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Involvement of monoamine oxidase B on models of postoperative and neuropathic pain in mice

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ABSTRACT

In this study we assessed the involvement of monoamine oxidase B (MAO-B), a key enzyme implicated in monoamine metabolism, on postoperative (plantar incision) and neuropathic (partial sciatic nerve ligation) pain models in mice. Paw incision submitted mice showed a significant decrease in mechanical threshold compared with the sham-operated mice, characterizing the development of mechanical allodynia. The selective and irreversible MAO-B inhibitor selegiline, at a dose sufficient to selectively inhibit MAO-B activity (10 mg/kg), showed an anti-allodynic effect from 0.5 to 6 h after incision. Likewise, partial sciatic nerve ligation submitted mice also developed mechanical allodynia, which was reversed by selegiline (10 mg/kg) from 2 to 6 h after treatment. In addition, a significant increase on striatal MAO-B activity was observed in neuropathic mice compared with the sham-operated animals, which was reversed by selegiline treatment. Taken together, our results showed that MAO-B seems to exert a critical role in the development of postoperative and neuropathic pain.

1. Introduction

Monoamine oxidase (MAO; EC 1.4.3.4) catalyses the oxidative deamination of biogenic amines, such as serotonin, norepinephrine and dopamine. MAO is a flavin adenine dinucleotide (FAD)-dependent enzyme located at the outer mitochondrial membrane of neuronal, glial, and other cells. On the basis of pharmacological, biochemical and genetics studies, two isoforms of MAO were proposed and designated MAO-A and MAO-B (Bach et al., 1988; Johnston, 1968). MAO-A preferentially deaminates serotonin and is inhibited by low concentrations of clorgyline, whereas MAO-B oxidizes β-phenylethylamine and benzylamine and is inactivated by low concentrations of selegiline (L-deprenyl). Dopamine, norepinephrine, epinephrine, tryptamine, and tyramine are oxidized by both isoforms of the enzyme in most species (Youdim et al., 2006).

Monoamines appear to play an important role on specific central nervous system structures implicated in pain modulation such as spinal cord, cerebral cortex, and striatum, and are involved in the antinociceptive mechanism of several drugs commonly used for the management of pain (Girard et al., 2006; Millan, 2002; Thor et al., 2007; Wood, 2008). Because of the key role played by the two MAO isoforms in the metabolism of monoamine neurotransmitters, MAO inhibitors represent a useful tool for the treatment of several neurological disorders, including depression and Parkinson's disease (PD) (Youdim et al., 2006). Interestingly, pain is a common symptom presented by certain patients with depression or PD pathologies (Bair et al., 2003; Beiske et al., 2009; Lee et al., 2006; Mongini et al., 2007; Nicholson and Verma, 2004; Tinazzi et al., 2006).

In this context, there are increasing evidences supporting an important role for MAO in nociception, as indicated by several studies showing the antinociceptive action of MAO-A inhibitors (Apaydin et al., 2001; Bianchi et al., 1992; Dina et al., 2008; Pirildar et al., 2003; Schreiber et al., 1998). Nonetheless, there are few studies investigating a possible involvement of MAO-B on pain and analgesia. For instance, Almay et al. (1987) reported that patients with neuropathic pain presented low platelet MAO-B activity, suggesting a possible relationship between this enzyme and pain sensation. Moreover, it was demonstrated recently an association of a functional polymorphism of MAO-B with postoperative pain intensity in humans, indicating a potential role of MAO-B in the perception of pain (Serý et al., 2006). In this regard, it is well known that postoperative and neuropathic painful disorders are conditions with debilitating symptoms such as allodynia (pain responses to non-noxious stimuli) and of difficult treatment, constituting still one of the most important health problems in the world (Gilron, 2006; Woolf and Mannion, 1999). In addition, studies towards mechanisms underlying these pain syndromes are decisive to the development of more efficacious analgesic drugs (Scholz and Woolf, 2002).

In the present study we evaluated the involvement of MAO-B on postsurgical and neuropathic pain. For this purpose, we

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examined the effects of the selective and irreversible MAO-B inhibitor selegiline on nociception as well as the MAO activity in different central nervous system regions of mice submitted to postoperative (plantar incision) or neuropathic (partial sciatic nerve ligation) pain models.

2. Materials and methods

2.1. Animals

Experiments were conducted using female Swiss mice (25–30 g) from our own colony. We have used female mice since sex did not influence neither incision nor neuropathy-induced pain behaviors in mice (Banik et al., 2006; Bortalanza et al., 2002; Li et al., 2009). Mice were maintained in polycarbonate cages with free access to food and water and on a 12 h alternating light–dark schedule in a temperature-controlled (22 ± 3 °C) room. Animals were allowed to adapt to the test environment for 2 h before testing. Mice were kept and used in accordance to the guidelines of the National Council for Control of Animal Experiments (CONCEA) and the National Institutes of Health guide for the care and use of Laboratory Animals. The number of animals and intensity of noxious stimuli used were the minimum necessary to demonstrate consistent effects of drug treatments. For behavioral tests, selegiline and vehicle were administered in random order and the behavioral measure was carried out by a blinded investigator.

2.2. Drugs

Selegiline hydrochloride (*R*-(−)-Deprenyl hydrochloride or *R*(−)-*N*-α-dimethyl-*N*-2-propynyl-benzeneethanamine hydrochloride; Sigma Chemical Co., St. Louis, USA) was dissolved in saline (vehicle) and administered orally by gavage (10 ml/kg) in all *in vivo* experiments. Clorgyline hydrochloride, kynuramine dihydrobromide (Sigma Chemical Co., St. Louis, USA) and selegiline hydrochloride were dissolved in incubation buffer when used for in vitro experiments. All the other reagents used were of analytical grade and were purchased from local suppliers.

2.3. Measurement of mechanical allodynia

In this study we used mechanical allodynia as a parameter of nociception, which was characterized by a significant decrease in the mechanical paw withdrawal threshold (PWT). The measurement of mechanical paw withdrawal threshold was carried out using the up-and-down paradigm as previously described by Chaplan et al. (1994). Briefly, mice were first acclimatized in individual clear Plexiglas boxes ($9 \times 7 \times 11$ cm) on an elevated wire mesh platform to allow the access to the plantar surface of the right hind paw. Filaments of von Frey of increasing stiffness (0.02–10 g) were applied to the mice hind paw plantar surface with a pressure causing the filament to bend. Absence of a paw lifting after 5 s led to the use of the next filament with increasing weight, whereas paw lifting indicated a positive response and led to the use of next weaker filament. This paradigm continued until a total of six measurements or until four consecutive positive or four consecutive negative responses occurred. All measurements were carried out in the paw ipsilateral to the surgical or sham procedure. The 50% mechanical paw withdrawal threshold response was then calculated from the resulting scores as previously described by Dixon (1980). The 50% paw withdrawal threshold was expressed in grams (g) and was evaluated before (baseline) and several times after treatments or surgical procedures.

2.4. Postoperative pain model

The postoperative pain model was carried out according to the procedure previously described (Milano et al., 2008; Pogatzki and Raja, 2003). Mice were anesthetized with 2% halothane using a nose cone. After anti-septic preparation of the right hind paw with 10% povidone–iodine solution, a 5-mm longitudinal incision was made with a number 11 blade through the skin and fascia of the plantar foot. The incision was started 2 mm from the proximal edge of the heel and extended towards the toes. The underlying muscle was elevated with a curved forceps, leaving the muscle origin and insertion intact. The skin was apposed with a single mattress suture of 6–0 nylon.

Mice were pretreated with selegiline (3 or 10 mg/kg), clorgyline (10 mg/kg) or vehicle 1 h before incision and mechanical sensitivity was measured 0.5, 1, 2, 4, 6, and 24 h after surgery procedure. Sham-operated mice were pretreated (1 h before procedure) with selegiline (10 mg/kg), clorgyline (10 mg/kg) or vehicle and mechanical sensitivity was verified from 0.5 to 6 h after procedure (pretreatment protocol). For the dose-response curve, mice were submitted to a pretreatment with selegiline (1, 3 or 10 mg/kg) or vehicle 1 h before incision and mechanical sensitivity measures were carried out 4 h after incision (5 h after treatment). In the post-treatment protocol, animals were submitted to the incisional procedure and mechanical sensitivity was determined 30 min after incision. Afterwards, animals were treated with selegiline (1, 3 or 10 mg/kg) or vehicle and the paw withdrawal threshold to mechanical stimuli was measured 4 h after treatment. For time-course curve, responses to mechanical stimuli were verified 0.5, 1, 2, 4, 6, and 24 h after treatment with selegiline (10 mg/kg) or vehicle.

2.5. Neuropathic pain model

For induction of chronic mononeuropathy, mice were first anesthetized by intraperitoneal injection of 90 mg/kg of ketamine plus 3 mg/kg of xylazine hydrochloride. Then, a partial ligation of the right sciatic nerve was made by tying one-third to one-half of the dorsal portion of the sciatic nerve, using a similar procedure to that previously described (Ferreira et al., 2005; Malmberg and Basbaum, 1998). In sham-operated mice, the nerve was exposed without ligation. Seven days after the surgical procedure, the mechanical sensitivity was measured to confirm the development of allodynia. Then, mice were treated with selegiline (10 mg/kg) or vehicle and mechanical sensitivity was measured 1, 2, 4, 6, and 24 h after treatment (time-course curve). For the dose-response curve, animals received a single injection of selegiline (1, 3 or 10 mg/kg) or vehicle 4 h before the nociceptive test.

2.6. Rota-rod test

Motor coordination was evaluated using the rota-rod test (Godoy et al., 2004). The apparatus consisted of a bar (3.7 cm in diameter) divided into three separate compartments, placed at a 25 cm height and rotating at a fixed velocity of 8 rpm. Twenty-four hours before testing, all animals were submitted to a training session until they could remain in the apparatus for 60 s without falling. On the test day, mice received a single injection of selegiline (10 mg/kg) or vehicle 4 or 5 h before testing. During the test session, the latency (s) for the first fall and the total number of fall during a 4 min period were observed.

2.7. Determination of MAO activity

For the postoperative pain model experiment, mice were pretreated with selegiline (10 mg/kg) or saline 1 h before incision

or sham procedure. Then, they were killed and different central nervous system structures (spinal cord, cerebral cortex, and striatum) were collected 4 h after incision or sham procedure. For the neuropathic pain model experiment, mice were treated with selegiline (10 mg/kg) or saline seven days after sham or partial sciatic nerve ligation procedure, and the central nervous system structures were collected 4 h after treatment. We also determined the MAO-B activity in mice treated with selegiline (1 mg/kg, p.o.) and the MAO-A activity in mice administered with clorgyline (10 mg/kg, p.o.). Mice were killed and the central nervous system structures were collected 5 or 3 h after treatment with selegiline (1 mg/kg, p.o.) or clorgyline (10 mg/kg, p.o.), respectively.

Spinal cord, cerebral cortex, and striatum were immediately separated and homogenized in assay buffer (16.8 mM Na_2HPO_4, 10.6 mM KH_2PO_4, 3.6 mM KCl, pH 7.4). MAO-A and MAO-B activities were measured in brain homogenates by a fluorometric method detecting the formation of the fluorescent product 4-hydroxyquinoline (4-HQ) from kynuramine substrate, as previously described (Matsumoto et al., 1985; Sant'Anna et al., 2009). Briefly, assays were performed in duplicate in a final volume of 500 μL containing 0.25 mg of protein and incubated at 37 °C for 30 min. Activities of the A and B isoforms were isolated pharmacologically by incorporating 250 nM selegiline (selective MAO-B inhibitor) or 250 nM clorgyline (selective MAO-A inhibitor) into the reaction mix. The reaction mixture was preincubated at 37 °C for 5 min and the reaction was started by the addition of 60 μM kynuramine. Results were expressed as nmol of 4-HQ/min/mg of protein.

2.8. Statistical analysis

Results were expressed as means ± S.E.M. Statistical analysis were carried out using GraphPad Prism 4.0 software. Significance of differences among groups was evaluated with unpaired *t*-test, one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test or two-way ANOVA followed by Bonferroni's test when appropriate. *F* values demonstrated in the text were obtained from two-way ANOVA with repeated measures and indicate the interaction between time and treatment factors. Significance was considered to be when $P < 0.05$.

3. Results

3.1. Effects of selegiline and clorgyline on postoperative pain model

Paw withdrawal threshold of sham-operated animals pretreated with selegiline (10 mg/kg) did not differ significantly from saline pretreated mice (Fig. 1A; $F(5, 60)=0.12$, $P=0.987$). The incisional procedure caused a significant decrease in paw withdrawal threshold at all time points measured (0.5–24 h) in saline treated mice compared with the baseline paw withdrawal threshold, characterizing the development of allodynia. The pretreatment

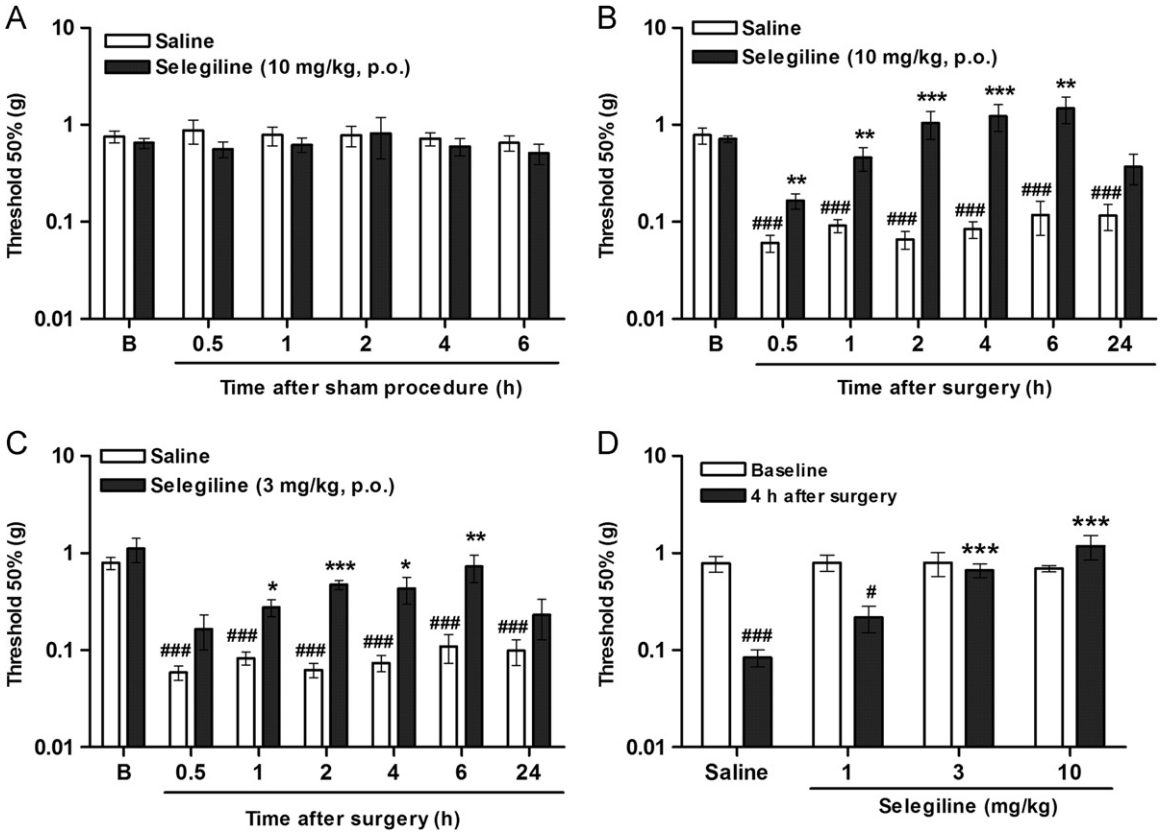

Fig. 1. Effect of selegiline pretreatment on mechanical nociceptive threshold in a mice postoperative pain model. (A) Time-course curve of paw withdrawal threshold (PWT) in sham-operated mice pretreated with selegiline (10 mg/kg, p.o.) or saline ($n=7$ per group). (B) Time-course curve of PWT in plantar incision submitted mice pretreated with selegiline (10 mg/kg, p.o.) or saline ($n=7$ per group). $^{###}P < 0.001$ compared with the baseline PWT (one-way ANOVA followed by the Student–Newman–Keuls test); $^{**}P < 0.01$ or $^{***}P < 0.001$ compared with the respective control group (unpaired *t*-test for each time point). (C) Time-course curve of PWT in plantar incision submitted mice pretreated with selegiline (3 mg/kg, p.o.) or saline ($n=7$–9 per group). $^{###}P < 0.001$ compared with the baseline PWT (one-way ANOVA followed by the Student–Newman–Keuls test); $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.001$ compared with the respective control group (unpaired *t*-test for each time point). (D) Dose-response curve of PWT in plantar incision submitted mice pretreated with selegiline (1, 3 or 10 mg/kg, p.o.) or saline ($n=6$–8 per group). $^{#}P < 0.05$ or $^{###}P < 0.001$ compared with the respective baseline PWT (paired *t*-test); $^{***}P < 0.001$ compared with the respective saline pretreated group (one-way ANOVA followed by the Student–Newman–Keuls test). Data are expressed as means ± S.E.M.

of mice with selegiline (10 mg/kg) prevented allodynia development, showing an antinociceptive effect from 0.5 to 6 h after incision (Fig. 1B; $F(6, 72)=7.63$, $P<0.001$). Furthermore, selegiline (3 mg/kg) also presented an anti-allodynic effect from 1 to 6 h after incision when administered 1 h before incision (Fig. 1C; $F(6, 84)=2.75$, $P<0.05$). However, selegiline at 1 mg/kg did present neither antinociceptive action (Fig. 1D) nor inhibitory effect on MAO-B activity (Table 2).

We also tested the effect of the irreversible and selective MAO-A inhibitor clorgyline on postoperative pain model. In sham-operated mice, clorgyline (10 mg/kg) did not alter the mechanical sensitivity compared with saline pretreated mice (Fig. 2A; $F(5, 70)=0.27$, $P=0.930$). In plantar incision-submitted mice, the pretreatment with clorgyline at 10 mg/kg, a dose that almost abolished MAO-A activity (Table 3), presented a modest antinociceptive effect at 0.5 and 2 h after incision (Fig. 2B). Since the antinociceptive effect of the MAO-B inhibitor was more pronounced and long-lasting than the effect produced by the MAO-A inhibitor, selegiline was chosen to carry out the following experiments.

Selegiline (10 mg/kg) was also able to reverse mechanical allodynia induced by incision when administered after the incisional procedure. This antinociceptive effect was observed from 0.5 to 6 h after treatment (Fig. 3A; $F(7, 91)=5.33$, $P<0.001$). Also, an anti-allodynic effect was observed 4 h after the administration of selegiline at 3 mg/kg, but not at 1 mg/kg (Fig. 3B; $F(6, 40)=5.82$, $P<0.001$).

As expected, the pretreatment of mice with selegiline (10 mg/kg) did not alter the MAO-A activity in striatum, cerebral cortex or spinal cord compared with the saline pretreated animals in both sham and incision groups (Fig. 4A). Moreover, selegiline (10 mg/kg) caused a pronounced inhibition of the MAO-B activity in both sham and incision groups compared with the respective saline pretreated mice in any analyzed structure (Fig. 4B). However, there was no difference in MAO-A and MAO-B activities between sham and incision groups in animals pretreated with saline (Fig. 4A and B).

3.2. Effects of selegiline on neuropathic pain model

Mechanical nociceptive thresholds were stable before and after the sham procedure in both saline and selegiline treated

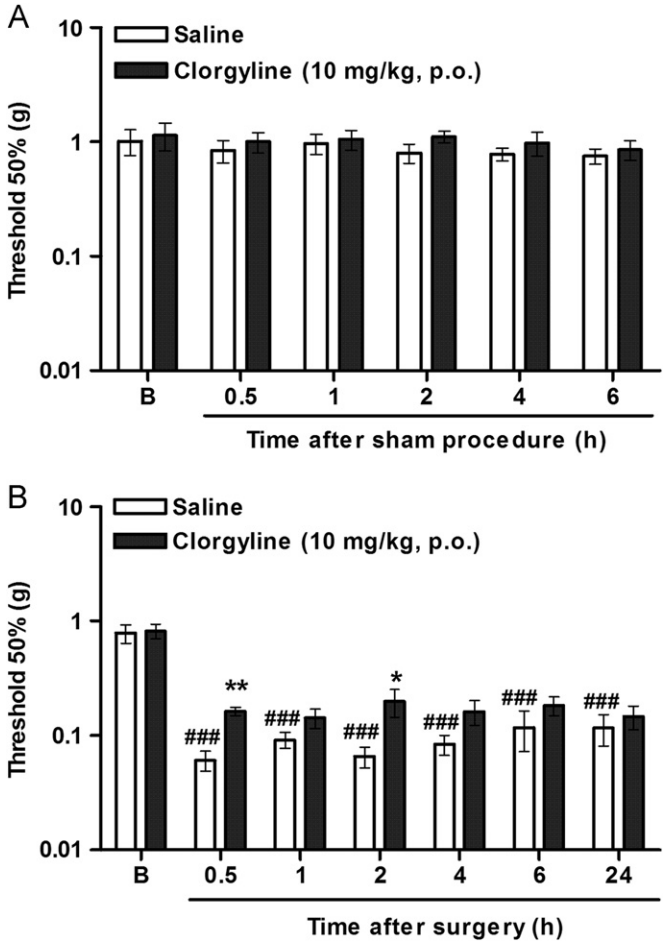

Fig. 2. Effect of clorgyline pretreatment on mechanical nociceptive threshold in a mice postoperative pain model. (A) Time-course curve of paw withdrawal threshold (PWT) in sham-operated mice pretreated with clorgyline (10 mg/kg, p.o.) or saline ($n=7$–9 per group). (B) Time-course curve of PWT in plantar incision submitted mice pretreated with clorgyline (10 mg/kg, p.o.) or saline ($n=7$ per group). $^{###}P<0.001$ compared with the baseline PWT (one-way ANOVA followed by the Student–Newman–Keuls test); $^{*}P<0.05$ or $^{**}P<0.01$ compared with the respective control group (unpaired t-test for each time point). Data are expressed as means ± S.E.M.

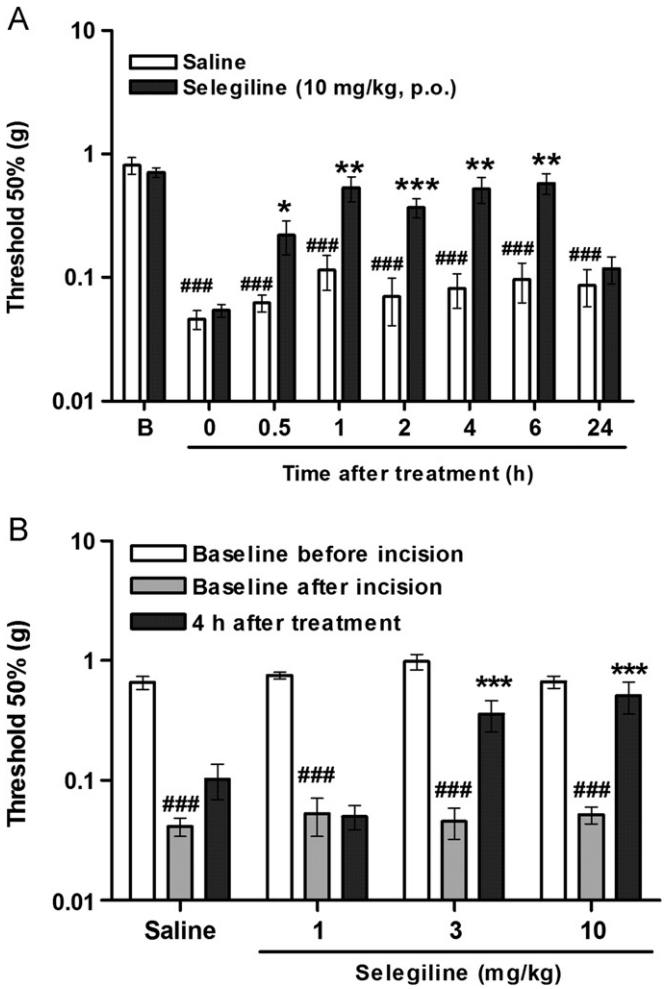

Fig. 3. Effect of selegiline post-treatment on mechanical nociceptive threshold in a mice postoperative pain model. (A) Time-course curve of paw withdrawal threshold (PWT) in plantar incision submitted mice post-treated with selegiline (10 mg/kg, p.o.) or saline ($n=7$–8 per group). $^{###}P<0.001$ compared with the respective baseline PWT (one-way ANOVA followed by the Student–Newman–Keuls test); $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ compared with respective control group (unpaired t-test for each time point). The point 0 on the x-axis represents the PWT measured immediately before drug treatment. (B) Dose-response curve of PWT for selegiline (1, 3 or 10 mg/kg, p.o.) or saline post-treatment in plantar incision submitted mice ($n=6$ per group). $^{###}P<0.001$ compared with the PWT of respective baseline before incision and $^{***}P<0.001$ compared with the PWT of respective baseline after incision (one-way ANOVA with repeated measures followed by the Student–Newman–Keuls test). Data are expressed as means ± S.E.M.

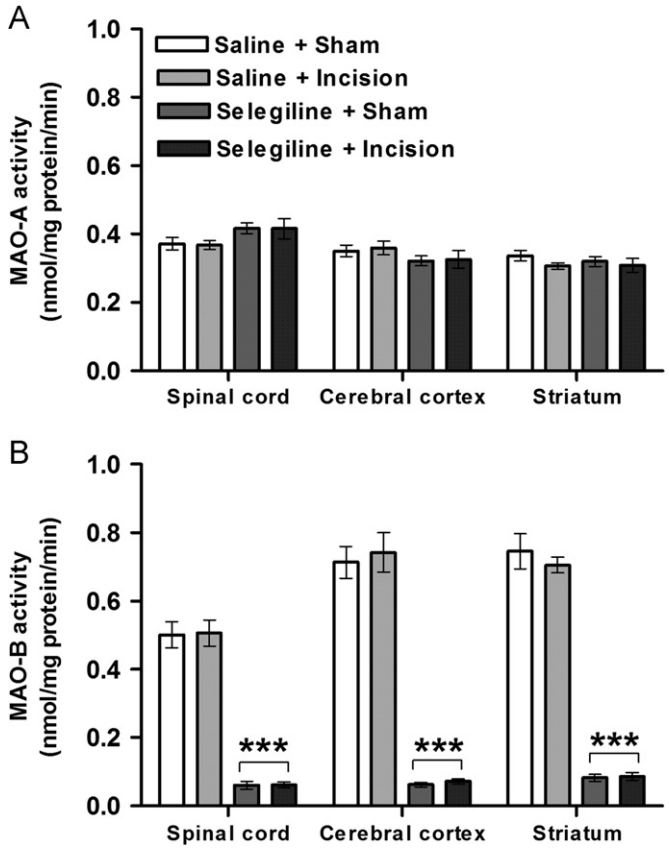

Fig. 4. Effect of pretreatment with selegiline (10 mg/kg, p.o.) or saline on monoamine oxidase (MAO) activity in spinal cord, cerebral cortex and striatum of sham-operated or plantar incision submitted mice. (A) MAO-A activity. (B) MAO-B activity. Data are expressed as means ± S.E.M. of six to seven animals per group. $^{***}P<0.001$ compared with Saline+Sham or Saline+Incision group (one-way ANOVA followed by the Student–Newman–Keuls test).

mice (Fig. 5A; $F(5, 60)=0.76$, $P=0.584$). Partial ligation of sciatic nerve produced a profound decrease in the mean paw withdrawal threshold seven days after surgery compared with the mean baseline paw withdrawal threshold (Fig. 5B). The mechanical allodynia produced by nerve injury was maintained throughout the experiment period in saline treated mice, but it was reversed by selegiline (10 mg/kg) from 1 to 6 h after treatment (Fig. 5B; $F(6, 84)=8.74$, $P<0.001$). An anti-allodynic effect was also observed 4 h after treatment with the dose of 3 mg/kg, but not with 1 mg/kg of selegiline (Fig. 5C; $F(6, 54)=6.39$, $P<0.001$).

As in the postoperative pain model, no difference was observed between groups on the MAO-A activity in striatum, cerebral cortex or spinal cord (Fig. 6A). Partial sciatic nerve ligation caused an increase in the MAO-B activity in striatum, but not in cerebral cortex or spinal cord of saline pretreated mice compared with the sham-operated animals (Fig. 6B). Likewise, selegiline (10 mg/kg) caused a pronounced inhibition of the MAO-B activity in both sham and partial sciatic nerve ligation groups compared with the respective saline pretreated mice in all examined structures (Fig. 6B).

3.3. Effects of selegiline in the rota-rod test

Treatment of mice with selegiline (10 mg/kg, 4 or 5 h before testing that corresponds to the time for the peak antinociceptive effect on neuropathic or postoperative pain models, respectively) caused no change in the motor coordination activity compared with the vehicle treated animals, as evaluated by both the latency

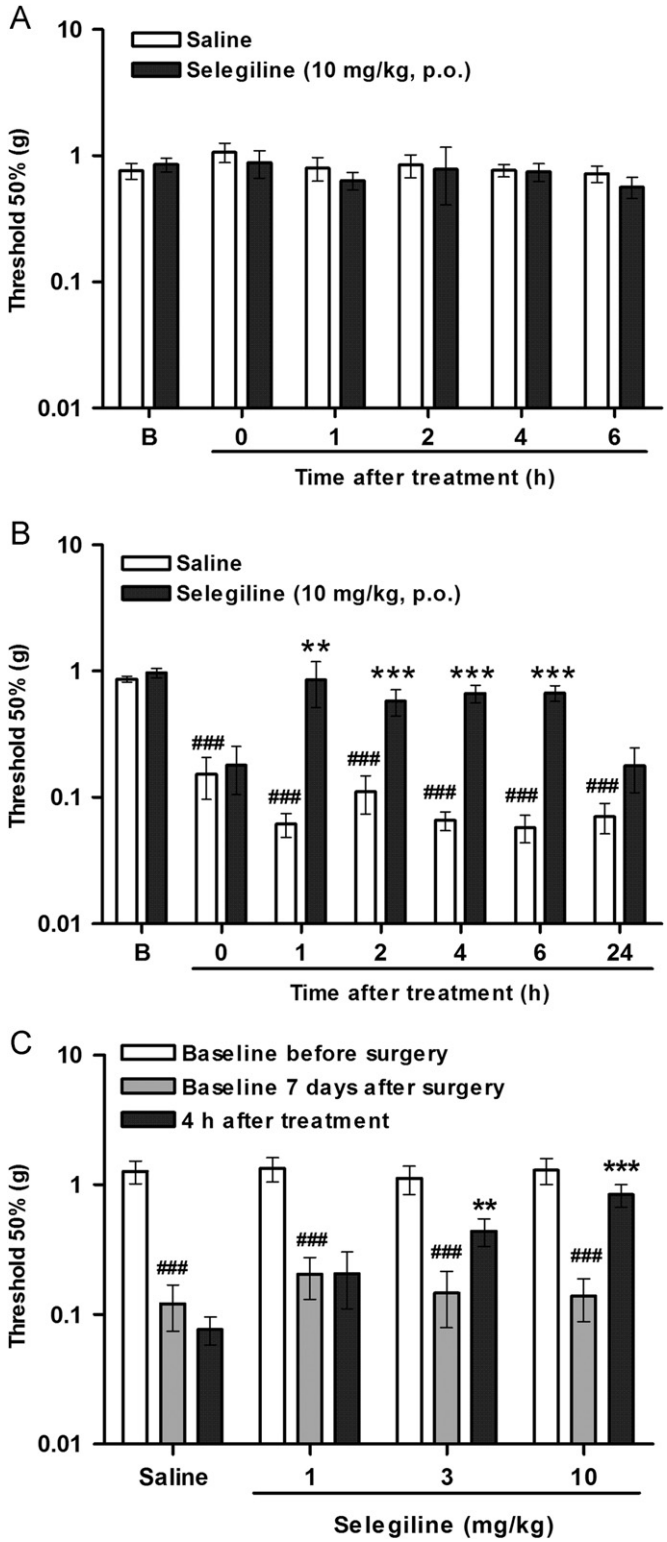

Fig. 5. Effect of selegiline treatment on mechanical nociceptive threshold in a mice neuropathic pain model. (A) Time-course curve of paw withdrawal threshold (PWT) in sham-operated mice treated with selegiline (10 mg/kg, p.o.) or saline ($n=7$ per group). (B) Time-course curve of PWT in partial sciatic nerve ligation submitted mice treated with selegiline (10 mg/kg, p.o.) or saline ($n=8$ per group). $^{###}P<P<0.001$ compared with baseline PWT (one-way ANOVA followed by the Student–Newman–Keuls test); $^{**}P<0.01$ or $^{***}P<0.001$ compared with respective control group (unpaired t-test for each time point). (C) Dose-response curve of PWT in partial sciatic nerve ligation submitted mice treated with selegiline (1, 3 or 10 mg/kg, p.o.) or saline ($n=4$–6 per group). $^{###}P<0.001$ compared with respective PWT before partial sciatic nerve ligation; $^{**}P<0.01$ or $^{***}P<0.001$ compared with respective PWT after partial sciatic nerve ligation (one-way ANOVA with repeated measures followed by the Student–Newman–Keuls test). Data are expressed as means ± S.E.M.

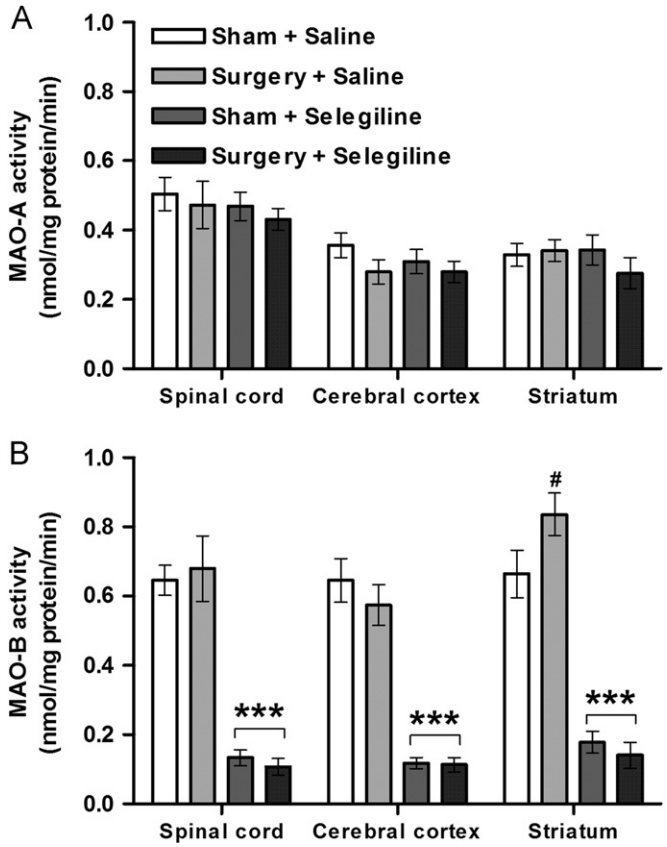

Fig. 6. Effect of selegiline (10 mg/kg, p.o.) or saline treatment on monoamine oxidase (MAO) activity in spinal cord, cerebral cortex and striatum of sham-operated or partial sciatic nerve ligation submitted mice. (A) MAO-A activity. (B) MAO-B activity. Data are expressed as means ± S.E.M. of seven to eight animals per group. $^{\#}P<0.05$ compared with Sham+Saline; $^{***}P<0.001$ compared with Sham+Saline or partial sciatic nerve ligation+saline group (one-way ANOVA followed by the Student–Newman–Keuls test).

Table 1
Effects of selegiline (10 mg/kg, p.o.) or saline treatment on latency for the first fall (s) and total number of falls in the rota-rod test in mice.

	4 h before test		5 h before test	
	Saline	**Selegiline**	**Saline**	**Selegiline**
Latency (s)	192 (15–240)	99 (17–240)	89 (43–240)	145 (12–240)
Fall number	1 (0–2)	1 (0–2)	2 (0–3)	1 (0–4)

Data are expressed as medians (interquartile ranges) of six animals per group.

(s) for the first fall and the total fall number in the rota-rod test (Table 1).

4. Discussion

Postsurgical pain is a common form of acute pain and several evidences indicate that an effective postoperative analgesia reduces morbidity following surgery, improves patient outcome, and reduces clinical expenses. However, surveys demonstrated that about 50–70% of patients experience moderate to severe pain after surgery indicating that, despite the development of new drugs and improved analgesic techniques, postsurgical pain remains still underestimated and poorly treated (Pogatzki-Zahn et al., 2007). This occurs because few studies are driven toward the mechanisms of acute postoperative pain (Zahn et al., 2002). In the present study, we evaluated the possible involvement of MAO

Table 2
Effect of selegiline (3 mg/kg, p.o.) or saline treatment on monoamine oxidase-B activity in spinal cord, cerebral cortex and striatum of mice.

	Saline	**Selegiline**
Spinal cord	0.52 ± 0.04	0.57 ± 0.02
Cerebral cortex	0.63 ± 0.04	0.65 ± 0.03
Striatum	0.67 ± 0.05	0.68 ± 0.03

Data are expressed as mean ± S.E.M. of six to seven animals per group.

Table 3
Effect of clorgyline (10 mg/kg, p.o.) or saline treatment on monoamine oxidase-A activity in spinal cord, cerebral cortex and striatum of mice.

	Saline	**Clorgyline**
Spinal cord	0.30 ± 0.03	0.03 ± 0.01***
Cerebral cortex	0.25 ± 0.03	0.07 ± 0.01***
Striatum	0.25 ± 0.04	0.04 ± 0.01***

Data are expressed as mean ± S.E.M. of seven to eight animals per group. $^{***}P<0.001$ compared with saline treated mice (unpaired *t*-test).

inhibition in a mouse model of postsurgical pain. The selective and irreversible MAO-B inhibitor selegiline (10 mg/kg) prevented the development of mechanical allodynia induced by plantar incision, showing an anti-allodynic effect from 0.5 to 6 h after incision. Selegiline was also able to reverse the decrease in paw withdrawal threshold produced by incision, showing a similar time-course profile as observed in the pretreatment experiments. Conversely, the selective and irreversible MAO-A inhibitor clorgyline, at a dose effective to largely inhibit MAO-A activity, presented a modest antinociceptive effect only at 0.5 and 2 h after incision. Although our study indicates that both MAO isoforms participates of the postoperative nociception, inhibitors of MAO-B seem to be better than MAO-A inhibitors as candidates to clinically treat painful conditions due to their better efficacy and long-lasting antinociceptive effect.

In accordance with our results, Serý et al. (2006) reported a relationship between a functional polymorphism of MAO-B and average intensity of postoperative pain. The authors observed that patients presenting the G genotype in intron 13 of MAO-B gene reported higher average intensity of postoperative pain than patients with the A genotype, suggesting that MAO-B could be involved in the perception of pain intensity. In this study, we determined the *ex vivo* MAO activity in mice pretreated with saline or selegiline (10 mg/kg) and submitted to incision or sham procedure. The plantar incision caused no alterations in the MAO-A or MAO-B activities compared with the sham submitted animals. As expected, selegiline pretreatment was able to cause a pronounced inhibition of the MAO-B activity, without affecting the MAO-A activity in sham and incision groups.

Moreover, in order to eliminate a possible false positive antinociceptive effect of selegiline owing to a motor impairment (Negus et al., 2006), mice were evaluated in the rota-rod test. Selegiline (10 mg/kg) caused no alterations on the latency for the first fall or the total number of falls, indicating that the antinociceptive effect was not mediated by an unspecific alteration on motor coordination activity.

The effects of selegiline on a neuropathic pain model in mice were also evaluated. Neuropathic pain is a debilitating condition that frequently results from partial injury to a peripheral nerve and is often resistant to common therapeutic interventions

(Woolf and Mannion, 1999). Here, we observed that the animals submitted to partial sciatic nerve ligation presented a significant decrease in mechanical threshold seven days after surgery, which was reversed by selegiline (10 mg/kg) from 1 to 6 h after treatment. Selegiline decreased the MAO-B activity in sham and partial sciatic nerve ligation submitted mice in all analyzed structures. As expected, the MAO-A activity was not altered by selegiline treatment. Differently from what occurred on postoperative acute pain model, partial sciatic nerve ligation submitted mice presented an enhanced MAO-B activity in striatum compared with the sham-operated mice, which was abolished by the selegiline treatment. In this context, brain imaging studies frequently show increased regional cerebral blood flow in the striatum during various types of painful stimulation (Coghill et al., 1999; Iadarola et al., 1998). Furthermore, it has been proposed that striatal dopamine may have an important role in pain regulation (Hagelberg et al., 2004). Because neural plasticity clearly underlies the pain hypersensitivity characteristic of chronic pain (Woolf and Salter, 2000), it is possible that this increase on striatal MAO-B activity could be due to an augment in the enzyme expression and may be relevant to the production of neuropathic pain.

MAO-B is an important enzyme for the metabolism of biogenic and trace amines, such as dopamine and phenylethylamine, respectively. Several studies indicate that dopamine plays an important role on pain and analgesia and dopaminergic agonists have antinociceptive effect in different animal models of pain (Altier and Stewart, 1999; Wood, 2008). Moreover, it has been demonstrated that phenylethylamine and its derivatives possess antinociceptive action in mice (Giardina, 1974; Matsuoka et al., 1988, 1993). For instance, Ukponmwan et al. (1986) demonstrated that the antinociceptive action of an enkephalinase inhibitor was potentiated by both the MAO-B inhibitor selegiline and the MAO-B substrate phenylethylamine in rats, suggesting that this amine could mediate the analgesic activity of endogenously released enkephalins. Therefore, the antinociceptive action of selegiline could be mediated by an increase in the levels of dopamine and phenylethylamine in the central nervous system. Nevertheless, further studies are required to investigate the participation of specific monoamines in the antinociceptive mechanism of selegiline.

With the purpose of investigate if a dose of selegiline without antinociceptive action could inhibit the MAO-B activity, we tested the effect of 1 mg/kg of selegiline on this enzyme activity. We observed that this dose, which was ineffective in producing antinociception, caused no inhibition on the MAO-B activity, suggesting that the MAO-B inhibition appears to be required for the antinociceptive action of selegiline. However, besides MAO-B inhibitory properties, selegiline is known to have other effects such as antioxidant and neuroprotective activities (Le et al., 1997; Maruyama and Naoi, 1999; Youdim and Bakhle, 2006; Zhu et al., 2008). Despite our results suggest that the antinociceptive effect of selegiline is related to its MAO-B inhibitory property, we cannot exclude the possibility that selegiline could be acting via a MAO-B independent mechanism.

Taken together, our results showed that selegiline presented antinociceptive effect on mice models of acute postoperative pain and chronic neuropathic pain, suggesting a possible involvement of MAO-B in the mechanism of these pain conditions and a potential utility of its inhibitors for the development of new therapeutic approaches.

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References

Almay, B.G., von Knorring, L., Oreland, L., 1987. Platelet MAO in patients with idiopathic pain disorders. J. Neural Transm. 69, 243–253.
Altier, N., Stewart, J., 1999. The role of dopamine in the nucleus accumbens in analgesia. Life Sci. 65, 2269–2287.
Apaydin, S., Goldeli, E., Uyar, M., Erhan, E., Yegul, I., Tuglular, I., 2001. The antinociceptive effect of moclobemide on the vocalization threshold to paw pressure in a rat model of unilateral mononeuropathy. Pharmacol. Res. 44, 503–507.
Bach, A.W.J., Lan, N.C., Johnson, D.L., Abell, C.W., Bembenek, M.E., Kwan, S.-W., et al., 1988. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. Proc. Natl. Acad. Sci. 85, 4934–4938.
Banik, R.K., Woo, Y.C., Park, S.S., Brennan, T.J., 2006. Strain and sex influence on pain sensitivity after plantar incision in the mouse. Anesthesiology 105, 1246–1253.
Bair, M.J., Robinson, R.L., Katon, W., Kroenke, K., 2003. Depression and pain comorbidity: a literature review. Arch. Intern. Med. 163, 2433–2445.
Beiske, A.G., Loge, J.H., Rønningen, A., Svensson, E., 2009. Pain in Parkinson's disease: prevalence and characteristics. Pain 141, 173–177.
Bianchi, M., Mantegazza, P., Panerai, A.E., 1992. Effects of two different reversible monoamine oxidase-A inhibitors on nociceptive thresholds in the rat. Eur. J. Pharmacol. 219, 113–116.
Bortalanza, L.B., Ferreira, J., Hess, S.C., Delle Monache, F., Yunes, R.A., Calixto, J.B., 2002. Anti-allodynic action of the tormentic acid, a triterpene isolated from plant, against neuropathic and inflammatory persistent pain in mice. Eur. J. Pharmacol. 453, 203–208.
Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M., Yaksh, T.L., 1994. Quantitative assessment of tactile allodynia in the rat paw. J. Neurosci. Methods 53, 55–63.
Coghill, R.C., Sang, C.N., Maisog, J.M., Iadarola, M.J., 1999. Pain intensity processing within the human brain: a bilateral, distributed mechanism. J. Neurophysiol. 82, 1934–1943.
Dina, O.A., Khasar, S.G., Alessandri-Haber, N., Bogen, O., Chen, X., Green, P.G., et al., 2008. Neurotoxic catecholamine metabolite in nociceptors contributes to painful peripheral neuropathy. Eur. J. Neurosci. 28, 1180–1190.
Dixon, W.J., 1980. Efficient analysis of experimental observations. Annu. Rev. Pharmacol. Toxicol. 20, 441–462.
Ferreira, J., Beirith, A., Mori, M.A., Araújo, R.C., Bader, M., Pesquero, J.B., et al., 2005. Reduced nerve injury-induced neuropathic pain in kinin B1 receptor knockout mice. J. Neurosci. 25, 2405–2412.
Giardina, W.J., 1974. Analgesic properties of phenylethylamine and phenylethanolamine in mice. Pharmacology 12, 1–6.
Gilron, I., 2006. Review article: the role of anticonvulsant drugs in postoperative pain management: a bench-to-bedside perspective. Can. J. Anaesth. 53, 562–571.
Girard, P., Coppé, M.C., Verniers, D., Pansart, Y., Gillardin, J.M., 2006. Role of catecholamines and serotonin receptor subtypes in nefopam-induced antinociception. Pharmacol. Res. 54, 195–202.
Godoy, M.C., Fighera, M.R., Souza, F.R., Flores, A.E., Rubin, M.A., Oliveira, M.R., et al., 2004. Alpha 2-adrenoceptors and 5-HT receptors mediate the antinociceptive effect of new pyrazolines, but not of dipyrone. Eur. J. Pharmacol. 496, 93–97.
Hagelberg, N., Jääskeläinen, S.K., Martikainen, I.K., Mansikka, H., Forssell, H., Scheinin, H., et al., 2004. Striatal dopamine D2 receptors in modulation of pain in humans: a review. Eur. J. Pharmacol. 500, 187–192.
Iadarola, M.J., Berman, K.F., Zeffiro, T.A., Byas-Smith, M.G., Gracely, R.H., Max, M.B., et al., 1998. Neural activation during acute capsaicin-evoked pain and allodynia assessed with PET. Brain 121, 931–947.
Johnston, J.P., 1968. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. Biochem. Pharmacol. 17, 1286–1297.
Le, W., Jankovic, J., Xie, W., Kong, R., Appel, S.H., 1997. (−)-Deprenyl protection of 1-methyl-4 phenylpyridium ion (MPP^+)-induced apoptosis independent of MAO-B inhibition. Neurosci. Lett. 224, 197–200.
Lee, M.A., Walker, R.W., Hildreth, T.J., Prentice, W.M., 2006. A survey of pain in idiopathic Parkinson's disease. J. Pain. Symptom Manage. 32, 462–469.
Li, L., Fan, X., Warner, M., Xu, X.J., Gustafsson, J.A., Wiesenfeld-Hallin, Z., 2009. Ablation of estrogen receptor alpha or beta eliminates sex differences in mechanical pain threshold in normal and inflamed mice. Pain 143, 37–40.
Malmberg, A.B., Basbaum, A.I., 1998. Partial sciatic nerve injury in the mouse as a model of neuropathic pain: behavioral and neuroanatomical correlates. Pain 76, 215–222.
Maruyama, W., Naoi, M., 1999. Neuroprotection by (-)-deprenyl and related compounds. Mech. Ageing Dev. 111, 189–200.
Matsumoto, T., Suzuki, O., Furuta, T., Asai, M., Kurokawa, Y., Rimura, Y., et al., 1985. A sensitive fluorometric assay for serum monoamine oxidase with kynuramine as substrate. Clin. Biochem. 18, 126–129.

Matsuoka, Y., Sakuma, M., Sugioka, T., Terawaki, Y., Uruno, T., Kubota, K., 1988. Characteristics of antinociception induced by noncatecholic phenylethylamine derivatives: the relation of endogenous norepinephrine to phenylethylamine analog-induced antinociception. Jpn. J. Pharmacol. 48, 263–272.

Matsuoka, Y., Sugioka, T., Terawaki, Y., Uruno, T., Kubota, K., 1993. Characteristics of antinociception induced by noncatecholic phenylethylamine derivatives: the involvement of alpha-2-adrenoceptors. Jpn. J. Pharmacol. 63, 101–108.

Milano, J., Rossato, M.F., Oliveira, S.M., Drewes, C., Machado, P., Beck, P., et al., 2008. Antinociceptive action of 4-methyl-5-trifluoromethyl-5-hydroxy-4, 5-dihydro-1*H*-pyrazole methyl ester in models of inflammatory pain in mice. Life Sci. 83, 739–746.

Millan, M.J., 2002. Descending control of pain. Prog. Neurobiol. 66, 355–474.

Mongini, F., Ciccone, G., Ceccarelli, M., Baldi, I., Ferrero, L., 2007. Muscle tenderness in different types of facial pain and its relation to anxiety and depression: a cross-sectional study on 649 patients. Pain 131, 106–111.

Negus, S.S., Vanderah, T.W., Brandt, M.R., Bilsky, E.J., Becerra, L., Borsook, D., 2006. Preclinical assessment of candidate analgesic drugs: recent advances and future challenges. J. Pharmacol. Exp. Ther. 319, 507–514.

Nicholson, B., Verma, S., 2004. Comorbidities in chronic neuropathic pain. Pain Med. 5, S9–S27.

Pirildar, S., Sezgin, U., Elbi, H., Uyar, M., Zileli, B., 2003. A preliminary open-label study of moclobemide treatment of pain disorder. Psychopharmacol. Bull. 37, 127–134.

Pogatzki, E.M., Raja, S.N., 2003. A mouse model of incisional pain. Anesthesiology 99, 1023–1027.

Pogatzki-Zahn, E.M., Zahn, P.K., Brennan, T.J., 2007. Postoperative pain—clinical implications of basic research. Best Pract. Res. Clin. Anaesthesiol. 21, 3–13.

Sant'Anna, G.S., Machado, P., Sauzem, P.D., Rosa, F.A., Rubin, M.A., Ferreira, J., et al., 2009. Ultrasound promoted synthesis of 2-imidazolines in water: a greener approach toward monoamine oxidase inhibitors. Bioorg. Med. Chem. Lett. 19, 546–549.

Scholz, J., Woolf, C.J., 2002. Can we conquer pain? Nat. Neurosci. 5, 1062–1067.

Schreiber, S., Getslev, V., Weizman, A., Pick, C.G., 1998. The antinociceptive effect of moclobemide in mice is mediated by noradrenergic pathways. Neurosci. Lett. 253, 183–186.

Serý, O., Hrazdilová, O., Didden, W., Klenerová, V., Staif, R., Znojil, V., et al., 2006. The association of monoamine oxidase B functional polymorphism with postoperative pain intensity. Neuro Endocrinol. Lett. 27, 333–337.

Thor, K.B., Kirby, M., Viktrup, L., 2007. Serotonin and noradrenaline involvement in urinary incontinence, depression and pain: scientific basis for overlapping clinical efficacy from a single drug, duloxetine. Int. J. Clin. Pract. 61, 1349–1355.

Tinazzi, M., Del Vesco, C., Fincati, E., Ottaviani, S., Smania, N., Moretto, G., et al., 2006. Pain and motor complications in Parkinson's disease. J. Neurol. Neurosurg. Psychiatry 77, 822–825.

Ukponmwan, O.E., Rupreht, J., Dzoljic, M., 1986. An analgesic effect of enkephalinase inhibition is modulated by monoamine oxidase-B and REM sleep deprivations. Naunyn Schmiedebergs Arch. Pharmacol. 332, 376–379.

Wood, P.B., 2008. Role of central dopamine in pain and analgesia. Expert Rev. Neurother. 8, 781–797.

Woolf, C.J., Mannion, R.J., 1999. Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet 353, 1959–1964.

Woolf, C.J., Salter, M.W., 2000. Neuronal plasticity: increasing the gain in pain. Science 288, 1765–1769.

Youdim, M.B., Bakhle, Y.S., 2006. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. Br. J. Pharmacol. 147, S287–S296.

Youdim, M.B., Edmondson, D., Tripton, K.F., 2006. The therapeutic potential of monoamine oxidase inhibitors. Nat. Rev. Neurosci. 7, 295–309.

Zahn, P.K., Pogatzki, E.M., Brennan, T.J., 2002. Mechanisms for pain caused by incisions. Reg. Anesth. Pain Med. 27, 514–516.

Zhu, W., Xie, W., Pan, T., Jankovic, J., Li, J., Youdim, M.B., et al., 2008. Comparison of neuroprotective and neurorestorative capabilities of rasagiline and selegiline against lactacystin-induced nigrostriatal dopaminergic degeneration. J. Neurochem. 105, 1970–1978.