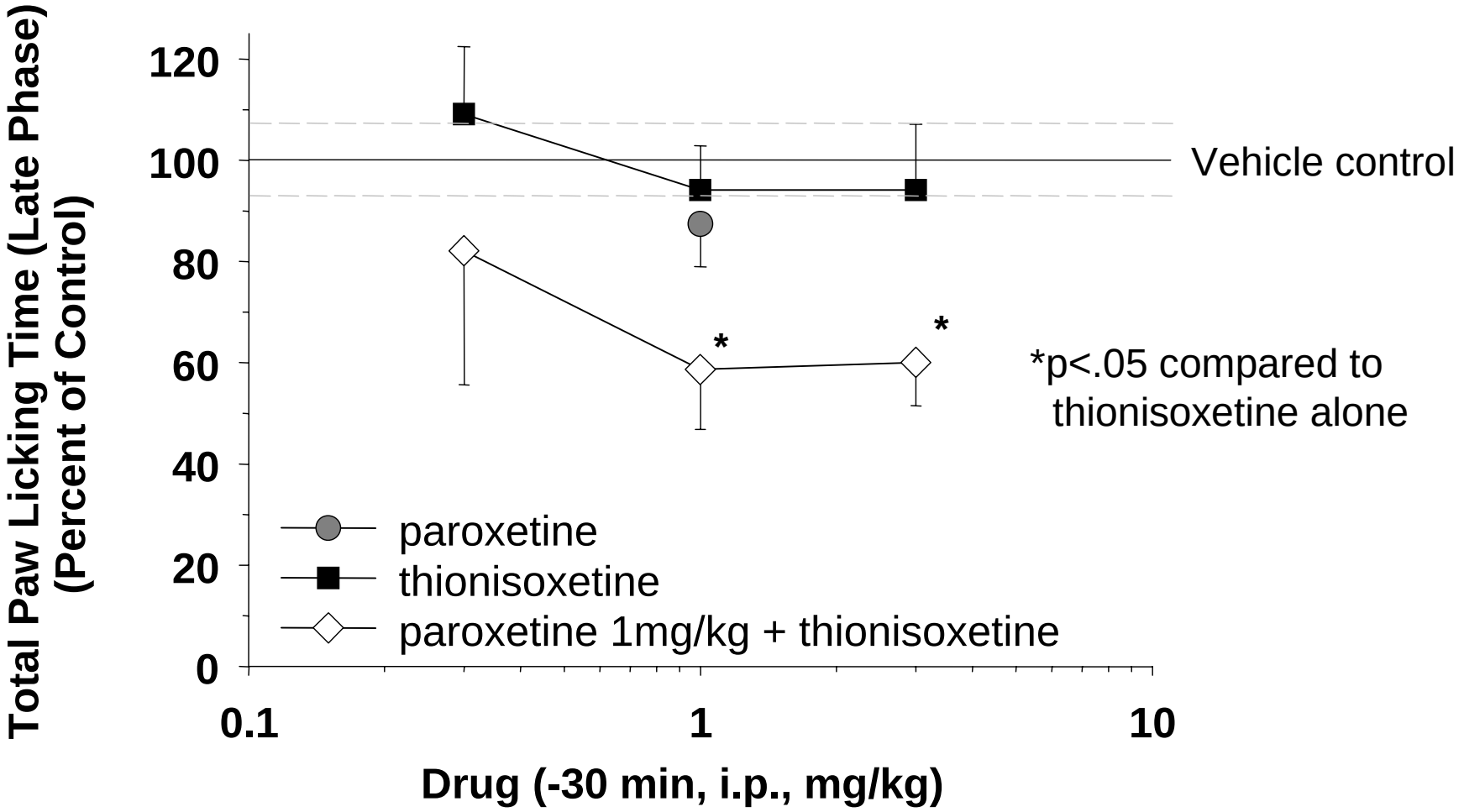

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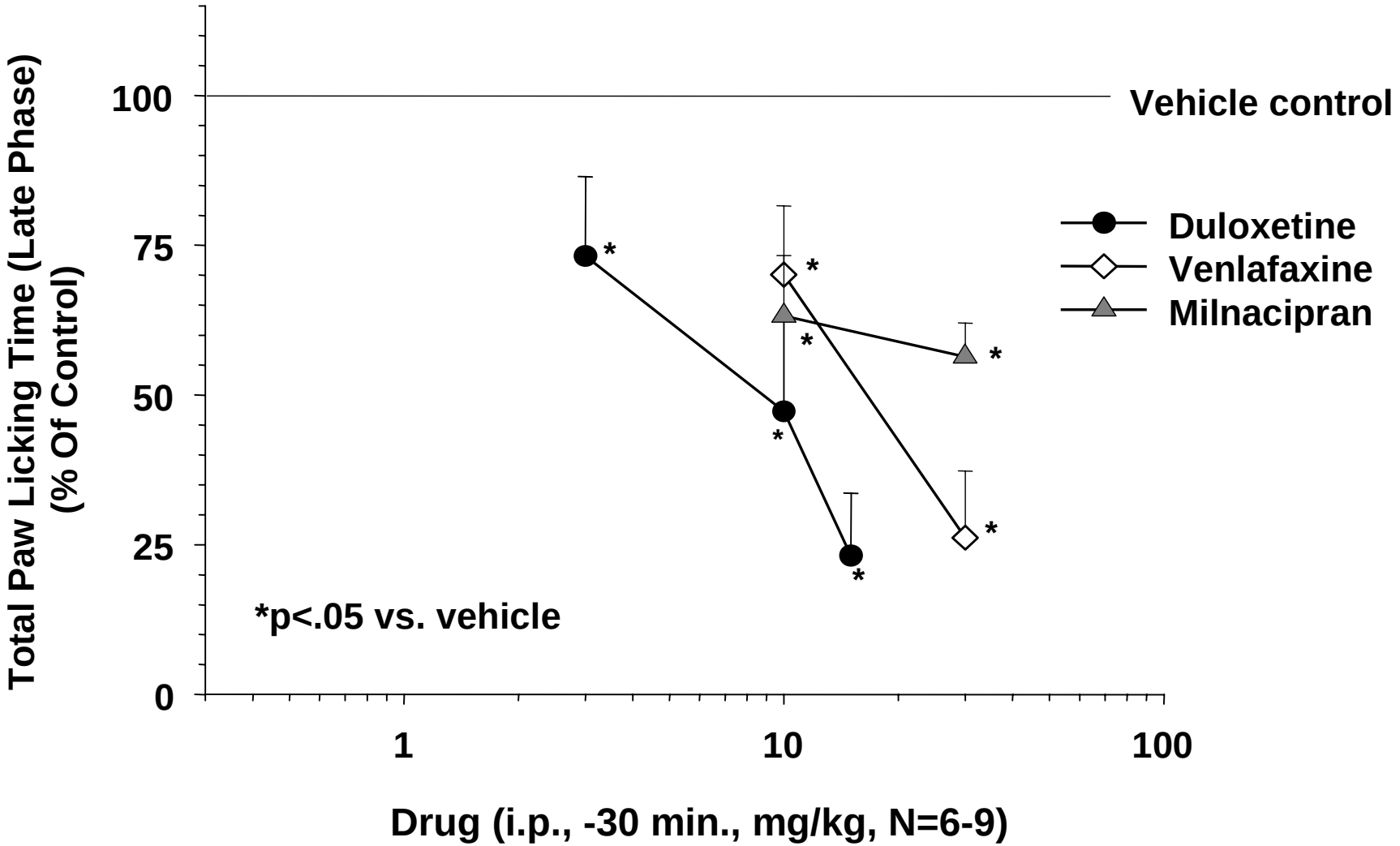

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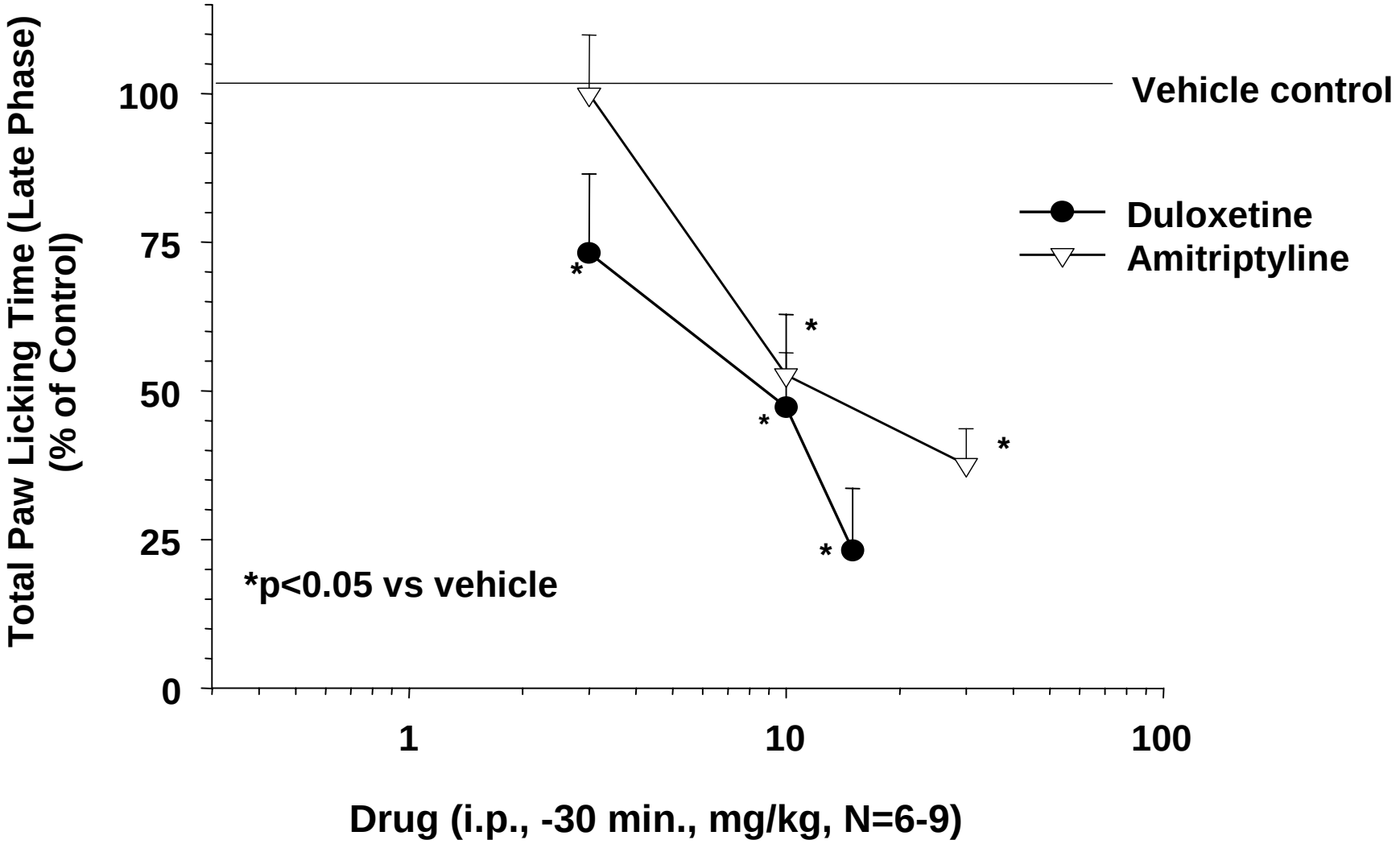

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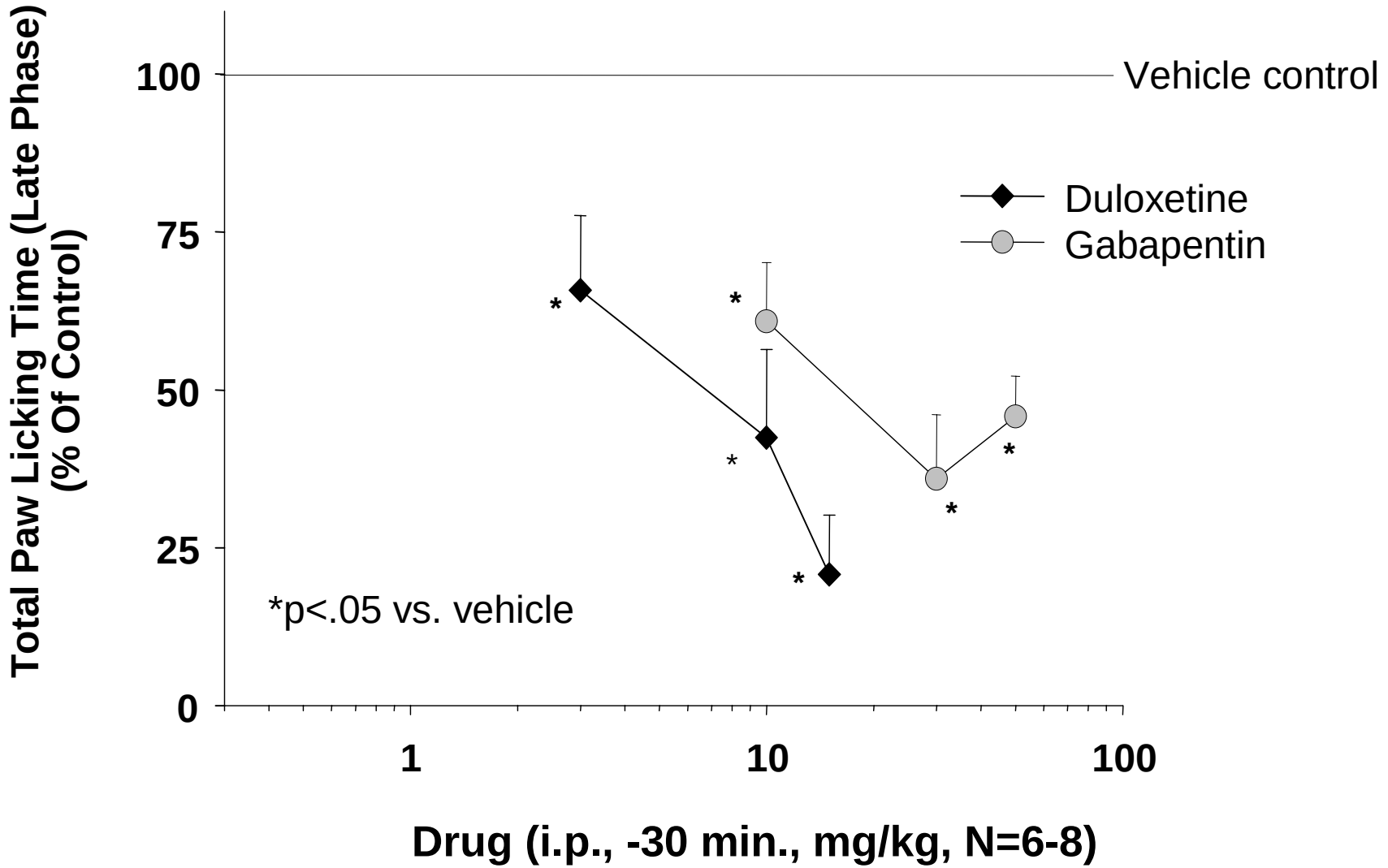

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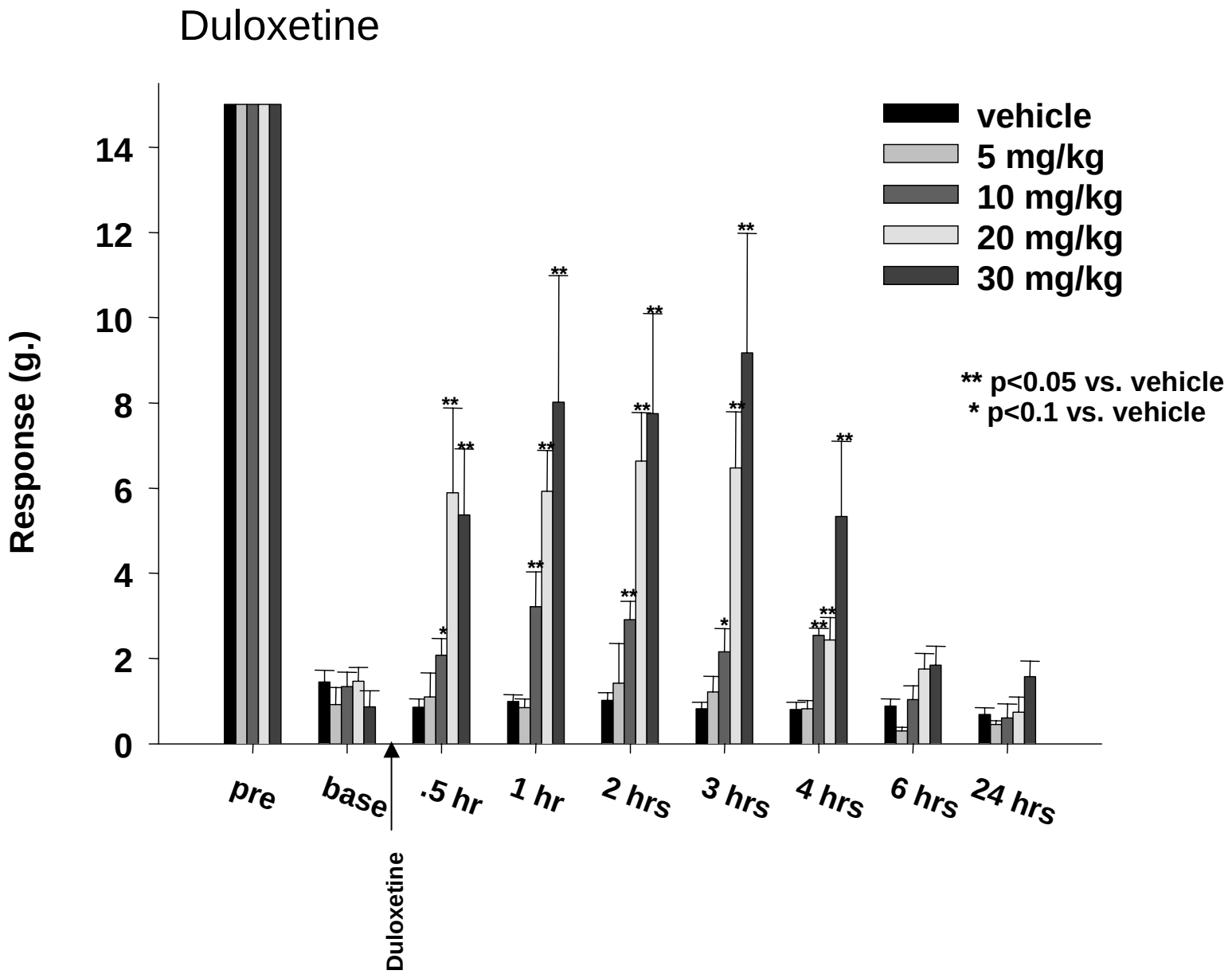

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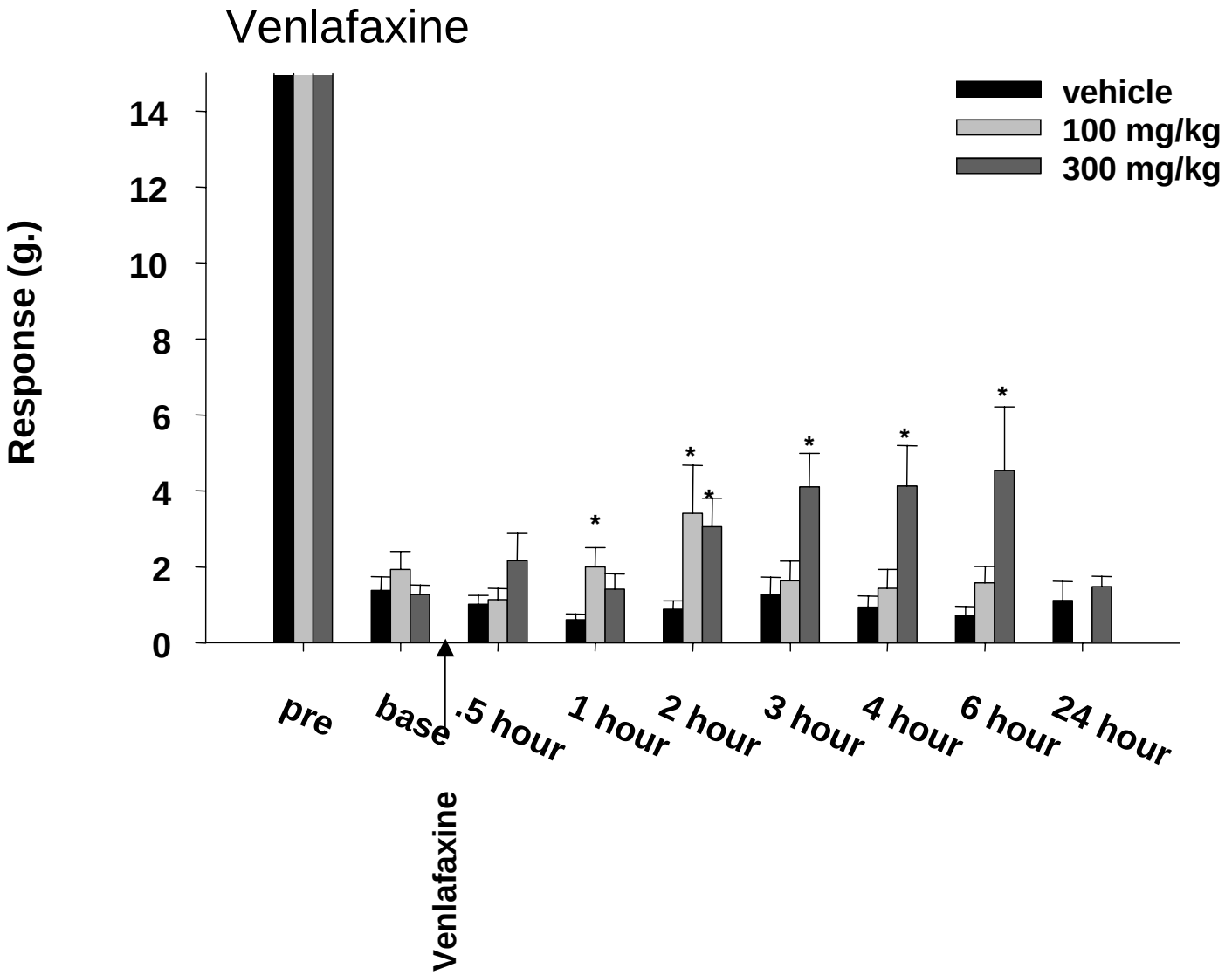

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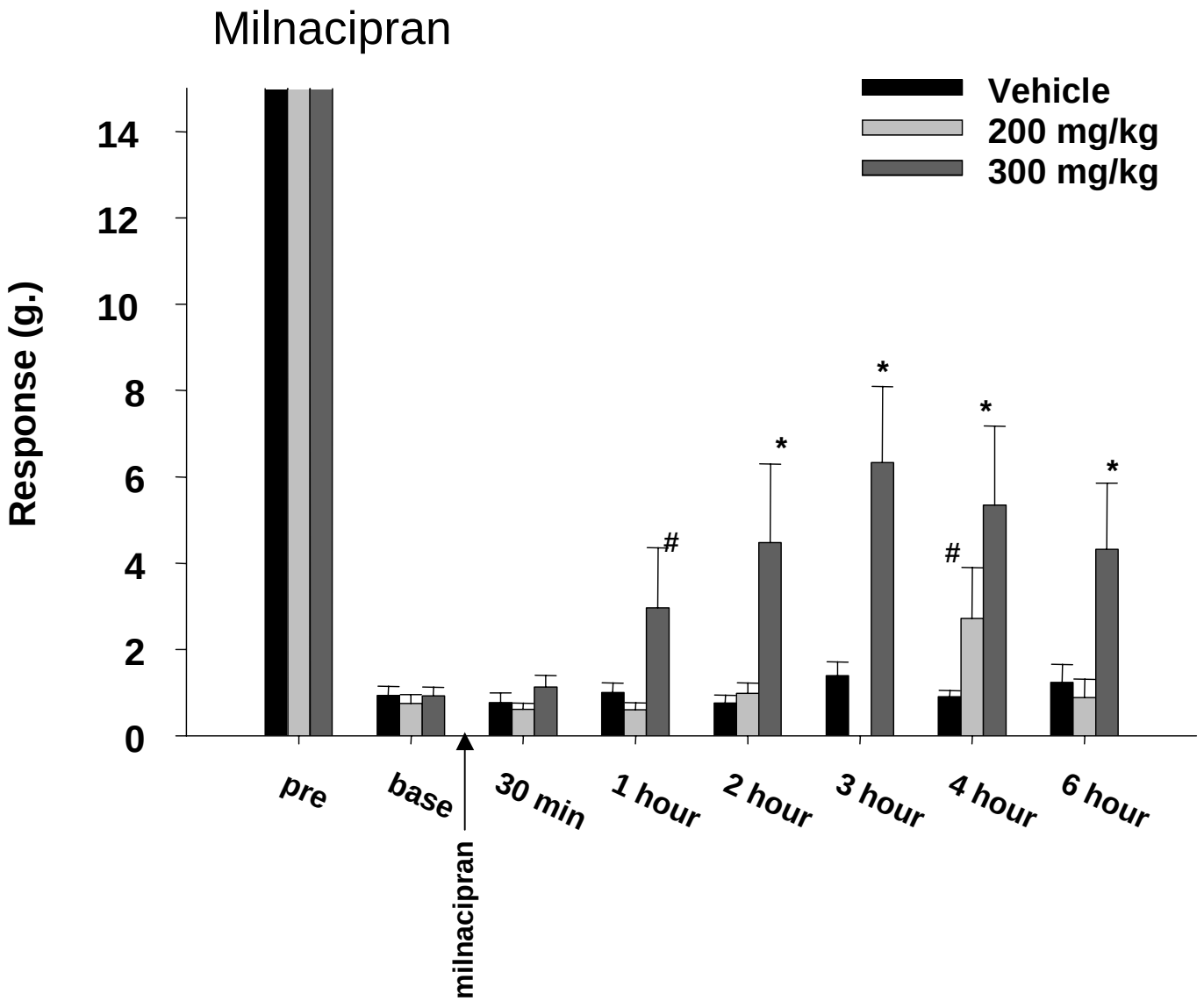

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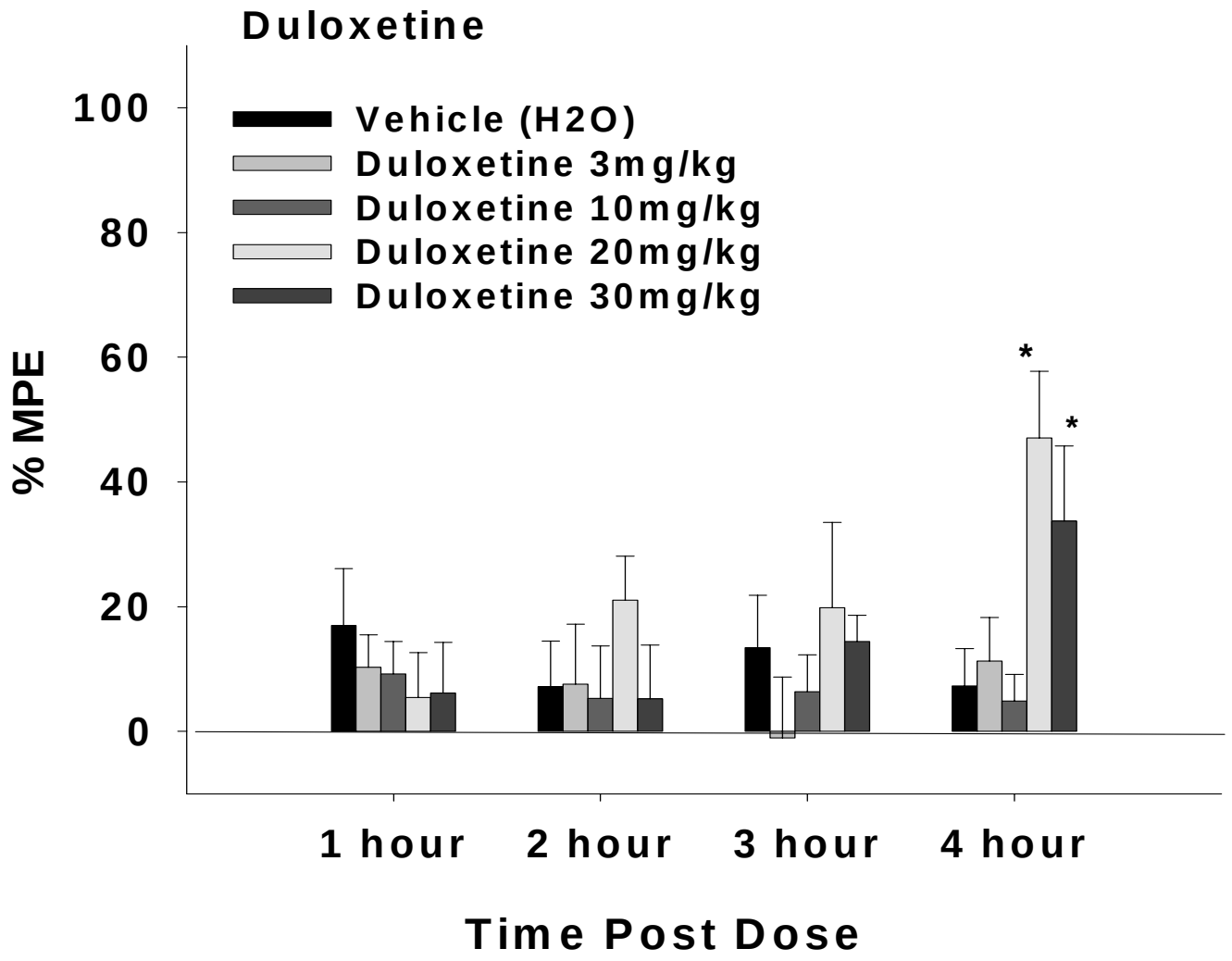

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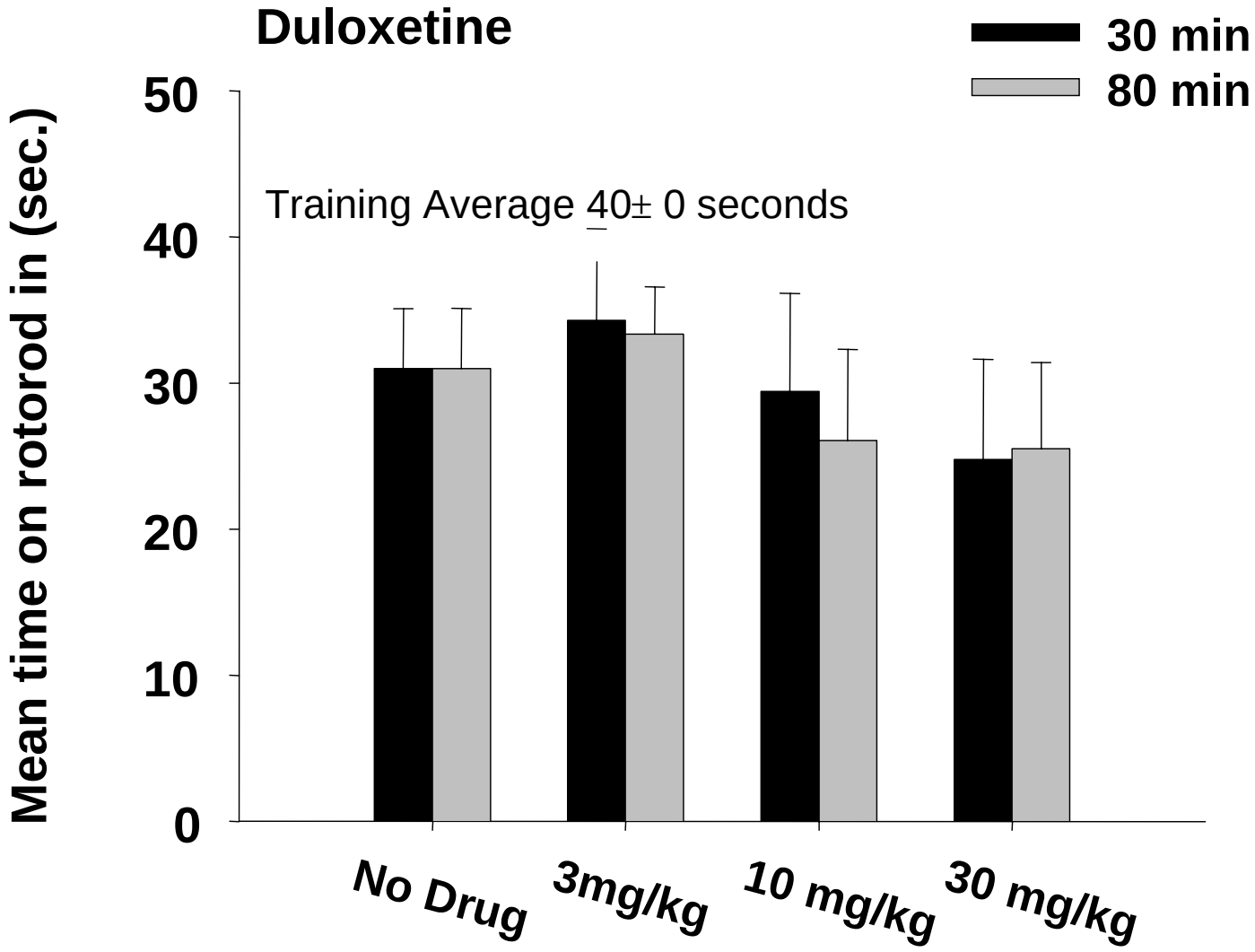

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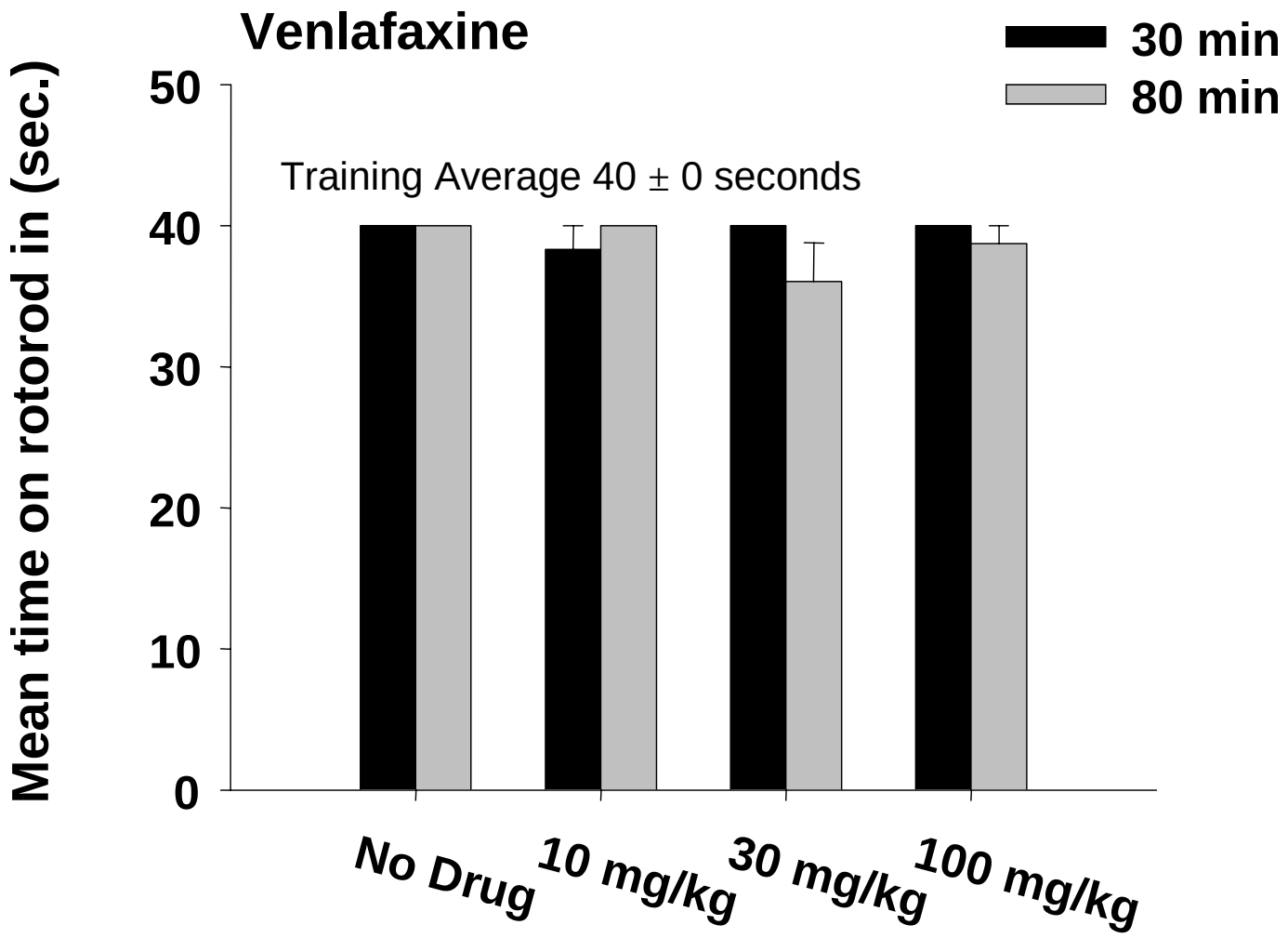

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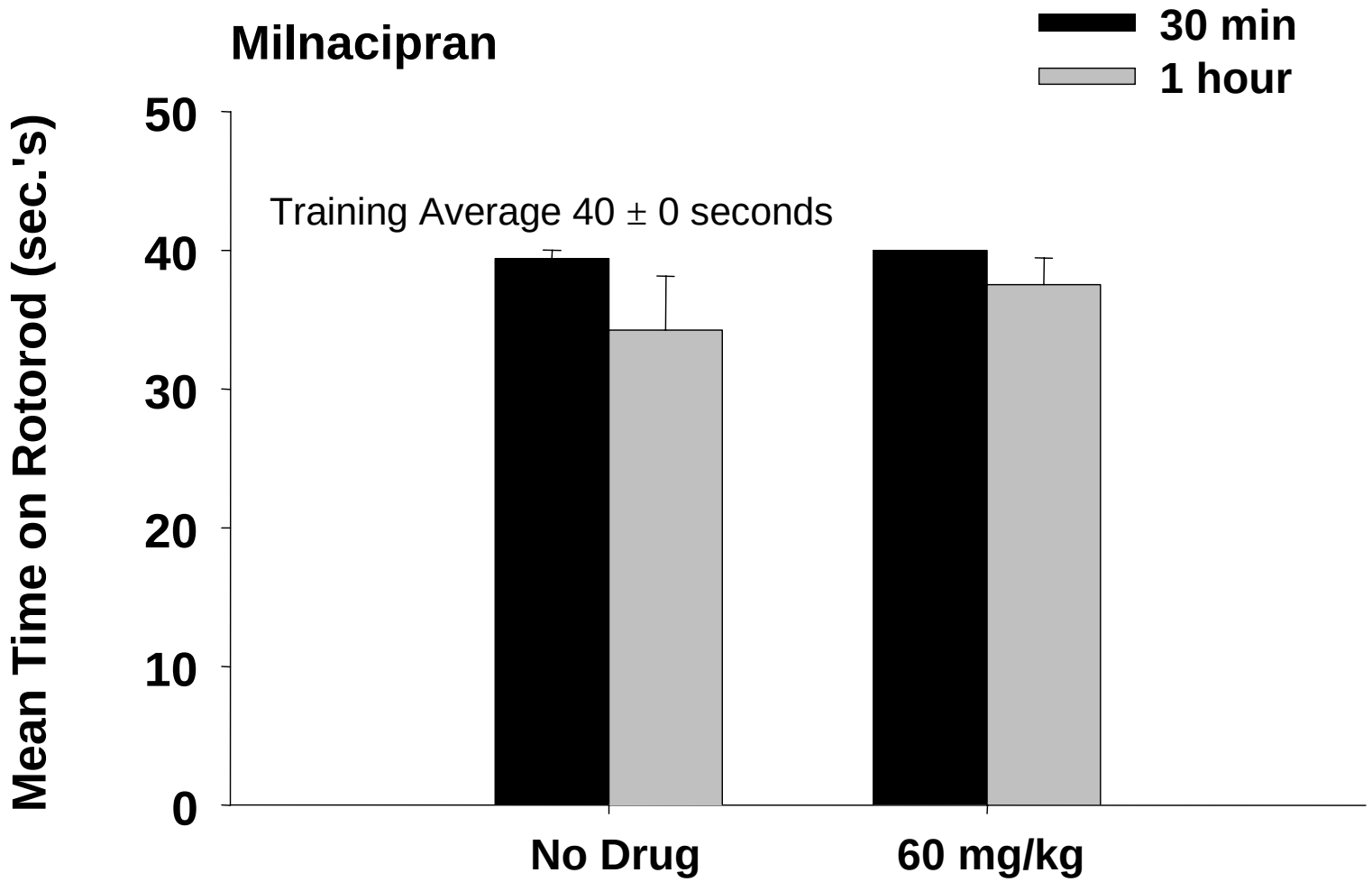

Figure 6d