Title: Operant self-administration of pregabalin in a mouse model of neuropathic pain

Running title: Pregabalin shows reinforcing properties in mice

Authors: S. A. Bura[#], D. Cabañero[#] and R. Maldonado^{#,δ}

[#]Laboratori de Neurofarmacologia, Departament de Ciències Experimentals i de la Salut,
Universitat Pompeu Fabra, PRBB Barcelona, Spain. ^δIMIM (Hospital del Mar Medical Research
Institute), Barcelona, Spain.

Corresponding Author:

Rafael Maldonado

Laboratori de Neurofarmacologia, Universitat Pompeu Fabra, Parc de Recerca Biomedica de
Barcelona (PRBB), C/ Dr. Aiguader, 88, 08003, Barcelona, Spain

E-mail: rafael.maldonado@upf.edu

Phone: +34933160824

Fax: +34933160901

Manuscript category: Original article

Funding Sources: This work was supported by the European Commission, FP7 (#HEALTH-F2-2013-602891), the Spanish Ministerio de Economía y Competitividad-MINECO (#SAF2014-59648-P/FEDER), Instituto de Salud Carlos III, RETICS-RTA (#RD12/0028/0023/FEDER), and the Generalitat de Catalunya, AGAUR (#2014-SGR-1547) and AGAUR (ICREA Acadèmia Award 2015) to R.M.

Conflicts of Interest Statement: The authors have no conflicts of interest to declare.

Significance: This study shows that mice with a nerve injury self-administer pregabalin at doses effective reducing nociceptive hypersensitivity and depressive-like behavior associated with the neuropathic pain model. Interestingly, mice without neuropathy also develop operant self-administration behavior, suggesting potential abuse liability of this first-line drug for neuropathic pain treatment.

1. Introduction

Pregabalin is an antiepileptic drug prescribed as first-line treatment for chronic neuropathic pain (Finnerup et al., 2015) mainly acting on the $\alpha 2\delta$ -1 calcium channel subunit, which is critical for functional expression of voltage-gated calcium channels (Field et al., 2006; Patel and Dickenson, 2016). Multiple preclinical studies have shown the efficacy of pregabalin alleviating hypernociception associated with neuropathic injuries (Field et al., 1999, 2006; Kremer et al., 2016; Nozaki-Taguchi et al., 2001). While some of these studies describe complete reversion of neuropathic hypersensitivity, it should be noted that the number of patients needed to treat for 50% pain relief (i.e. patients that need to be treated to obtain one with a 50% of pain relief) is estimated between 6.5 and 9.4 (Finnerup et al., 2015). This discordance between clinical data and preclinical studies may be related to the measurement of evoked sensitivity in the current preclinical studies. While one of the most prevalent symptoms in chronic neuropathic syndromes is spontaneous pain (96% of the cases (Backonja and Stacey, 2004)), studies estimating the efficacy of pregabalin alleviating spontaneous manifestations of neuropathic pain models in mice are lacking.

Different clinical studies (Bossard et al., 2016; Evoy et al., 2017; Papazisis and Tzachanis, 2014; Schjerner et al., 2016) and individual reports (Filipetto et al., 2010; Halaby et al., 2015; Papazisis et al., 2013) have pointed out the potential abuse liability of pregabalin, particularly when treating psychiatric disorders and in patients with concomitant abuse of other medications. Surprisingly, few studies have used animal models to estimate the ability of pregabalin to induce abuse or to alleviate spontaneous pain (Table 1). A first study failed to show pregabalin-induced conditioned place preference (CPP) after oral treatment in rats (Andrews et al., 2001), whereas a more recent study showed CPP after intraperitoneal administration to rats without pain (Rutten et al., 2011). In the context of pain, pregabalin reduced conditioned place aversion in a rat model of carrageenan-induced inflammation,

which seemed related to its capability to alleviate pain manifestations (Rutten et al., 2011). Higher doses of pregabalin induced rewarding effects in carraggenan-treated rats, although it could not be distinguished whether the reward was associated to pain relief or to psychotropic drug effects (Rutten et al., 2011). These previous results showed the effects of pregabalin facilitating context-drug associative learning. However, the ability of pregabalin to induce drug-taking behavior in the context of chronic neuropathic pain [models](#) or in the absence of pain has never been evaluated. Self-administration models in which animals are required to elicit an operant response to obtain a drug represent an approach with high face validity, useful to estimate drug efficacy alleviating spontaneous, non-evoked pain (Bura et al., 2013; Gutierrez et al., 2011). At the same time, operant drug self-administration shows high predictive validity assessing the reinforcing properties of drugs in rodents and non-human primates which are related to its abuse liability potential (O'Connor et al., 2011). The combination of both approaches could serve to estimate the ability of pregabalin to elicit an operant drug-taking behavior selectively associated to [relief of spontaneous pain-related manifestations](#) or to reinforcing properties related to its psychoactive effects. Previous studies evaluating the efficacy of pregabalin in mouse models of neuropathic pain have revealed incomplete efficacy of the drug inhibiting other aspects of chronic neuropathic syndromes, including anxiety-like behavior, cognitive impairment or decreased motivation for food (Galan-Arriero et al., 2014; La Porta et al., 2016).

Here we used an operant self-administration paradigm to evaluate the reinforcing effects of pregabalin in a mouse model of neuropathic pain. This paradigm mimics self-medication with painkillers in humans, in the sense that an effective analgesic drug will reinforce the response of taking the drug in individuals with pain. At the same time, pregabalin-induced reinforcement was measured in mice free of neuropathic [injury](#). The maintenance of an operant behavior for pregabalin in these mice would be indicative of abuse potential. In our experimental paradigm, sensitivity to mechanical and thermal stimuli was assessed before and

after the [drug self-administration](#) period to confirm the antinociceptive activity of pregabalin, and emotional consequences of the self-treatment were estimated by measuring depressive-like behavior [at the end of the experimental sequence](#).

2. Methods

2.1 Animals

C57BL/6J male mice (Charles River, Domaine des Oncins, BP, 0109, L'Arbresle, France), weighing 22–24 g at the beginning of the experiments, were used. Mice were housed in a temperature (21 ± 1 °C) and humidity-controlled ($55 \pm 10\%$) room, and handled during the dark phase of a 12-h light/dark reverse cycle (light off at 08:00 a.m., light on at 8:00 p.m.). Before starting the experimental procedure, mice were single housed and handled/habituated during 7 days. Food and water were available ad libitum except during the training period for food-maintained operant behavior, when mice were exposed to a restricted diet during 8 days. The experimental procedure (Fig. 1) and the animal husbandry were conducted according to standard ethical guidelines (European Community Guidelines on the Care and Use of Laboratory Animals 86/609/EEC) and were approved by the local ethical committee (Comité Etico Experimental Animal, Instituto Municipal de Asistencia Sanitaria/Universitat Pompeu Fabra).

2.2. Drugs

Pregabalin (generously provided by Laboratorios Dr. Esteve, Barcelona, Spain) was dissolved in physiological sterile saline solution (0.9%) and self-administered intravenously (i.v.) at 1.5 or 3 mg/kg/infusion in an infusion volume of 23.5 µl per injection. [These doses were selected on the basis of previous studies reporting behavioral effects of pregabalin in mice \(Vartanian et al., 2006; Wang et al., 2015\).](#) Higher pregabalin concentrations were avoided to prevent sedation or other possible non-specific effects that could interfere with operant responding. Thiopental was dissolved in saline and administered through the implanted catheter at a dose of 10 mg/kg.

2.3. Drug self-administration procedure.

Mice were first trained for operant food self-administration to facilitate drug self-administration to alleviate the spontaneous manifestations of the neuropathic pain model, as previously described (Bura et al., 2013) with some modifications. Briefly, mice were food-restricted for three days to reach 90% of their initial weight (Fig. 1). Then, mice were trained in operant chambers (model ENV-307A-CT, Med Associates Inc., Georgia, VT, USA) for 5 days (1 hour session per day) to acquire an operant behavior to obtain food pellets. A fixed ratio 1 schedule of reinforcement (FR1) was used during the whole training period, i.e., one nose-poke on the active hole resulted in the delivery of one reinforcer together with a stimulus-light for 2 sec (associated-cue). Nose-poking on the inactive hole had no consequence. Each session started with a priming delivery of one reinforcer and a timeout period of 10 sec after each reinforcer delivery, where no cues were presented and no reward was provided following active nose-pokes. Food self-administration sessions lasted 1 hour or until mice nose-poked 100 times on the active hole, whichever happened first. After completion of food training, mice underwent a partial sciatic nerve ligation (PSNL) or sham surgery, and 4 days later an i.v. catheter was implanted to allow i.v. self-administration. After catheter implantation, mice started the drug self-administration sessions on day 7. The food reinforcer was substituted by drug/saline infusions in operant training sessions. Self-administration sessions were conducted during 10 consecutive days, and mice received pregabalin (1.5 or 3 mg/kg) or saline under FR1. Sessions lasted 1 hour or until 60 active nose-pokes. Active and inactive nose-pokes were recorded after each session.

2.4. Partial Sciatic Nerve Ligation

PSNL was performed at mid-thigh level to induce neuropathic pain, as previously described (Bura et al., 2008; Malmberg and Basbaum, 1998). Briefly, mice were anaesthetized with

isoflurane (induction, 5%; surgery, 2%) and the sciatic nerve was exposed at the level of the mid-thigh of the right hind paw. At ~1 cm proximally to the nerve trifurcation, a tight ligature was created around 33–50% of the sciatic nerve using a 9–0 non-absorbable virgin silk suture (Alcon® Surgical Inc., Fort Worth, TX, USA), leaving the rest of the nerve undamaged. The muscle was then stitched with 6-0 silk (Alcon®Surgical Inc.), and the incision was closed with wound clips. Sham-operated mice underwent the same surgical procedure except that the sciatic nerve was not ligated.

2.5. Catheterization

Mice were implanted with indwelling i.v. silastic catheter, as previously reported (Soria et al., 2005). Briefly, a 6-cm length of silastic tubing (0.3-mm inner diameter, 0.64-mm outer diameter) (Silastic®, Dow Corning Europe, Seneffe, Belgium) was fitted to a 22-gauge steel cannula (Semat Technical Ltd., Herts, UK) that was bent at a right angle and then embedded in a cement disk (Dentalon Plus, Heraeus Kulzer, Wehrheim, Germany) with an underlying nylon mesh. The catheter tubing was inserted 1.3 cm into the right jugular vein and anchored with suture. The remaining tubing ran subcutaneously to the cannula, which exited at the midscapular region. All incisions were sutured and coated with antibiotic ointment (Bactroban, GlaxoSmithKline, Madrid, Spain).

2.6 Nociception

Hypersensitivity to noxious thermal stimuli and punctate mechanical stimulation were used as outcome measures of the neuropathic pain model. The behavioral manifestations of neuropathic pain were evaluated the day before, 3 and 6 days after the PSNL, as well as after the last drug self-administration session on day 16. Hypersensitivity to heat was assessed by evaluating the hind paw withdrawal latency in response to radiant heat with the plantar test

apparatus (Ugo Basile, Varese, Italy), as previously reported (Hargreaves et al., 1988). Punctate mechanical sensitivity was quantified by measuring the hind paw withdrawal response to von Frey filament stimulation through the up-down paradigm, as previously reported (Chaplan et al., 1994).

2.8 Depressive-like behavior

Depressive-like behavior was evaluated the day after the last drug self-administration session (day 17) using the tail suspension test (Steru et al., 1985). Briefly, mice were suspended 50 cm above the floor by means of an adhesive tape, placed approximately 1 cm from the tip of the tail. Two min after being hanged, time of immobility was quantified for 4 min. Mice were considered immobile only when they hung passively and motionless.

2.9 Statistics

Time courses of active and inactive operant responding were analyzed with a Repeated Measures ANOVA with “session” (session 4-10) and “nose poke” (active, inactive) as within-subject factors, and “dose” and “surgery” as between-subject factors, followed by Fisher’s least significant difference post-hoc test (LSD) when appropriate. Areas Under the Curve (AUCs) of time-course curves and depressive-like behavior were analyzed using a two-way ANOVA (surgery and treatment) followed by Fisher’s LSD. Nociceptive behavior (Plantar, von Frey) of nerve-injured and sham-operated mice were analyzed by a two-way repeated measures ANOVA (surgery and treatment), followed by Bonferroni post-hoc test. IBM SPSS 19 (SPSS Inc., Chicago, IL, USA) and GraphPad (GraphPad Software, La Jolla, CA, USA) were used to analyze the data, and differences were considered statistically significant when the p value was below 0.05.

3. Results

3.1. Pregabalin self-administration in sham-operated and nerve-injured mice.

Mice were first trained to self-administer food pellets in operant chambers to acquire an operant behavior maintained by food. This step was necessary for mice to reach interest for the active sensor before the surgery as previously reported (Bura et al., 2013). All groups of mice displayed similar operant responding for food, showing averages of 59 ± 2.5 active responses and 5 ± 0.5 inactive responses (Mean $\pm$ SEM) on the last day of the training (Fig. 2a, Shaded areas). After food training, mice were sham-operated or subjected to the PSNL surgery, and four days later jugular catheterization was conducted to allow operant drug self-administration (Fig. 1). Three days after catheter implantation, sham and PSNL mice were placed back in their chambers and started the i.v. self-administration sessions (1 hour/day, 10 days). In these sessions, the food reinforcer was substituted by saline, pregabalin 1.5 mg/kg/inf or pregabalin 3 mg/kg/inf. After the last self-medication session, all mice received a thiopental dose to check the patency of the catheter. This revealed 13, 10 and 10 mice preserving the catheter in sham groups receiving saline, pregabalin 1.5 and 3 mg/kg/inf, respectively, and 8, 6 and 13 mice in PSNL groups receiving the same drug treatments. Data from the first three days of drug self-administration were discarded to avoid the interference of food-maintained operant behavior. Analysis of the last 7 drug self-administration sessions using a repeated measures ANOVA followed by Fisher's LSD posthoc test, revealed that mice receiving pregabalin at 3 mg/kg/inf showed higher active responding than mice receiving saline, either when the PSNL injury was inflicted (Fig. 2a, sessions 4-5-6) or when the sham operation was conducted (Fig. 1a, session 7). Hence, on specific days, mice exposed to pregabalin 3 mg/kg/inf showed more active responding than mice exposed to saline (Fig. 2a). In addition, sham-operated mice receiving pregabalin 3 mg/kg/inf showed significantly less inactive nose pokes compared to sham mice receiving saline ($P < 0.05$, Figure 2), which suggests better

discrimination for the drug in sham mice receiving the high dose. Interestingly, mice receiving pregabalin 1.5 mg/kg/inf exhibited higher active responding when were sham-operated than being nerve-injured (Fig. 1a, session 4). Thus, both sham and PSNL-operated mice showed high active responding for pregabalin, albeit the difference vs. saline was more apparent in nerve-injured than in sham-operated mice (Fig. 2a).

Areas Under the Curve (AUCs) of drug self-administration curves for active responding were significantly higher in PSNL-injured mice exposed to pregabalin 3 mg/kg/inf than in PSNL mice exposed to saline (Fig. 2b). Sham-operated mice showed the same tendency, although the differences did not reach statistical significance (Fig. 2b). Interestingly, AUCs for inactive responding showed the opposite trend, with mice exposed to pregabalin 3 mg/kg/inf exhibiting less inactive responding than mice exposed to saline (Fig. 2b, N.S. differences). In agreement, the discrimination between active and inactive sensors was higher in PSNL-injured mice treated with either dose of pregabalin than in nerve-injured mice receiving saline, as shown by the AUCs of the discrimination indices $[(\text{Active} - \text{Inactive}) / (\text{Active} + \text{Inactive})]$ for the self-administration sessions (Fig. 2c). Sham-operated mice receiving pregabalin at the high dose showed a non significant trend for better discrimination (Fig. 2c). Hence, nerve injury markedly increased the ability to discriminate the effects of pregabalin.

The presence of a potential sedative effect of pregabalin could interfere with operant responding. Representation of the accumulated number of nose-pokes (active + inactive) along the 60-min self-administration sessions (Fig. 2d) showed similar levels of operant responding in saline-treated mice and mice treated with the different doses of pregabalin. This pattern of operant responding revealed the absence of sedative effects that could interfere operant responding at these doses of pregabalin.

3.2. Antinociceptive effects of pregabalin self-administration

The antinociceptive effects of pregabalin self-administration were evaluated using the plantar and the von Frey tests. Nociceptive responses were evaluated before the PSNL/sham surgery (day 0), after the surgery before drug self-administration (days 3 and 6 after PSNL/Sham), and after the last pregabalin self-administration session (day 16). Basal nociception (day 0) was similar in all groups of mice in the plantar and von Frey test (Fig. 3a and 3b).

Nerve injury consistently decreased withdrawal latency to heat stimulation in the ipsilateral paw on days 3, 6 and 16 ($***p < 0.001$ vs. sham-operated mice, two-way ANOVA followed by bonferroni post-hoc test for multiple comparisons, Fig. 3a). Pregabalin self-administration at 1.5 and 3 mg/kg/infusion significantly decreased the thermal hypersensitivity observed on days 3 and 6, showing a significant reduction in heat sensitivity compared to PSNL-mice treated with saline on day 16 (Fig. 3a, $^{\star}p < 0.05$ 1.5 mg/kg/infusion vs. saline and $^{\star\star}p < 0.01$ for 3 mg/kg/infusion vs. saline, two-way ANOVA). Thermal latencies of contralateral paws of all mice and ipsilateral paws of sham-operated mice remained stable thorough the whole experiment (Fig. 3a). Therefore, self-administration of pregabalin at 1.5 and 3 mg/kg/inf induced similar decreases in heat hypersensitivity associated to the nerve injury.

As expected, sciatic nerve injury also induced ipsilateral mechanical hypersensitivity (Fig. 3b). PSNL-operated mice experienced sharp decreases in mechanical thresholds, significant on days 3, 6 and 16 after the surgery (Fig. 3b, $***p < 0.001$ vs. sham-operated mice, two-way ANOVA). Pregabalin self-administration had antinociceptive effect when administered at 3 mg/kg/inf, significantly reducing on day 16 the mechanical sensitivity observed on days 3 and 6 (Fig. 3b, $^{\star\star\star}p < 0.001$ vs. nerve-injured mice receiving saline or $####p < 0.001$ vs. pregabalin at 1.5 mg/kg, 2-way RM ANOVA). Conversely, pregabalin at 1.5 mg/kg/inf was ineffective relieving mechanical hypersensitivity (Fig. 3b). Mechanical thresholds of contralateral paws and ipsilateral paws of sham-operated mice appeared stable thorough the complete experiment

(Fig. 3a). Hence, the effect of pregabalin **alleviating mechanical sensitivity** was present when the drug was self-administered at 3, but not at 1.5 mg/kg/inf.

3.3. Effects of pregabalin self-administration on emotional-like manifestations of the neuropathic pain model

The effects of pregabalin **self-administration** on depressive-like behavior associated to **the peripheral neuropathy** were evaluated in the tail-suspension test the day after discontinuation of the treatment. Nerve-injured mice treated with saline showed a significant increase of immobility time when compared to sham-operated mice treated with saline (Fig. 4, $^{**}p<0.01$). Conversely, nerve-injured mice treated with pregabalin at 3 mg/kg/inf showed immobility times similar to sham mice treated with saline. This emotional impairment was significantly reversed by pregabalin (3 mg/kg/inf) when compared to the depressive-like phenotype expressed by nerve-injured mice treated with saline (Fig. 4, $^{*}p<0.05$). Conversely, PSNL mice treated with pregabalin 1.5 mg/kg/inf still showed depressive-like behavior associated to the nerve injury (Fig. 4, $^{*}p<0.05$ vs. sham mice treated with saline). Overall, pregabalin administered at 3 mg/kg/inf reversed depressive-like behavior associated to the nerve-injury.

4. Discussion

This work describes the attenuation of the nociceptive and emotional-like manifestations of a neuropathic pain model in mice receiving an operant pregabalin self-administration with pregabalin to alleviate the spontaneous symptoms of this chronic pain. The self-administration paradigm showed that nerve-injured mice exposed to a pregabalin treatment exhibited higher active responding and discrimination indices than injured mice receiving saline, suggesting that pregabalin is able to elicit a drug-taking behavior associated to relief of spontaneous neuropathic pain. In agreement, a previous CPP/CPA study performed in rats with carrageenan-induced inflammation revealed that low doses of pregabalin (1 mg/kg) inhibited the aversion to chambers paired with the carrageenan treatment (Rutten et al., 2011). Importantly, these pregabalin doses inhibiting place aversion did not modify the behavior in subjects without pain. Although CPP/CPA and self-administration paradigms provide distinct information about reward and reinforcement (Bardo and Bevins, 2000; Wise, 2004), both methods represent indirect approaches to estimate the presence of spontaneous pain relief in chronic pain models (Mogil and Crager, 2004), suggesting that pregabalin has efficacy alleviating non-evoked spontaneous pain. In our study, mice without neuropathy also showed a significant self-administration of pregabalin at the high dose. Previous CPP studies investigating the presence of reward associated to pregabalin treatments failed to show such an effect after oral administration (Andrews et al., 2001). In contrast, intraperitoneal administration of high doses of pregabalin (3.16 and 10 mg/kg) induced preference for the chambers paired with the drug (Table 1; Rutten et al., 2011). In our operant experimental conditions, higher doses of pregabalin could not be evaluated due to the complexity of the experimental paradigm, since a possible non specific motor effect of such a high dose of pregabalin would certainly interfere with the performance of the operant responding. Our data also suggest that high doses of pregabalin could be reinforcing, independently of its pain-relieving effects. These reinforcing effects raise possible concerns about the potential abuse

liability of pregabalin. Indeed, several clinical studies are reporting increasing cases of abuse liability mainly in cases of concomitant opioid treatments (Baird et al., 2014; Häkkinen et al., 2014). Pregabalin has also been reported to modify the reinforcing effects of other drugs of abuse. Different studies described reduction of cocaine or alcohol self-administration in rats and humans treated with pregabalin (de Guglielmo et al., 2013; Stopponi et al., 2012). This reduced drug self-administration after pregabalin could be interpreted as a decrease of the dose needed to obtain the rewarding effects of the drug. Indeed, a percentage of abusers admit using gabapentinoids to potentiate the 'high' obtained from opioid drugs (Baird et al., 2014). Our self-administration experiments showed certain levels of active responding in saline-receiving mice. A participation of food craving on this active responding cannot be ruled out from our data, since food pellets were previously delivered by operating the same active sensor. However, the initial food operant training is mandatory on this experimental approach since mice will not primarily self-administer a drug to alleviate pain in these experimental conditions (Bura et al., 2013), in agreement with a previous research group that has also used similar operant approaches (Gutierrez et al., 2013). PNL mice showed a tendency to decrease operant responding (Fig. 2b). A general decrease in operant responding may be related to anhedonic effects or cognitive impairment induced by the neuropathic injury (La Porta et al., 2016). Interestingly, these effects did not reduce operant responding in nerve-injured mice exposed to pregabalin.

A partial reversal of thermal and mechanical hypersensitivity was shown after pregabalin self-administration. This is in agreement with a number of publications assessing the antinociceptive effects of pregabalin in neuropathic pain models in rodents (Field et al., 1999, 2006; Kremer et al., 2016; Nozaki-Taguchi et al., 2001; La Porta et al., 2016). It has been suggested that pregabalin can activate the descending noradrenergic system to alleviate both mechanical and heat sensitivity associated with the nerve injury (Takeuchi et al., 2007). In our study, inhibition of thermal sensitivity was found at high and low pregabalin doses (3 and 1.5

mg/kg/inf). Conversely, alleviation of mechanical hypersensitivity was only found after [self-administration](#) of the high pregabalin dose. Those differences are in line with other studies using mouse models of peripheral neuropathic pain suggesting distinct modulation of mechanical and thermal [hypersensitivity](#) after pregabalin administration (Takeuchi et al., 2007; Wang et al., 2015). Thus, knockout mice for $\alpha 2\delta$ receptors, the main site of action of pregabalin, showed increased neuropathic mechanosensitivity, whereas thermal hyperalgesia was unaffected (Patel et al., 2013). [In addition, pregabalin did not modify mechanical and thermal thresholds in sham-operated mice, as expected from previous studies showing unchanged nociceptive thresholds after different pregabalin treatments \(Kremer et al., 2016; La Porta et al., 2016\).](#)

[Pregabalin self-administration](#) at the high dose significantly decreased the emotional-like manifestations associated to neuropathic pain in nerve-injured mice. Pregabalin has shown preventive effect inhibiting depressive-like behavior after chronic restraint stress in rodents (Valente et al., 2012) and demonstrates clinical efficacy reducing ratings of anxiety and depression (Montgomery et al., 2008; Stein et al., 2008). Conversely, non-contingent intraperitoneal administration of pregabalin did not modify the depressive-like manifestations induced by the complex Freund's adjuvant or the PSNL [models of chronic pain](#) (Maciel et al., 2013; La Porta et al., 2016).

The present model of i.v. pregabalin [self-administration](#) may be more efficient mimicking clinical outcomes, since the active mouse operant responding required to alleviate the pain manifestations could lead to different motivational responses that would influence the final effects of the treatment. A trend for increased tail suspension immobility was also observed in sham-operated mice receiving pregabalin at the high dose. It needs to be noted that depressive-like behavior was evaluated the day after discontinuation of the treatment, and a paradoxical withdrawal response might have been elicited after the abrupt cessation of

pregabalin treatment. Clinical treatments have a taper phase in which pregabalin doses are gradually decreased, and normally report absence of withdrawal signs after treatments for neuropathic pain or generalized anxiety disorder (Feltner et al., 2003; Frampton and Foster, 2006; Owen, 2007; Pande et al., 2003; Rosenstock et al., 2004). This taper schedule could not be applied in our mouse model. Further studies are required to clarify the consequences at the emotional level of the withdrawal of a chronic pregabalin treatment.

Our results show that mice maintain a contingent pregabalin self-administration at a dose effective alleviating mechanical and thermal hypersensitivity. This dose of pregabalin inhibits the depressive-like manifestations associated to this [model of](#) chronic neuropathic pain and also shows reinforcing effects in mice not exposed to chronic pain. Conversely, a lower dose of pregabalin, able to reduce thermal hyper[nociception](#), but without effects on mechanical sensitivity or depressive-like behavior is insufficient to induce significant operant responding for the drug. These findings suggest that analgesic drug intake associated to pain relief depends not only on the alleviation of specific nociceptive responses, but rather on the inhibition of a multiplicity of symptoms that constitute the complexity of neuropathic pain syndromes. The multifaceted experimental approach used in this study allows the simultaneous evaluation of spontaneous pain relief, emotional and safety consequences of pregabalin treatment. The characterization of all these complementary aspects required for evaluating the effectiveness and safety of an analgesic treatment provides an important translational value of these preclinical findings and underlines the need of using these new experimental models to improve the predictive value of the preclinical data.

6. Acknowledgements

This work was supported by the European Commission, FP7 (#HEALTH-F2-2013- 602891), Spanish Ministerio de Economía y Competitividad-MINECO (#SAF2014- 59648-P) and Instituto de Salud Carlos III, RETICS-RTA (#RD12/0028/0023) and Generalitat de Catalunya, AGAUR (#2014-SGR-1547). We acknowledge the financial support of the AGAUR (ICREA Acadèmia Award 2015) to R.M. FEDER partial funding is also acknowledged. The authors would like to thank Roberto Cabrera and Cristina Fernández for their excellent technical expertise.

7. Author contributions

In this work, Dr. Bura conducted the experiments, Drs. Cabañero and Maldonado wrote the manuscript, and Drs. Bura and Maldonado conceived the experimental design.

References

- Andrews, N., Loomis, S., Blake, R., Ferrigan, L., Singh, L., McKnight, A. (2001). Effect of gabapentin-like compounds on development and maintenance of morphine-induced conditioned place preference. *Psychopharmacology (Berl)* 157, 381–387.
- Backonja, M.-M., Stacey, B. (2004). Neuropathic pain symptoms relative to overall pain rating. *J Pain* 5, 491–497.
- Baird, C.R.W., Fox, P., Colvin, L.A. (2014). Gabapentinoid abuse in order to potentiate the effect of methadone: a survey among substance misusers. *Eur Addict Res* 20, 115–118.
- Bardo, M.T., Bevins, R.A. (2000). Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl)* 153, 31–43.
- Bossard, J.-B., Ponté, C., Dupouy, J., Lapeyre-Mestre, M., Jouanjus, E. (2016). Disproportionality Analysis for the Assessment of Abuse and Dependence Potential of Pregabalin in the French Pharmacovigilance Database. *Clin Drug Investig* 36, 735–742.
- Bura, A.S., Guegan, T., Zamanillo, D., Vela, J.M., Maldonado, R. (2013). Operant self-administration of a sigma ligand improves nociceptive and emotional manifestations of neuropathic pain. *Eur J Pain* 17, 832–843.
- Bura, A.S., Nadal, X., Ledent, C., Maldonado, R., Valverde, O. (2008). A2A adenosine receptor regulates glia proliferation and pain after peripheral nerve injury. *Pain* 140, 95–103.
- 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.
- Evoy, K.E., Morrison, M.D., Saklad, S.R. (2017). Abuse and Misuse of Pregabalin and Gabapentin. *Drugs*.
- Feltner, D.E., Crockatt, J.G., Dubovsky, S.J., Cohn, C.K., Shrivastava, R.K., Targum, S.D., Liu-

Dumaw, M., Carter, C.M., Pande, A.C. (2003). A randomized, double-blind, placebo-controlled, fixed-dose, multicenter study of pregabalin in patients with generalized anxiety disorder. *J Clin Psychopharmacol* 23, 240–249.

Field, M.J., Cox, P.J., Stott, E., Melrose, H., Offord, J., Su, T.-Z., Bramwell, S., Corradini, L., England, S., Winks, J., Kinloch, R.A., Hendrich, J., Dolphin, A.C., Webb, T., Williams, D. (2006). Identification of the $\alpha 2$ -delta-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin. *Proc Natl Acad Sci U S A* 103, 17537–17542.

Field, M.J., McCleary, S., Hughes, J., Singh, L. (1999). *Pain* 80, 391–398.

Filipetto, F.A., Zipp, C.P., Coren, J.S. (2010). Potential for pregabalin abuse or diversion after past drug-seeking behavior. *J Am Osteopath Assoc* 110, 605–607.

Finnerup, N.B., Attal, N., Haroutounian, S., McNicol, E., Baron, R., Dworkin, R.H., Gilron, I., Haanpää, M., Hansson, P., Jensen, T.S., Kamerman, P.R., Lund, K., Moore, A., Raja, S.N., Rice, A.S.C., Rowbotham, M., Sena, E., Siddall, P., Smith, B.H., Wallace, M. (2015). Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol* 14, 162–173.

Frampton, J.E., Foster, R.H. (2006). Pregabalin: in the treatment of generalised anxiety disorder. *CNS Drugs* 20, 685-93-5.

Galan-Arriero, I., Avila-Martin, G., Ferrer-Donato, A., Gomez-Soriano, J., Bravo-Esteban, E., Taylor, J. (2014). Oral administration of the p38 α MAPK inhibitor, UR13870, inhibits affective pain behavior after spinal cord injury. *Pain* 155, 2188–2198.

de Guglielmo, G., Cippitelli, A., Somaini, L., Gerra, G., Li, H., Stopponi, S., Ubaldi, M., Kallupi, M., Ciccocioppo, R. (2013). Pregabalin reduces cocaine self-administration and relapse to cocaine

seeking in the rat. *Addict Biol* 18, 644–653.

Gutierrez, T., Crystal, J.D., Zvonok, A.M., Makriyannis, A., Hohmann, A.G. (2011). Self-medication of a cannabinoid CB2 agonist in an animal model of neuropathic pain. *Pain* 152, 1976–1987.

Häkkinen, M., Vuori, E., Kalso, E., Gergov, M., Ojanperä, I. (2014). Profiles of pregabalin and gabapentin abuse by postmortem toxicology. *Forensic Sci Int* 241, 1–6.

Halaby, A., Kassm, S.A., Naja, W.J. (2015). Pregabalin dependence: a case report. *Curr Drug Saf* 10, 184–186.

Hargreaves, K., Dubner, R., Brown, F., Flores, C., Joris, J. (1988). A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 32, 77–88.

Kremer, M., Yalcin, I., Nexon, L., Wurtz, X., Ceredig, R.A., Daniel, D., Hawkes, R.A., Salvat, E., Barrot, M. (2016). The antiallodynic action of pregabalin in neuropathic pain is independent from the opioid system. *Mol Pain* 12.

Maciel, I.S., Silva, R.B.M., Morrone, F.B., Calixto, J.B., Campos, M.M. (2013). Synergistic effects of celecoxib and bupropion in a model of chronic inflammation-related depression in mice. *PLoS One* 8, e77227.

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.

Mogil, J.S., Crager, S.E. (2004). What should we be measuring in behavioral studies of chronic pain in animals? *Pain* 112, 12–15.

Montgomery, S., Chatamra, K., Pauer, L., Whalen, E., Baldinetti, F. (2008). Efficacy and safety of pregabalin in elderly people with generalised anxiety disorder. *Br J Psychiatry* 193, 389–394.

- Nozaki-Taguchi, N., Chaplan, S.R., Higuera, E.S., Ajakwe, R.C., Yaksh, T.L. (2001). Vincristine-induced allodynia in the rat. *Pain* 93, 69–76.
- O'Connor, E.C., Chapman, K., Butler, P., Mead, A.N. (2011). The predictive validity of the rat self-administration model for abuse liability. *Neurosci Biobehav Rev* 35, 912–938.
- Owen, R.T. (2007). Pregabalin: Its efficacy, safety and tolerability profile in generalized anxiety. *Drugs of Today* 43, 601.
- Pande, A.C., Crockatt, J.G., Feltner, D.E., Janney, C.A., Smith, W.T., Weisler, R., Londborg, P.D., Bielski, R.J., Zimbrow, D.L., Davidson, J.R.T., Liu-Dumaw, M. (2003). Pregabalin in generalized anxiety disorder: a placebo-controlled trial. *Am J Psychiatry* 160, 533–540.
- Papazisis, G., Garyfallos, G., Sardeli, C., Kouvelas, D. (2013). Pregabalin abuse after past substances seeking behavior. *Int J Clin Pharmacol Ther* 51, 441–442.
- Papazisis, G., Tzachanis, D. (2014). Pregabalin's abuse potential: a mini review focusing on the pharmacological profile. *Int J Clin Pharmacol Ther* 52, 709–716.
- Patel, R., Bauer, C.S., Nieto-Rostro, M., Margas, W., Ferron, L., Chaggar, K., Crews, K., Ramirez, J.D., Bennett, D.L.H., Schwartz, A., Dickenson, A.H., Dolphin, A.C. (2013). $\alpha 2\delta$ -1 gene deletion affects somatosensory neuron function and delays mechanical hypersensitivity in response to peripheral nerve damage. *J Neurosci* 33, 16412–16426.
- [Patel, R., Dickenson, A.H. \(2016\). Mechanisms of the gabapentinoids and \$\alpha 2\delta\$ -1 calcium channel subunit in neuropathic pain. *Pharmacol Res Perspect*, 4: e00205.](#)
- La Porta, C., Lara-Mayorga, I.M., Negrete, R., Maldonado, R. (2016). Effects of pregabalin on the nociceptive, emotional and cognitive manifestations of neuropathic pain in mice. *Eur J Pain* 20, 1454–1466.
- Rosenstock, J., Tuchman, M., LaMoreaux, L., Sharma, U. (2004). Pregabalin for the treatment

of painful diabetic peripheral neuropathy: a double-blind, placebo-controlled trial. *Pain* 110, 628–638.

Rutten, K., De Vry, J., Robens, A., Tzschentke, T.M., van der Kam, E.L. (2011). Dissociation of rewarding, anti-aversive and anti-nociceptive effects of different classes of anti-nociceptives in the rat. *Eur J Pain* 15, 299–305.

Schjerner, O., Rosenzweig, M., Pottegård, A., Damkier, P., Nielsen, J. (2016). Abuse Potential of Pregabalin. *CNS Drugs* 30, 9–25.

Soria, G., Mendizábal, V., Touriño, C., Robledo, P., Ledent, C., Parmentier, M., Maldonado, R., Valverde, O. (2005). Lack of CB1 cannabinoid receptor impairs cocaine self-administration. *Neuropsychopharmacology* 30, 1670–1680.

Stein, D.J., Baldwin, D.S., Baldinetti, F., Mandel, F. (2008). Efficacy of pregabalin in depressive symptoms associated with generalized anxiety disorder: a pooled analysis of 6 studies. *Eur Neuropsychopharmacol* 18, 422–430.

Steru, L., Chermat, R., Thierry, B., Simon, P. (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)* 85, 367–370.

Stopponi, S., Somaini, L., Cippitelli, A., de Guglielmo, G., Kallupi, M., Cannella, N., Gerra, G., Massi, M., Ciccocioppo, R. (2012). Pregabalin reduces alcohol drinking and relapse to alcohol seeking in the rat. *Psychopharmacology (Berl)* 220, 87–96.

Takeuchi, Y., Takasu, K., Ono, H., Tanabe, M. (2007). Pregabalin, S-(+)-3-isobutylgaba, activates the descending noradrenergic system to alleviate neuropathic pain in the mouse partial sciatic nerve ligation model. *Neuropharmacology* 53, 842–853.

Valente, M.M., Bortolotto, V., Cuccurazzu, B., Ubezio, F., Meneghini, V., Francese, M.T., Canonico, P.L., Grilli, M. (2012). $\alpha 2\delta$ ligands act as positive modulators of adult hippocampal

neurogenesis and prevent depression-like behavior induced by chronic restraint stress. *Mol Pharmacol* 82, 271–280.

Vartanian, M.G., Radulovic, L.L., Kinsora, J.J., Serpa, K.A., Vergnes, M., Bertram, E., Taylor, C.P. (2006). Activity profile of pregabalin in rodent models of epilepsy and ataxia. *Epilepsy Res* 68, 189-205.

Wang, T.-X., Yin, D., Guo, W., Liu, Y.-Y., Li, Y.-D., Qu, W.-M., Han, W.-J., Hong, Z.-Y., Huang, Z.-L. (2015). Antinociceptive and hypnotic activities of pregabalin in a neuropathic pain-like model in mice. *Pharmacol Biochem Behav* 135, 31–39.

Wise, R.A. (2004). Dopamine, learning and motivation. *Nat Rev Neurosci* 5, 483–494.

Figure 1. Experimental procedure. Mice were food-restricted (days -9 to -2), and trained for 5 days to acquire an operant self-administration behavior for food (-6 to -2). Afterwards, mice were subjected to a PSNL or sham surgery (day 0). Following catheter i.v. implantation on day 4, mice were trained again in the operant chambers on day 7 to acquire an operant behavior maintained by i.v. infusions of pregabalin at 1.5 or 3 mg/kg or saline, during 10 days (from days 7 to 16). The thiopental test (i.v. injection of thiopental) served to check the patency of the catheter at the end (day 16). Mice were habituated to nociceptive tests the day before the PSNL/sham surgery. Nociceptive tests consisted on plantar and von Frey tests to measure heat and mechanical sensitivity, respectively. Nociception was evaluated before (days 0, 3, 6) and after the [drug self-administration](#) period (day 16). Depressive-like behavior was assessed the day after the last [self-administration](#) session (day 17) using the tail suspension test.

Figure 2. Reinforcing properties of pregabalin. a) Time-course of pregabalin self-administration in sham and PSNL-operated mice. Active (filled squares) and inactive nose-pokes (empty squares) are plotted for the last food training session (shaded areas) and for the last 7 drug self-administration sessions (white areas). Sham-operated mice receiving pregabalin at 3 mg/kg/inf showed higher active responding (Session 7) and lower inactive responding (Session 2) than sham mice receiving saline. Similarly, PSNL-operated mice treated with pregabalin 3 mg/kg/inf showed higher active responding than PSNL mice receiving saline (Sessions 4,5,6). On session 4, mice receiving pregabalin 1.5 mg/kg/inf showed higher active responding than PSNL mice receiving the same treatment. $^{\star}p<0.05$ Pregabalin vs. Saline; $^{\star}p<0.05$ PSNL vs. Sham; RM ANOVA followed by Fisher's LSD test. **b) Areas Under the Curve (AUCs) of drug self-administration time-course series for active (upper graph) and inactive responding (lower graph).** Overall active responding was significantly higher in PSNL-injured mice exposed to pregabalin than in PSNL mice exposed to saline ($^{\star}p<0.05$, two-way ANOVA followed by Fisher's LSD test). **c) AUCs of Discrimination Indices for the time-course series.** PSNL-injured mice treated with either those of pregabalin showed better discrimination indices than nerve injured mice receiving saline ($^{\star\star}p<0.01$, two-way ANOVA). **d) Cumulative number of nose-pokes for pregabalin during the last operant session (session 10).** The pattern of operant responding the 60-min sessions suggest absence of motor impairment or sedative effects associated with the doses of pregabalin tested.

Figure 3. Nociceptive manifestations before and after pregabalin self-administration. Time-courses show withdrawal latency to heat stimulation in the plantar test **(a)** and mechanical thresholds to von Frey filaments **(b)**. Measures are shown before PSNL/Sham surgery (day 0), after surgery before **drug self-administration** (days 3 and 6), and after surgery and **self-administration** (day 16). Values of ipsilateral (left panels) and contralateral paws (right panels) are plotted for each experimental group. **(a) Sensitivity to heat.** Plantar test showed a persistent decrease of the withdrawal latency to heat stimulation in ipsilateral paws of all PSNL-operated mice (days 3, 6 and 16, filled shapes, ** $p < 0.01$ or *** $p < 0.001$ vs. sham-operated mice, two-way ANOVA followed by bonferroni post-hoc test). After **drug self-administration** (day 16), PSNL-mice treated with pregabalin at 1.5 or 3 mg/kg/inf showed partial recovery of withdrawal latencies (* $p < 0.05$ and ** $p < 0.01$ vs. nerve-injured mice receiving saline). **(b) Mechanical sensitivity.** Von Frey filaments revealed a sharp decrease of mechanical thresholds in all PSNL-operated mice (days 3, 6 and 16, filled shapes, *** $p < 0.001$ vs. sham-operated mice). After **drug self-administration**, PSNL mice treated with pregabalin at 3 mg/kg/inf showed partial recovery (*** $p < 0.001$ vs. nerve-injured mice receiving saline or ### $p < 0.001$ vs. pregabalin at 1.5 mg/kg/inf).

Figure 4. Emotional-like manifestations of neuropathic pain after pregabalin self-administration. Times of immobility in the tail suspension test one day after termination of the operant drug self-administration. PSNL-operated mice treated with saline showed a significant increase of immobility compared to sham-operated mice receiving saline (** $p < 0.01$, 2-way ANOVA followed by Fisher's LSD test). PSNL-operated mice treated with pregabalin at 1.5 mg/kg/inf still showed increased depressive-like behavior (* $p < 0.05$). Conversely, nerve-injured mice treated with pregabalin 3 mg/kg/inf did not show depressive-like behavior (* $p < 0.05$ vs. PSNL mice treated with saline).

Fig. 1

Fig.2

		Pregabalin dose (mg/kg)						
Species	Condition	0.3	1	3	10	30	Administration Procedure	
Rat	No Pain (naïve)			No CPP			- Four drug-paired conditioning session - p.o. before session	Andrews et al., 2001
Rat	No Pain (i.pl. vehicle)		No CPP	CPP			- One drug-paired conditioning session - i.p. before session	Rutten et al., 2011
	Pain (i.pl. carrageenan)	No CPA inhibition	No CPP	CPP				
		No CPP	CPA inhibition	CPA inhibition				

Table 1. Summary of previous rodent studies exploring the ability of pregabalin to induce abuse or to alleviate spontaneous pain. Conditioned Place Preference (CPP) suggests abuse potential, whereas inhibition of Conditioned Place Aversion (CPA) would be indicative of pain relief. i.pl.: Intraplantar. p.o.: per os, oral. i.p.: intraperitoneal.