1. Title: Operant self-administration of a sigma ligand improves nociceptive and emotional manifestations of neuropathic pain

2. Running title: Sigma ligand and chronic pain

3. The authors' names: Andreea S.Bura[1], Thomas Guegan[1], Daniel Zamanillo[2], José M. Vela[2] and Rafael Maldonado[1]

4. [1]Laboratori de Neurofarmacologia, Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, C/Doctor Aiguader 80, 08003 Barcelona, Spain.

[2]Esteve, Drug Discovery and Preclinical Development, Av. Mare de Déu de Montserrat, 221, 08041 Barcelona,

Spain

5. Corresponding author : Dr. Rafael Maldonado, Laboratori de Neurofarmacologia, Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, PRBB Barcelona, Spain

Dr. Aiguader 88, 08003 Barcelona, Spain;

rafael.maldonado@upf.edu

Telephone: +34 933160824, fax: +34 933160901

6. The category for which the manuscript is being submitted: original article

7. Funding sources: S1RA was provided by Esteve as a gift within research projects funded by the CENIT program (CEN-20061005) from the Centro para el Desarollo Technológico Industrial from the Spanish Ministry of Science and Innovation (#SAF2007-64062), “Redes temáticas de investigación cooperativa en salud (RETICS) del Instituto de Salud Carlos III (RD06/001/001). Red de trastornos adictivos (RTA)”, the Catalan Government (SGR2009-00131), the ICREA Foundation (ICREA Academia-2008) and the DG Research of the European Commission (PHECOMP, #LSHM-CT-2007-037669).

8. DZ and JMV are employees of the company “Esteve”.

9. What's already known about this topic?

A recent study has demonstrated the acquisition of an operant behaviour to self-administer a CB2 agonist in rats exposed to neuropathic pain, which underlines the relevance of these operant methods for identifying novel analgesics that suppress spontaneous neuropathic pain with limited abuse liability in humans.

What does this study add?

The operant model described in the present study opens new perspectives given the possibility to evaluate the emotional component of pain and to use genetically modified mice to identify specific genes involved in the relationships between pain and addiction.

Abstract

Background

The treatment of neuropathic pain is unsatisfactory at the present moment and the sigma 1 receptor has been identified as a new potential target for neuropathic pain. The aim of this study was to use an operant self-administration model to reveal the potential interest of a new sigma 1 receptor antagonist, S1RA, in chronic pain that was developed in mice by a partial ligation of the sciatic nerve.

Methods

Once that chronic pain had reached a steady-state, mice were trained to maintain an operant behaviour to self-administer S1RA. The possible abuse liability of the analgesic compound was determined by evaluating operant self-administration in sham-operated mice. The influence of S1RA on the anhedonic state related to chronic pain was also evaluated by measuring the preference for palatable drink (2 % sucrose solution) using a recently validated and highly sensitive behavioural device.

Results

Nerve injured mice, but not sham-operated animals, acquired the operant responding to obtain S1RA (6 mg/kg/infusion). After 10 days of S1RA self-administration, neuropathic pain was significantly reduced in nerve injured mice. In addition, an anhedonic state was revealed in nerve injured mice by a decreased consumption of palatable drink, which was significantly attenuated by S1RA (25 mg/kg).

Conclusions

These results reveal the analgesic efficacy of the sigma antagonist, S1RA, in neuropathic pain associated to an improvement of the emotional negative state and that was devoided of reinforcing effects. The operant responses evaluated in this new mouse

model can have a high predictive value to estimate the clinical benefit/risk ratio of new analgesic compounds to treat chronic pain, such as S1RA.

Key words: sigma receptor, S1RA, self-administration, anhedonia, allodynia, hyperalgesia, self-medication

1. Introduction

Neuropathic pain is defined by the International Association for the Study of Pain (IASP) as a clinical entity initiated or caused by a primary lesion or dysfunction in the nervous system that is often associated with hyperalgesia, allodynia, spontaneous pain and emotional alterations. Several compounds are currently used to treat neuropathic pain (Banos et al., 2003; Dickenson and Ghandehari, 2007; Woolf and Mannion, 1999). However, these compounds have a limited efficacy and present side effects that can limit their use. Some of the most important side effects are abuse liability and sedative effects that represent serious limitations for the clinical use of multiple analgesic compounds. Nowadays severe pain treatment remains an open issue to deal with and there is an urgent need for more effective drugs and new animal models with high predictive value to evaluate the analgesic and side effects of new compounds.

The relationships between chronic pain and addiction are largely recognised (Ballantyne and LaForge, 2007; Flugsrud-Breckenridge et al., 2007). Both chronic pain and addiction involve sensitization and synaptic plasticity, which alter the responses of the nerve circuits to sensory inputs, including the painful stimuli (Ji et al., 2003). At present, the most reliable technique to evaluate the relationships between chronic pain and addiction in preclinical research is the operant drug self-administration paradigm. Thus, chronic pain altered drug self-administration in rats (Colpaert et al., 2001; Lyness et al., 1989), and heroin and methadone were more effective in maintaining self-administration when administered at analgesic doses in rats exposed to neuropathic pain, whereas lower doses were similarly self-administered in both nerve-injured and control animals (Martin et al., 2007). A recent study has demonstrated the acquisition of an operant behaviour to self-administer a CB2 agonist in rats exposed to neuropathic pain, which underlines the relevance of these operant methods for identifying novel

analgesics that suppress spontaneous neuropathic pain with limited abuse liability in humans (Gutierrez et al., 2011). A possible operant model developed in mice could open new perspectives given the possibility to identify specific genes involved in the relationships between pain and addiction thanks to the new lines of genetically modified mice now available.

Emotional alterations such as depressive-like symptoms are often associated with chronic pain (Campbell et al., 2006). Pain can cause or worsen symptoms or depression and similarly, depression may amplify the pain experience leading to a decrease in life quality (Campbell et al., 2006; Ong and Keng, 2003). Because depression may interfere with pain treatment an appropriate medication of both emotional and painful symptoms is essential to improve the quality of life of patients with chronic pain.

The involvement of the sigma 1 receptor (σ1R) in pain modulation has received a particular attention in the last years. σ1Rs are expressed in key areas involved in pain control, such as the superficial layers of the dorsal horn, periaqueductal gray matter, locus coeruleus and rostroventral medulla (Diaz et al., 2009). Genetical studies using σ1R knockout mice and pharmacological blockade of these receptors revealed decrease manifestations of neuropathic pain after partial sciatic nerve ligation (PSNL) (de la Puente et al., 2009). Furthermore, σ1Rs exert a modulatory role on NMDA receptors, a key receptor involved in central sensitization associated to chronic pain (Kim et al., 2006; Kim et al., 2008). In agreement, the σ1R antagonist S1RA, inhibits neuropathic pain and neuronal activity-induced nociceptive sensitization in mice (Romero et al., 2012).

The aim of this study was to evaluate the effects of the σ1R antagonist S1RA in the nociceptive and emotional manifestations of neuropathic pain, and the motivation of mice to obtain this analgesic compound using a new operant model of drug self-

administration that can be useful to evaluate the therapeutic potential of novel compounds for neuropathic pain. Chronic pain was developed by a PSNL and mice were trained to maintain an operant behaviour to self-administer S1RA once that pain reached a steady-state. The possible abuse liability of the compound was identified by evaluating self-administration behaviour in sham-operated mice. The anhedonic state related to chronic pain was also evaluated by measuring the preference for palatable drink using a recently described and highly sensitive behavioural device (Bura et al., 2010).

2. Materials and methods

2.1 Animals

C57BL/6 male mice (Charles River, France) weighting 22–24 g at the beginning of the experiments were used. Mice were housed individually in a temperature (21 ± 1°C) and humidity-controlled (55 ± 10%) room. Mice were tested during the dark phase of a 12 h light/dark reverse cycle (light off at 08:00 AM, light on at 8:00 PM). Food and water were available *ad libitum* except during the training for the food maintained operant behaviour, when mice were exposed to a restricted diet. Mice were isolated in individual cages, habituated to their new environment and handled for 1 week before starting the experimental procedure. All experimental procedures were conducted according to standard ethical guidelines (European Community Guidelines on the Care and Use of Laboratory Animals 86/609/EEC) and approved by the local ethical committee (Comité Etico Experimental Animal–Instituto Municipal de Asistencia Sanitaria/Universitat Pompeu Fabra). The observer was blind to treatment in all the experiments.

2.2 Drugs

The σ1R antagonist, S1RA, (Laboratorios Dr. Esteve, Barcelona, Spain) was dissolved in physiological sterile saline solution (0.9%) and administered intravenously (i.v.) in the self-administration paradigm and intraperitonealy (i.p.) in the behavioural paradigm used to evaluate anhedonia.

2.3 Operant model to evaluate analgesic effects in neuropathic pain

Mice were first trained to acquire an operant behaviour to obtain food. In a second step, mice were operated for the PSNL and then intravenous catheters were implanted to

allow training the animals to acquire an operant responding maintained by drug self-administration. The nociceptive behavioural tests were performed as indicated in Figure 1.

2.3.1 Acquisition of an operant responding maintained by food

After 7 days of habituation, mice were food deprived during 3 days until reaching 85% of their initial weight. The same food deprivation regime was maintained during the whole period of evaluation of food-operant behaviour. Water was available *ad libitum* during the whole experiment. Three days after starting food deprivation, mice were trained in mouse operant chambers (Model ENV-307A-CT, Med Associates, Georgia, VT, USA) to nose-poke for food pellets, as previously reported (Barbano et al., 2009; Soria et al., 2005). The experimental chambers were made of aluminium and acrylic, had grid floors (EVV-414, Med. Associates Inc., St Albans, USA), and were housed in sound and light-attenuated boxes equipped with fans to provide ventilation and white noise. The chambers contained two manipulanda (holes of 1.2 cm diameter), one was selected as active hole for delivering the reinforcer and the other as inactive hole. Nose-poking in the active hole resulted in a pellet delivery (standard pellet) together with a stimulus-light during two sec (associated-cue), while nose-poking in the inactive hole had no consequences. A food dispenser equidistant between the two holes permitted delivery of food pellets when required. The beginning of each operant responding session was signalled by turning on a house light placed on the ceiling of the box for 3 sec that was then turn off during the remaining duration of the session. The side of the active and inactive hole was counterbalanced between animals. Mice were trained during 10 days under a FR1 schedule of reinforcement,-i.e., one nose-poke resulted in one food pellet delivery whilst inactive nose-poking had no consequences. Each session

started with a priming delivery of one pellet. A time-out period of 10 sec was established after each pellet delivery where no cues were presented and no reward was provided following an active nose-poke. Responses on the inactive hole and all the responses performed during the 10 sec time-out period were also recorded. The session was finished after 100 reinforces were delivered or after 1 hour whichever occurred first. After the last session of food maintained operant behaviour, food and water were available *ad libitum* during the remaining phases of the experiment.

2.3.2 Partial sciatic nerve ligation

Next day after the last session of food operant training, animals were habituated for the nociceptive assays used to evaluate neuropathic pain, 2 hours to each different experimental test. The following day, basal values were measured and then a partial ligation of the sciatic nerve was performed at mid-thigh level to induce neuropathic pain, as previously described (Bura et al., 2008; Castane et al., 2006; Malmberg and Basbaum, 1998). Briefly, mice were anaesthetized with isofluorane (induction, 5%; surgery, 2%) and the common 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 18-inch (9–0) non-absorbable virgin silk suture (Alcon® surgical, Texas, USA), leaving the rest of the nerve 'undamaged'. The muscle was then stitched, and the incision was closed with wound clips. Sham-operated mice underwent the same surgical procedure except that the sciatic nerve was not ligated.

2.3.3 Nociceptive behavioural tests

Hyperalgesia to noxious thermal stimulus and allodynia to cold and mechanical stimuli were used as outcome measures of neuropathic pain. The behavioural manifestations of neuropathic pain were evaluated the day before and 3 days after the PSNL, as well as the day before and 11 days after drug self-administration by using the following behavioural models:

2.3.3.1 Plantar test

Thermal hyperalgesia was assessed in the plantar test (Ugo Basile, Varese, Italy) by measuring paw withdrawal latency in response to radiant heat as previously reported (Malmberg and Basbaum, 1998). Mice were placed in Plexiglas® cylinders (20 cm high, 9 cm diameter) positioned on a glass surface and were habituated to the environment for 30 min before testing in order to allow an appropriate behavioural immobility. The mean paw withdrawal latencies for the ipsilateral and contralateral hind paws were determined from the average of 3 separate trials, taken at 5-10 min intervals to prevent thermal sensitization and behavioural disturbances. A cut-off time of 20 sec was used to prevent tissue damage in absence of response.

2.3.3.2 Von Frey paradigm

Mechanical allodynia was quantified by measuring the hind paw withdrawal response to von Frey filament stimulation, as previously reported (Chaplan et al., 1994). Briefly, animals were placed in Plexiglas® cylinders (20 cm high, 9 cm diameter) positioned on a grid surface through which the von Frey calibrated filaments (North Coast Medical, Inc., San Jose, CA, USA) were applied by using the up-down paradigm. The threshold of response was then calculated by using the *up-down Excel* program generously

provided by Dr. A. Basbaum (UCSF, San Francisco, USA). Clear paw withdrawal, shaking or licking was considered as nociceptive-like response. Both ipsilateral and contralateral hind paws were tested.

2.3.3.3 Cold plate test

Thermal allodynia to a cold stimulus was assessed by using the hot/cold-plate analgesia meter (Colombus, OH, USA), as previously described (Bennett and Xie, 1988). The number of elevations of each hind paw was recorded in the mice exposed to the cold plate (5 ± 0.5 C) during 5 min. A score was calculated for each animal as the difference of number of elevations between ipsilateral and contralateral paw.

2.3.4 Acquisition of drug self-administration

Three days after sciatic nerve surgery and following the evaluation of the behavioural manifestations of neuropathic pain, mice were implanted with indwelling intravenous silastic catheter, as previously reported (Soria et al., 2005). Briefly, a 6-cm length of silastic tubing (0.3 mm inner diameter, 0.6 mm outer diameter) (Silastic®, Dow Corning, Houdeng-Goegnies, Belgium) was fitted to a 22-gauge steel cannula (Semat, 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 exits at the midscapular region. All incisions were sutured and coated with antibiotic ointment (Bactroban, GlaxoSmithKline, Madrid, Spain). Food and water were available *ad libitum* during this experimental phase. Drug self-administration sessions were conducted for 10 consecutives days as describe above for food-maintained responding, except that responses were maintained by drug delivery, S1RA 6 mg/kg/infusion in a volume of 23.5 µl over 2 sec. Stable acquisition of self-administration

behaviour was achieved when mice followed all the next criteria for at least three consecutive sessions: (1) less than 20% of deviation from the mean of the total number of responses in active hole (80% of stability), (2) 85% of discrimination between holes, (3) a minimum of four infusions per session. After 10 days of drug self-administration, mice were tested on a progressive ratio (PR) schedule. In this schedule, the first active nose-poke resulted in one drug delivery. The second drug infusion is delivered after two nose-pokes in the active hole and the number of nose-pokes necessary to get one infusion is progressively increasing escalated according to the following series: 1-2-3-5-12-18-27-40-60-90-135-200-300-450-675-1000. The breaking point, defined as the last number of nose-poke necessary to get one drug-injection completed before self-administration behaviour extinguished in a 2-hours session, was determined for each animal once. Only the values of mice that reached the acquisition criteria were considered. After the PR session neuropathic pain manifestations were measured and subsequently, the patency of i.v. catheters was evaluated by infusion of 0.1 ml of tiobarbital (Figure 1). Animals in which the patency of catheter was negative were removed.

2.4 Anhedonia model

A different group of mice was individualized and habituated in a room with a reversed light/dark cycle with food and water *ad libitum*. The anhedonic state associated to chronic pain and the effects of S1RA treatment on this emotional response were evaluated using a new food and drink monitoring system recently validated in the laboratory (http://www. panlab.com/ panlabWeb/ Hardware/ php/ display Hard.php? campo=Metabolism&nameHard =PHECOMP) that allows to evaluate with an extremely high sensitivity (less than 0.02 g for both food and drink) the preference for a palatable food or/and drink (Bura et al., 2010). Each animal cage of this food and drink

monitoring system is associated to 4 external units for food and drink registering (2 drink dispensers and 2 food dispensers). In our study, two different kinds of drinking solutions were used in the drink dispensers: water and sucrose solution 2%. The anhedonic state was evaluated by measuring the preference for sucrose solution 2% as a palatable drink of mice exposed to neuropathic pain (Figure 7). After one week of habituation to the reversed light/dark cycle, mice were subjected to four sessions (4 hours each) during 1 week (days 1, 3, 5 and 7) in the monitoring boxes in order to be familiarised with the new environment and drink taste. After each session, mice were replaced to their home cage. After this habituation period, baseline values of drink intake were measured. Mice were deprived for 2 hours before starting the 4 hours session in the monitoring box. One day after the baseline measurement, mice were exposed to PSNL or sham-operation and were then subjected every second day to the 2 hours deprivation followed by the 4 hours session in the monitoring box during 16 days. The chronic treatment with S1RA started 7 days after PSNL. Mice were injected twice daily (at 8:00 AM and 8:30 PM) with S1RA (25 mg/kg i.p) or saline during 10 days. On the days of testing (figure 6), mice received the morning injection 30 min before starting the 4 hours session and the evening injection immediately before the light period of the reversed cycle started. This dose and schedule of chronic administration of S1RA has been reported to reduce the nociceptive manifestations of neuropathic pain in mice (Romero et al., 2012).

2.5 Statistical analysis

Data obtained in the plantar test, cold plate test and von Frey filament paradigm were compared each experimental day by using two-way ANOVA (surgery and treatment as between factors of variation), followed by Newman Keuls *post hoc* comparisons when

required. The same statistical analysis was used for the data obtained the last day of drug self-administration, the PR and the mean of 10 days of sucrose preference on the anhedonia model. Data obtained in food self-administration paradigm were analysed using three-way ANOVA with repeated measures (surgery and treatment as between-subjects factors and day as within-subjects factor of variation). Pearson's χ^2 test was used to compare the percentage of sham and PSNL mice receiving saline or S1RA that acquired the criteria. The differences between means were considered statistically significant when the p value was below 0.05. SPSS statistical package was used.

3. Results

3.1 Acquisition of an operant responding maintained by food

Three-way ANOVA, calculated for number of nose-pokes for the active and inactive hole, showed significant main effects of day ($F_{(9, 342)} = 19.288$, $p < 0.001$; and $F_{(9,342)} = 19.964$, $p < 0.001$ respectively), no effect of surgery ($F_{(1, 38)} = 2.405$, N.S.; and $F_{(1, 38)} = 0.863$, N.S. respectively) or treatment ($F_{(1, 38)} = 0.653$, N.S.; and $F_{(1, 38)} = 1.359$, N.S. respectively) and no interaction among these factors ($F_{(9, 342)} = 0.540$, N.S.; and $F_{(9,342)} = 0.709$, N.S. respectively). The absence of significant effect of the factors surgery and treatment reveals that all groups were homogenous at the beginning of the drug self-administration training (data not shown).

3.2 Mice exposed to PSNL self-administer S1RA

On day 10 of drug self-administration, the number of operant responses to obtain S1RA (6 mg/kg/infusion) was significantly enhanced in PSNL mice compared to sham-operated mice trained to obtain the same treatment. Indeed, two-way ANOVA (treatment and surgery as between-subjects factors) revealed a significant effect of treatment ($F_{(1,38)} = 6.877$, $p < 0.05$) and surgery ($F_{(1,38)} = 4.933$, $p < 0.05$). *Post hoc* Newman Keuls revealed that the number of responses of animals self-administering S1RA (6 mg/kg/infusion) was significantly higher in PSNL mice when compared to sham-operated mice trained to obtain the same treatment ($p < 0.05$). Significant differences were also observed between mice with PSNL self-administering S1RA (6 mg/kg/infusion) or saline ($p < 0.05$) (Figure 2A). No differences were observed between sham-operated groups, or between groups that received saline.

No differences were observed in the responses in the inactive hole. Thus, two-way ANOVA revealed no significant effect of treatment ($F_{(1,38)}$ = 1.627, N.S.) or surgery ($F_{(1,38)}$ = 0.289, N.S.) (Figure 2B).

Mice were tested on PR schedule 11 days after PSNL. Two-way ANOVA did not reveal significant differences between the different experimental groups. In sham-operated animals, the breaking point values were similar for mice self-administering S1RA or saline (3.9 ± 2.7 and 5 ± 5), whereas these values were higher in PSNL mice self-administering S1RA (6.2±2.7) then in those PSNL mice receiving saline (0.22 ± 0.22). The percentage of sham and PSNL mice receiving saline or S1RA that acquired criteria was compared using the Pearson's χ^2 test. χ^2 showed in the percentage of sham-operated mice that reached the acquisition criteria was similar when self-administering S1RA (6 mg/kg/infusion) or saline (χ^2 = 2.800, p = N.S.). In contrast, the percentage of PSNL mice that reached the acquisition criteria was different in animals self-administering S1RA (6 mg/kg/infusion) or saline (χ^2 = 7.500, $p < 0.01$).

3.3 S1RA self-administration decreases neuropathic pain manifestation after intravenous

3.3.1 Mechanical allodynia was significantly decreased after S1RA self-administration

Sciatic nerve injury led to a profound decrease of the threshold for evoking withdrawal of the hind ipsilateral paw to a mechanical stimulus and this response was significantly attenuated in animals that have self-administered S1RA (6 mg/kg/infusion) (Figure 3). Baseline values were similar in all the groups, as revealed by two-way ANOVA calculated for ipsilateral and contralateral paw (Table 1). Nerve injury led to a significant decrease of the threshold for evoking paw withdrawal to mechanical

stimulation on the injured side, as revealed by two-way ANOVA (Table 1). The threshold for evoking withdrawal of the ipsilateral paw to a mechanical stimulus was significant on day 3 ($p < 0.001$), day 6 ($p < 0.001$) and day 17 ($p < 0.001$ for saline group and $p < 0.05$ for 6 mg/kg/infusion group) after surgery when compared to sham-operated mice (*post hoc* Newmann Keuls). However, a significant decrease of mechanical allodynia was observed on day 17 after PSNL in mice self-administering S1RA when compared to the saline group ($p < 0.001$ *post hoc* Newmann Keuls). Withdrawal latencies of the contralateral paw were not modified in any experimental group during the whole experiment (Table 1).

3.3.2 Hyperalgesia was significantly decreased after S1RA self-administration

Sciatic nerve injury decreased ipsilateral paw withdrawal latency to thermal stimulus and this response was significantly attenuated in animals that have self-administered S1RA (6 mg/kg/infusion) (Figure 4). Baseline values in ipsilateral paw and contralateral paw were similar in all the groups, as revealed by two-way ANOVA (Table 1). A marked decrease of the paw withdrawal latencies was observed in the ipsilateral paw of mice exposed to sciatic nerve injury, as showed by two-way ANOVA (Table 1). Comparison of paw withdrawal latencies for ipsilateral side between sham and PSNL animals were significant on day 3 ($p < 0.001$) and day 6 ($p < 0.001$) after surgery, (post hoc Newmann Keuls). On day 17, a decrease in paw withdrawal latency to thermal stimulus was only revealed in mice self-administering saline ($p < 0.01$) when compared with sham-operated animals, and this response was abolished in mice receiving the S1RA (N.S.). The comparison between PSNL mice self-administering S1RA or saline was also significant on day 17 ($p < 0.05$, post hoc Newman Keuls).

Withdrawal latencies of the contralateral paw were not modified in any of the experimental groups (Table 1).

3.3.3 Thermal allodynia was significantly reduced in mice self-administering S1RA

Sciatic nerve injury enhanced the score values (see materials and methods and table 2 for ipsilateral and contralateral values) obtained during the cold thermal stimulation, as revealed by two-way ANOVA (table 1). A significant difference of the score values was displayed in animals exposed to PSNL when compared with sham-operated animals (*post hoc* Newmann Keuls) on day 3 ($p < 0.001$) both groups and day 6 in mice receiving saline ($p < 0.01$) or S1RA (6 mg/kg/infusion) ($p < 0.05$). On day 17, a significant increase in the score values of PSNL mice receiving saline ($p < 0.01$) was observed when compared with sham-operated animals (*post hoc* Newmann Keuls). This response was suppressed in mice self-administering S1RA (N.S.), and the response of these mice was significantly different compared with PSNL mice receiving saline ($p < 0.05$, *post hoc* Newman Keuls). Baseline score values were similar in all these groups, as showed by two-way ANOVA (Table 1) (Figure 5).

3.4 Chronic S1RA administration improved the anhedonic state associated to neuropathic pain

Neuropathic pain induced an anhedonic-like state in animals treated with saline that were previously exposed to PSNL, as revealed by the decrease of sucrose preference in a free choice paradigm. Chronic non-contingent administration of S1RA (6 mg/kg/infusion) during 10 days improved this emotional deficit. Indeed, two-way ANOVA (treatment and surgery) of the mean of sucrose preference during the whole treatment period (10 days) revealed a significant effect of surgery ($F_{(1,44)} = 6.025$, $p <$

0.05) and treatment ($F_{(1,44)} = 7.813$, $p < 0.01$). Subsequent *post hoc* analysis (Newmann Keuls) indicated that animals exposed to nerve injury receiving saline had a lower preference for sucrose ($p < 0.01$) compared with sham-operated animals. The anhedonic-like state disappeared in mice receiving S1RA exposed to neuropathic pain. Indeed, mice previously exposed to nerve injury that received S1RA twice daily during 10 days (25 mg/kg), showed a higher preference for sucrose ($p < 0.05$) compared with the corresponding PSNL saline group (Newman Keuls *post hoc*) (Figure 7).

4. Discussion

In this study, we revealed the attenuation of the nociceptive and emotional manifestations of neuropathic pain in mice that have acquired an operant behaviour to self-administer the σR1 antagonist, S1RA. σR1 has been previously reported to be an interesting target for the treatment of chronic pain. Indeed,σ1R antagonism at the spinal cord level inhibits spinal excitability following prolonged noxious stimulation, such as capsaicin or formalin administration (Cendan et al., 2005; Entrena et al., 2009), and persistent abnormal afferent by sciatic nerve injury (Diaz et al., 2009; Romero et al., 2012). Furthermore, the analgesic effects reported on neuropathic pain manifestations by the pharmacological blockade of σ1R with S1RA are consistent with the role of σ1Rs in the central sensitization and pain hypersensitivity (Romero et al., 2012).

One of the most important side effects that can limit the use of pharmacological compounds to treat neuropathic pain is the potential abuse liability. In addition, sedative effects represents a serious limitation for some of the drugs currently used to treat neuropathic pain, such as the gabapentinoids and the serotonin norepinephrine reuptake inhibitors (Dworkin et al., 2007; Finnerup et al., 2005). Both potential side effects can be easily evaluated for novel therapeutic agents in this operant model of contingent self-administration of analgesic compounds.

The relationships between chronic pain treatment and addiction are largely recognised and are difficult to be explored in the currently available behavioural models (Ballantyne and LaForge, 2007; Flugsrud-Breckenridge et al., 2007). Studies using a yoking procedure have demonstrated that the neurobiological consequences of drug intake can differ depending on whether the animal self-administers the drug or if the drug is passively administered (Martin and Ewan, 2008). In this sense, drug self-administration, unlike non-contingent infusions, merge many additional features of the

drug different from its analgesic properties, including reinforcing effects and the consequent motivation to seek for the drug. Our operant model allows the animal exposed to the chronic neuropathic pain to seek for drug delivery in order to alleviate the pain, but also permits to evaluate the possible reinforcing effects of the drug by comparing the operant responses between sham-operated and animals exposed to neuropathic pain. Mice were first trained to acquire a food-maintained operant behaviour and neuropathic pain was then developed by PSNL. Once that chronic pain had reached a steady-state, mice were trained to maintain an operant behaviour to self-administer the new analgesic compound, S1RA, which has been previously reported to alleviate neuropathic pain manifestations (Romero et al., 2012). The sciatic nerve ligation led to a neuropathic pain syndrome characterised by mechanical and thermal allodynia, and thermal hyperalgesia present from the first day of measurement and maintained during the whole experimental sequence. Intravenous self-administration of S1RA decreased thermal and mechanical allodynia and thermal hyperalgesia induced by neuropathic pain. These results are in accordance with the analgesic effects reported on the manifestation of neuropathic pain after the non-contingent administration of this σ1R antagonist (Romero et al., 2012). In contrast, previous studies showed that thermal hyperalgesia during neuropathic pain was not modified after the decrease of σR1 activity (Roh et al., 2008). The use of different route of administration (intrathecal) and neuropathic pain model (chronic constriction injury) could explain these differences since different neurobiological systems are involved in pain manifestations in these two models (Malmberg and Basbaum, 1998).

Preliminary results obtained in our laboratory have revealed that a previous operant training to seek for food is necessary in order to obtain a contingent self-administration of analgesic compounds under chronic pain states (data not shown). Therefore, the

responses of the animals during the first days of drug self-administration were influenced by the previous acquisition of the food maintained operant behaviour and cannot be considered for the analysis of the drug operant responding. On the last day of training, mice that developed neuropathic pain maintained an operant behaviour to self-administer the σR1 antagonist. The self-administration of this analgesic alleviates neuropathic pain, as revealed by the significant attenuation of both hyperalgesia and allodynia on the last day of training to self-administer S1RA. In contrast, control mice exposed to sham surgery did not maintain such an operant behaviour to self-administer the sigma ligand. These results clearly reveal that mice exposed to chronic neuropathic pain maintained an operant behaviour to self-administer the sigma ligand in order to alleviate pain. No differences in the percentage of acquisition of operant responding to obtain saline or S1RA were found in sham-operated animals, whereas, the acquisition of mice exposed to neuropathic pain was higher when trained to obtain S1RA in comparison with saline. These results revealed that sham-operated animals display the same incentive to seek for the S1RA than saline, whereas this incentive to seek the σ1R antagonist was enhanced in mice exposed to neuropathic pain.

The present operant self-administration model also permits to assess the possible sedative effects of the analgesic compounds, another common limitation of the current analgesic compounds, by evaluating the number of responses in the inactive-hole of mice self-administrating the compound. In the present study, no differences in the number of inactive-hole responses were revealed when comparing the different groups self-administering S1RA or saline. These results revealed that the self-administration of S1RA did not impair locomotor activity and did not produce behavioural disturbances that would prevent the mice from nose-poking or maintaining activity to obtain drug infusion.

Chronic pain is often associated to several emotional alterations, such as depressive-like symptoms (Campbell et al., 2006) that impair the quality of life and difficult the therapeutic approach (Ong and Keng, 2003). Therefore, an appropriate treatment of both emotional components and painful symptoms may improve additional beneficial effects in chronic pain patients (Campbell et al., 2006). The consequences of non-contingent S1RA administration on the anhedonic state induced by the sciatic nerve ligation were also evaluated. This emotional component was assessed by measuring the preference for sucrose solution in a highly sensitive monitoring device recently validated (Bura et al., 2010). A non-contingent administration of the analgesic compound is required to perform this complex experimental procedure. Indeed, the experimental paradigm cannot be used together with operant self-administration procedures since animals would be daily exposed to two different environmental conditions that can promote stress and difficult the responses of mice in both experimental paradigms. In addition, food deprivation used for the evaluation of the anhedonic state could interfere with mice responses in the self-administration model (Carr, 2002). The consumption and preference for highly palatable sweet solutions have been reported to be decreased during anhedonic states (Papp et al., 1991; Strekalova and Steinbusch, 2010). In agreement, we found that anhedonia induced by the exposure to neuropathic pain diminishes the sucrose intake in control mice receiving saline. Non-contingent administration of S1RA improved this emotional response in mice exposed to chronic neuropathic pain. Indeed, animals exposed to PSNL receiving S1RA displayed the same preference for sucrose solution as sham-operated mice. These data show that S1RA improves both nociceptive and emotional manifestations of neuropathic pain pointing to the high potential interest of this new compound for the treatment of chronic pain.

Our results revealed the analgesic efficacy of the σR1 antagonist, S1RA, in the treatment of neuropathic pain using a new operant model of self-administration of the analgesic compound. S1RA was devoided of reinforcing and sedative effects in this operant paradigm. This analgesic effect was observed together with an improvement of the emotional consequences associated to the presence of chronic pain. The operant responses evaluated in this mouse operant animal model have a high predictive value to estimate the clinical benefit/risk ratio of new analgesic compounds to treat chronic pain, such as S1RA.

Acknowledgements

S1RA was provided by Esteve as a gift within research projects funded by the CENIT program (CEN-20061005) from the Centro para el Desarollo Technológico Industrial from the Spanish Ministry of Science and Innovation (#SAF2007-64062), "Redes temáticas de investigación cooperativa en salud (RETICS) del Instituto de Salud Carlos III (RD06/001/001). Red de trastornos adictivos (RTA)", the Catalan Government (SGR2009-00131), the ICREA Foundation (ICREA Academia-2008) and the DG Research of the European Commission (PHECOMP, #LSHM-CT-2007-037669).

All authors discussed the results and commented on the manuscript.

Reference List

Ballantyne,J.C., and LaForge,K.S. (2007). Opioid dependence and addiction during opioid treatment of chronic pain. Pain *129*, 235-255.

Banos,J.E., Sanchez,G., Berrendero,F., and Maldonado,R. (2003). Neuropathic pain: some clues for future drug treatments. Mini. Rev. Med. Chem. *3*, 719-727.

Barbano,M.F., Castane,A., Martin-Garcia,E., and Maldonado,R. (2009). Delta-9-tetrahydrocannabinol enhances food reinforcement in a mouse operant conflict test. Psychopharmacology (Berl) *205*, 475-487.

Bennett,G.J., and Xie,Y.K. (1988). A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain *33*, 87-107.

Bura,S.A., Burokas,A., Martin-Garcia,E., and Maldonado,R. (2010). Effects of chronic nicotine on food intake and anxiety-like behaviour in CB(1) knockout mice. Eur. Neuropsychopharmacol. *20*, 369-378.

Bura,S.A., Nadal,X., Ledent,C., Maldonado,R., and Valverde,O. (2008). A 2A adenosine receptor regulates glia proliferation and pain after peripheral nerve injury. Pain *140*, 95-103.

Campbell,J.N., Basbaum,A.I., Dray,A., Dubner,R., Dworkin,R.H., and Sang,N.C. (2006). Emerging strategies for the treatment of neuropathic pain (Seattle: IASP Press).

Carr,K.D. (2002). Augmentation of drug reward by chronic food restriction: behavioral evidence and underlying mechanisms. Physiol Behav. *76*, 353-364.

Castane,A., Celerier,E., Martin,M., Ledent,C., Parmentier,M., Maldonado,R., and Valverde,O. (2006). Development and expression of neuropathic pain in CB1 knockout mice. Neuropharmacology *50*, 111-122.

Cendan,C.M., Pujalte,J.M., Portillo-Salido,E., Montoliu,L., and Baeyens,J.M. (2005). Formalin-induced pain is reduced in sigma(1) receptor knockout mice. Eur. J. Pharmacol. *511*, 73-74.

Chaplan,S.R., Bach,F.W., Pogrel,J.W., Chung,J.M., and Yaksh,T.L. (1994). Quantitative assessment of tactile allodynia in the rat paw. J. Neurosci. Methods *53*, 55-63.

Colpaert,F.C., Tarayre,J.P., Alliaga,M., Bruins Slot,L.A., Attal,N., and Koek,W. (2001). Opiate self-administration as a measure of chronic nociceptive pain in arthritic rats. Pain *91*, 33-45.

de la Puente, Nadal,X., Portillo-Salido,E., Sanchez-Arroyos,R., Ovalle,S., Palacios,G., Muro,A., Romero,L., Entrena,J.M., Baeyens,J.M., Lopez-Garcia,J.A., Maldonado,R., Zamanillo,D., and Vela,J.M. (2009). Sigma-1 receptors regulate activity-induced spinal sensitization and neuropathic pain after peripheral nerve injury. Pain. *145*, 294-303.

Diaz,J.L., Zamanillo,D., Corbera,J., Baeyens,J.M., Maldonado,R., Pericas,M.A., Vela,J.M., and Torrens,A. (2009). Selective sigma-1 (sigma1) receptor antagonists: emerging target for the treatment of neuropathic pain. Cent. Nerv. Syst. Agents Med. Chem. *9*, 172-183.

Dickenson,A.H., and Ghandehari,J. (2007). Anti-convulsants and anti-depressants. Handb. Exp. Pharmacol. 145-177.

Dworkin,R.H., O'Connor,A.B., Backonja,M., Farrar,J.T., Finnerup,N.B., Jensen,T.S., Kalso,E.A., Loeser,J.D., Miaskowski,C., Nurmikko,T.J., Portenoy,R.K., Rice,A.S., Stacey,B.R., Treede,R.D., Turk,D.C., and Wallace,M.S. (2007). Pharmacologic management of neuropathic pain: evidence-based recommendations. Pain *132*, 237-251.

Entrena,J.M., Cobos,E.J., Nieto,F.R., Cendan,C.M., Baeyens,J.M., and Del,P.E. (2009). Antagonism by haloperidol and its metabolites of mechanical hypersensitivity induced by intraplantar capsaicin in mice: role of sigma-1 receptors. Psychopharmacology (Berl). *205*, 21-33.

Finnerup,N.B., Otto,M., McQuay,H.J., Jensen,T.S., and Sindrup,S.H. (2005). Algorithm for neuropathic pain treatment: an evidence based proposal. Pain *118*, 289-305.

Flugsrud-Breckenridge,M.R., Gevirtz,C., Paul,D., and Gould,H.J., III (2007). Medications of abuse in pain management. Curr. Opin. Anaesthesiol. *20*, 319-324.

Gutierrez,T., Crystal,J.D., Zvonok,A.M., Makriyannis,A., and Hohmann,A.G. (2011). Self-medication of a cannabinoid CB(2) agonist in an animal model of neuropathic pain. Pain. *152*, 1976-1987.

Ji,R.R., Kohno,T., Moore,K.A., and Woolf,C.J. (2003). Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci. *26*, 696-705.

Kim,H.W., Kwon,Y.B., Roh,D.H., Yoon,S.Y., Han,H.J., Kim,K.W., Beitz,A.J., and Lee,J.H. (2006). Intrathecal treatment with sigma1 receptor antagonists reduces formalin-induced phosphorylation of NMDA receptor subunit 1 and the second phase of formalin test in mice. Br. J. Pharmacol. *148*, 490-498.

Kim,H.W., Roh,D.H., Yoon,S.Y., Seo,H.S., Kwon,Y.B., Han,H.J., Kim,K.W., Beitz,A.J., and Lee,J.H. (2008). Activation of the spinal sigma-1 receptor enhances NMDA-induced pain via PKC- and PKA-dependent phosphorylation of the NR1 subunit in mice. Br. J. Pharmacol. *154*, 1125-1134.

Lyness,W.H., Smith,F.L., Heavner,J.E., Iacono,C.U., and Garvin,R.D. (1989). Morphine self-administration in the rat during adjuvant-induced arthritis. Life Sci. *45*, 2217-2224.

Malmberg,A.B., and 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.

Martin,T.J., and Ewan,E. (2008). Chronic pain alters drug self-administration: implications for addiction and pain mechanisms. Exp. Clin. Psychopharmacol. *16*, 357-366.

Martin,T.J., Kim,S.A., Buechler,N.L., Porreca,F., and Eisenach,J.C. (2007). Opioid self-administration in the nerve-injured rat: relevance of antiallodynic effects to drug consumption and effects of intrathecal analgesics. Anesthesiology *106*, 312-322.

Ong,K.S., and Keng,S.B. (2003). The biological, social, and psychological relationship between depression and chronic pain. Cranio. *21*, 286-294.

Papp,M., Willner,P., and Muscat,R. (1991). An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. Psychopharmacology (Berl). *104*, 255-259.

Roh,D.H., Kim,H.W., Yoon,S.Y., Seo,H.S., Kwon,Y.B., Kim,K.W., Han,H.J., Beitz,A.J., Na,H.S., and Lee,J.H. (2008). Intrathecal injection of the sigma(1) receptor antagonist BD1047 blocks both mechanical allodynia and increases in spinal NR1 expression during the induction phase of rodent neuropathic pain. Anesthesiology *109*, 879-889.

Romero,L., Zamanillo,D., Nadal,X., Sanchez-Arroyos,R., Rivera-Arconada,I., Dordal,A., Montero,A., Muro,A., Bura,A., Segales,C., Laloya,M., Hernandez,E., Portillo-Salido,E., Escriche,M., Codony,X., Encina,G., Burgueno,J., Merlos,M., Baeyens,J., Giraldo,J., Lopez-Garcia,J., Maldonado,R., Plata-Salaman,C., and Vela,J. (2012). Pharmacological properties of S1RA, a new sigma-1 receptor antagonist that inhibits neuropathic pain and activity-induced spinal sensitization. Br. J. Pharmacol. 10-5381.

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

Strekalova,T., and Steinbusch,H.W. (2010). Measuring behavior in mice with chronic stress depression paradigm. Prog. Neuropsychopharmacol. Biol. Psychiatry. *34*, 348-361.

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

FIGURE LEGENDS

Figure 1. Experimental schedule for the operant self-administration of analgesic compounds in mice exposed to chronic neuropathic pain.

Figure 2. Operant responses to obtain S1RA (6 mg/kg/infusion) or saline after 10 days of self-administration training in mice exposed to chronic neuropathic pain (PSNL) or sham-operated. Data are expressed as mean ± SEM of nose–pokes in active (A) and inactive (B) holes (n = 8-15 mice per experimental group).

★ $P < 0.05$, S1RA vs saline group (*post hoc* Newman Keuls). ☆ $P < 0.05$ sciatic nerve injury vs. sham–operated animals (*post hoc* Newman Keuls).

Figure 3. Development of mechanical allodynia in the ipsilateral and contralateral paw after sciatic nerve injury in mice trained to self-administer S1RA (6 mg/kg/infusion) or saline during 11 days (from day 7 to 17 after surgery). Mechanical allodynia was evaluated by using the von Frey model. The behavioural responses were determined under basal conditions and on day 3, 6, and 17 after sciatic nerve surgery. Data are expressed as mean ± SEM in mice exposed to sciatic nerve injury (black) and sham-operated mice (white) receiving saline (circle) and S1RA (6 mg/kg/infusion) (triangles); (n = 8-15 animals per experimental group) ★ $P < 0.05$, ★★★ $P < 0.001$ PSNL vs. sham-operated (*post hoc* Newman Keuls). ☆☆☆ $P < 0.001$ (S1RA treated mice vs. saline) (*post hoc* Newman Keuls).

Figure 4. Development of thermal hyperalgesia in the ipsilateral and contralateral paw after sciatic nerve injury in mice trained to self-administer S1RA (6 mg/kg/infusion) or saline during 11 days (from day 7 to 17 after surgery). Thermal hyperalgesia was evaluated by using the plantar test. The behavioural responses were determined under basal conditions and on day 3, 6, and 17 after sciatic nerve surgery. Data are expressed as mean ± SEM in mice exposed to sciatic nerve injury (black) and sham-operated mice

(white) receiving saline (circle) and S1RA (6 mg/kg/infusion) (triangles); n = (8-15 animals per experimental group) ★★ $P < 0.01$, ★★★ $P < 0.001$ PSNL vs. sham-operated (*post hoc* Newman Keuls). ☆ $P < 0.05$, S1RA treated mice vs. saline (*post hoc* Newman Keuls).

Figure 5. Development of thermal allodynia in the ipsilateral paws after sciatic nerve injury in mice trained to self-administer S1RA (6 mg/kg/infusion) or saline during 11 days (from day 7 to 17 after surgery). Thermal allodynia was evaluated in the cold-plate test (score). The behavioural responses were determined under basal conditions and on day 3, 6, and 17 after sciatic nerve surgery. Data are expressed as mean ± SEM in mice exposed to sciatic nerve injury (black) and sham- operated mice (white) receiving saline (circle) and S1RA (6 mg/kg/infusion) (triangles); (n = 8-15 animals per experimental group) ★ $P < 0.05$, ★★ $P < 0.01$, ★★★ $P < 0.001$ PSNL vs. sham-operated (*post hoc* Newman Keuls). ☆ $P < 0.05$, S1RA treated mice vs. saline (*post hoc* Newman Keuls).

Figure 6. Experimental schedule to evaluate anhedonic manifestations in mice exposed to chronic neuropathic pain

Figure 7. Sucrose preference of mice exposed to chronic neuropathic pain (PSNL) or sham-operated during 10 days of evaluation in the monitoring device (Phecomp). Mice received a non-contingent chronic treatment with S1RA (25 mg/kg) or saline during 10 days. Data are expressed as mean ± SEM of sucrose preference calculated for the whole treatment period (10 days). ☆$P < 0.05$ (sham-operated vs sciatic nerve injury in saline group) (*post hoc* Newman Keuls). ★ $P < 0.05$ saline vs. S1RA, 25 mg/kg, sciatic nerve injury group (Newman Keuls *post hoc* analysis).

Table

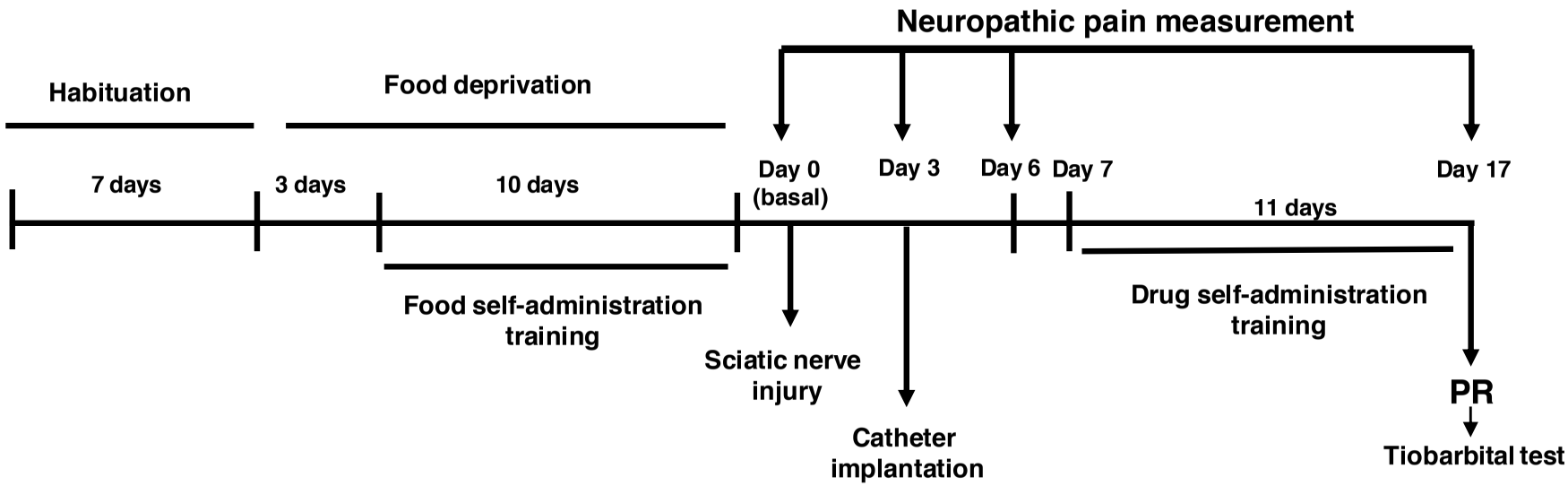
TABLE 1. The effect of intravenous self-administration of S1RA on the maintenance of the neuropathic pain manifestations

	Baseline		Day 3		Day 6		Day 17	
	F-value (1, 38)	P <	F-value (1, 38)	P <	F-value (1, 38)	P<	F-value (1, 38)	P <
von Frey test								
Ipsilateral paw								
Surgery	0.225	N.S.	901.225	0.001	101.639	0.001	43.898	0.001
treatment	0.863	N.S.	2.530	N.S.	0.172	N.S.	5.452	0.05
Interaction	0.225	N.S.	1.185	N.S.	0.300	N.S.	13.294	0.001
Controlateral paw								
Surgery	1.415	N.S.	0.404	N.S.	1.882	N.S.	0.342	N.S.
treatment	0.262	N.S.	0.458	N.S.	0.782	N.S.	2.270	N.S.
Interaction	0.012	N.S.	0.618	N.S.	1.672	N.S.	1.462	N.S.
Plantar test								
Ipsilateral paw								
Surgery	1.398	N.S.	70.421	0.001	53.688	0.001	5.195	0.05
treatment	0.651	N.S.	0.536	N.S.	2.529	N.S.	1.152	N.S.
Interaction	0.797	N.S.	0.009	N.S.	3.315	N.S.	6.588	0.05
Contralateral paw								
Surgery	0.271	N.S.	2.102	N.S.	2.101	N.S.	0.000	N.S.
treatment	0.014	N.S.	1.648	N.S.	0.238	N.S.	0.332	N.S.
Interaction	1.375	N.S.	1.161	N.S.	0.354	N.S.	0.386	N.S.
Cold plate test (score)								
Surgery	0.070	N.S.	20.174	0.001	16.936	0.001	10.327	0.01
treatment	0.261	N.S.	0.276	N.S.	1.304	N.S	0.132	N.S.
Interaction	0.004	N.S.	1.297	N.S.	1.987	N.S.	7.732	0.01

Table

TABLE 2. Cold plate test: number of paw elevations (group values):				
	Basal	**Day 3**	**Day 6**	**Day 17**
Group	**Mean S.E.M**	**Mean S.E.M**	**Mean S.E.M**	**Mean S.E.M**
Sham-operated				
Saline Ipsilateral	6.125 1.302	3.500 0.666	3.500 1.414	2.375 1.975
Saline Contralateral	8.625 2.442	4.625 1.153	5.375 1.781	4.285 2.360
S1RA 6 mg/kg Ipsilateral	4.700 1.125	5.300 1.366	5.600 1.739	3.000 1.316
S1RA 6 mg/kg Contralateral	6.100 0.809	6.700 1.738	4.300 1.164	2.700 1.164
Sciatic nerve injury				
Saline Ipsilateral	5.333 1.213	9.555 2.280	7.333 0.940	3.666 1.067
Saline Contralateral	6.000 1.364	4.333 1.213	2.333 0.600	0.888 0.771
S1RA 6 mg/kg Ipsilateral	5.333 0.969	10.266 1.725	6.800 1.138	2.400 0.607
S1RA 6 mg/kg Contralateral	6.533 0.716	2.533 0.716	2.133 0.748	1.800 0.750

Figure 1


Neuropathic pain measurement
Habituation
Food deprivation
7 days
3 days
10 days
Day 0
(basal)
Day 3
Day 6
Day 7
Day 17
11 days
Food self-administration
training
Drug self-administration
training
Sciatic nerve
injury
Catheter
implantation
PR
Tiobarbital test

Figure

Figure 2

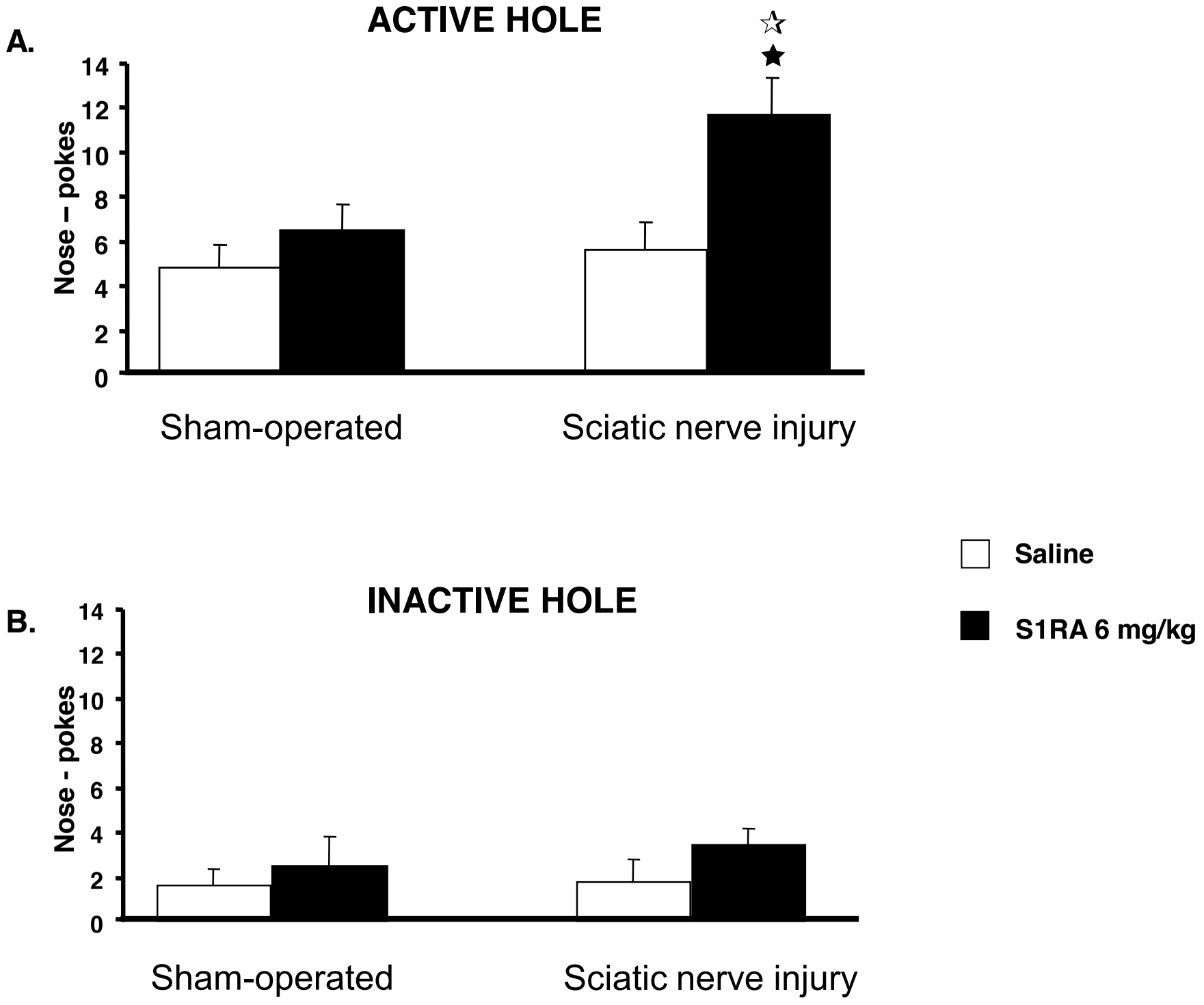


Figure 3

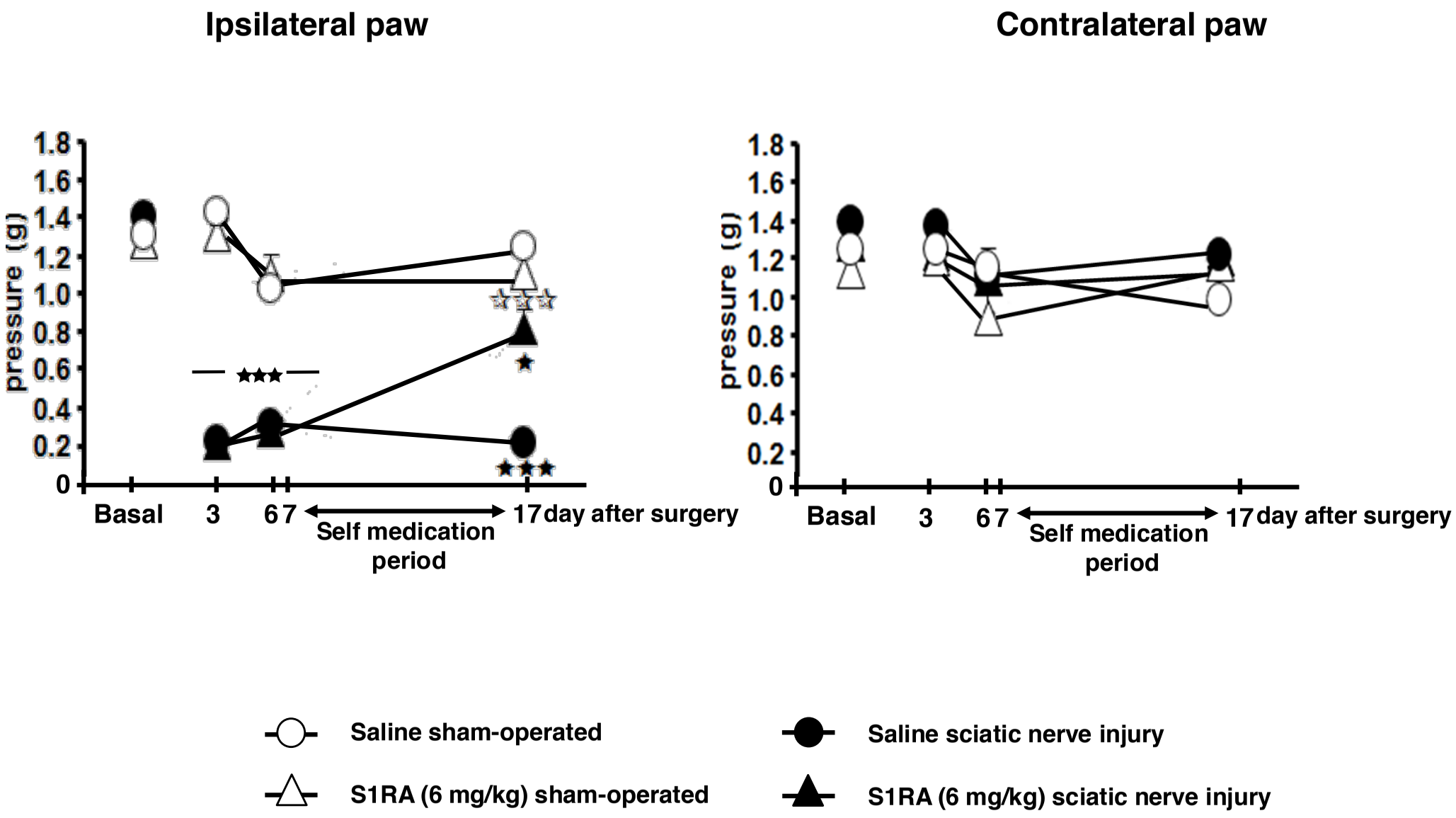

Ipsilateral paw
Contralateral paw
pressure (g)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Basal
3
6 7
17 day after surgery
Self medication period
Saline sham-operated
S1RA (6 mg/kg) sham-operated
Saline sciatic nerve injury
S1RA (6 mg/kg) sciatic nerve injury

Figure 4

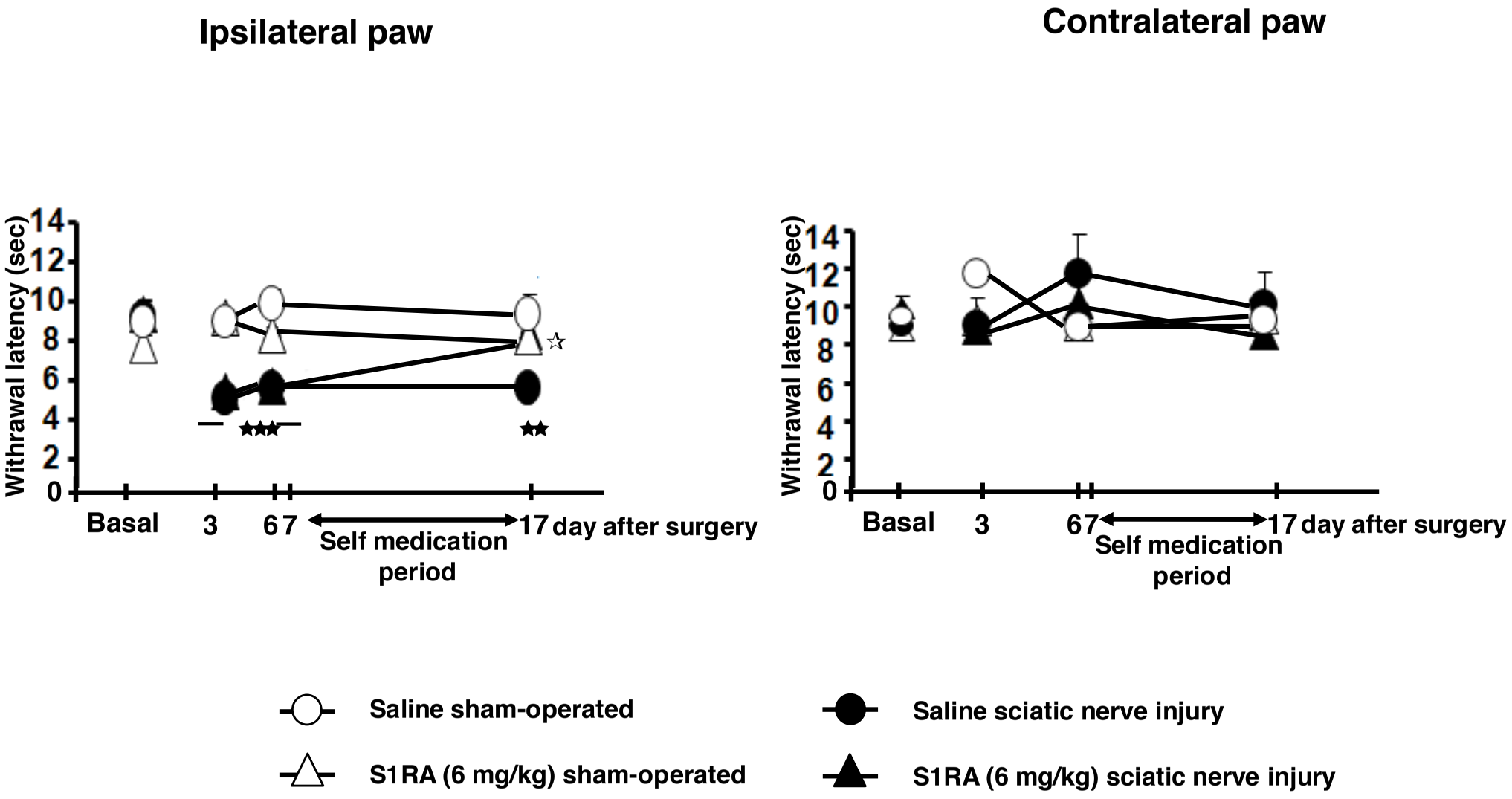

Ipsilateral paw
Contralateral paw
Withrawal latency (sec)
0
2
4
6
8
10
12
14
Basal
3
6 7
17 day after surgery
Self medication period
Saline sham-operated
S1RA (6 mg/kg) sham-operated
Saline sciatic nerve injury
S1RA (6 mg/kg) sciatic nerve injury

Figure 5

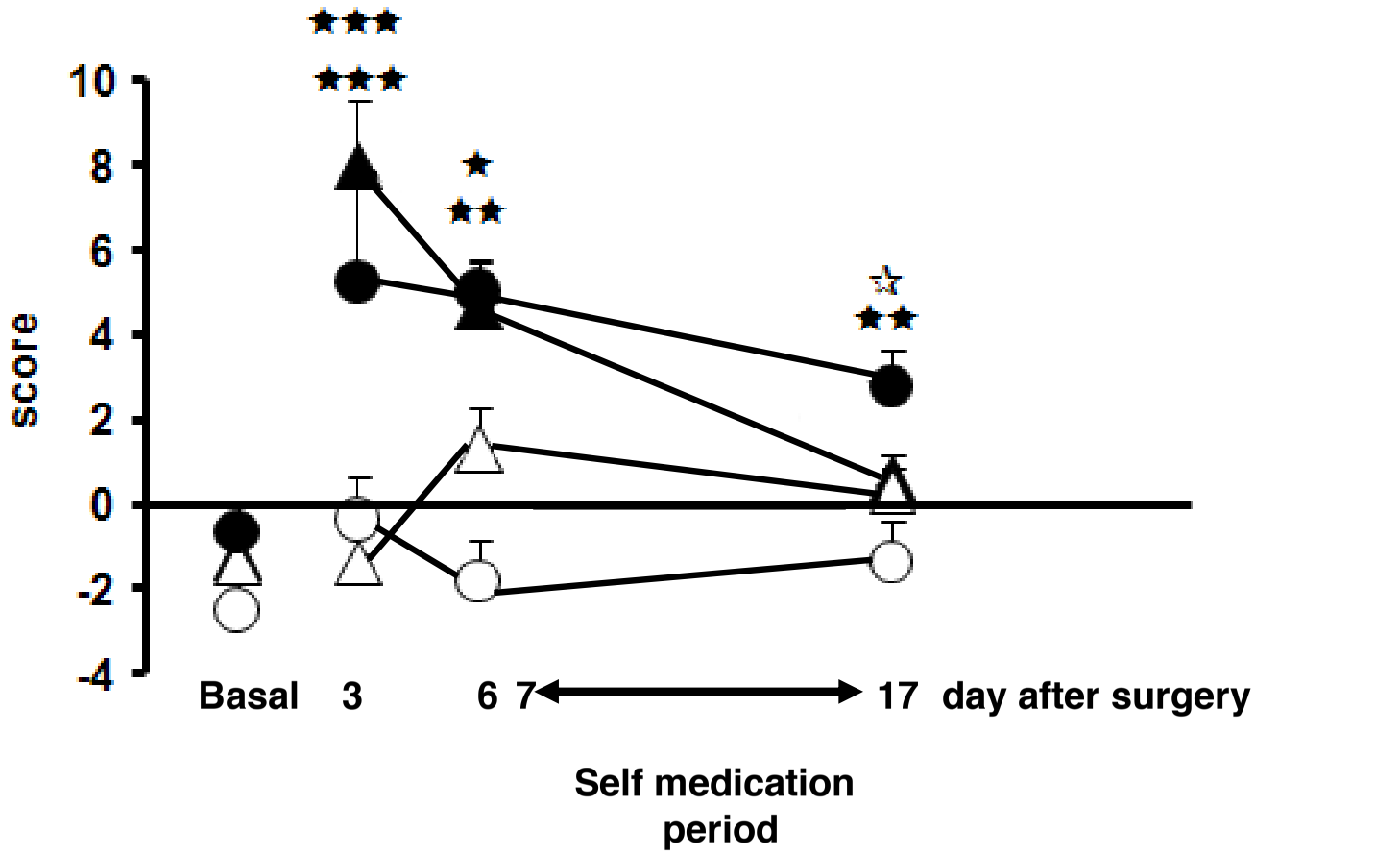

score
10
8
6
4
2
0
-2
-4
Basal
3
6
7
17
day after surgery
Self medication period
Saline sham-operated
Saline sciatic nerve injury
S1RA (6 mg/kg) sham-operated
S1RA (6 mg/kg) sciatic nerve injury

Figure 6

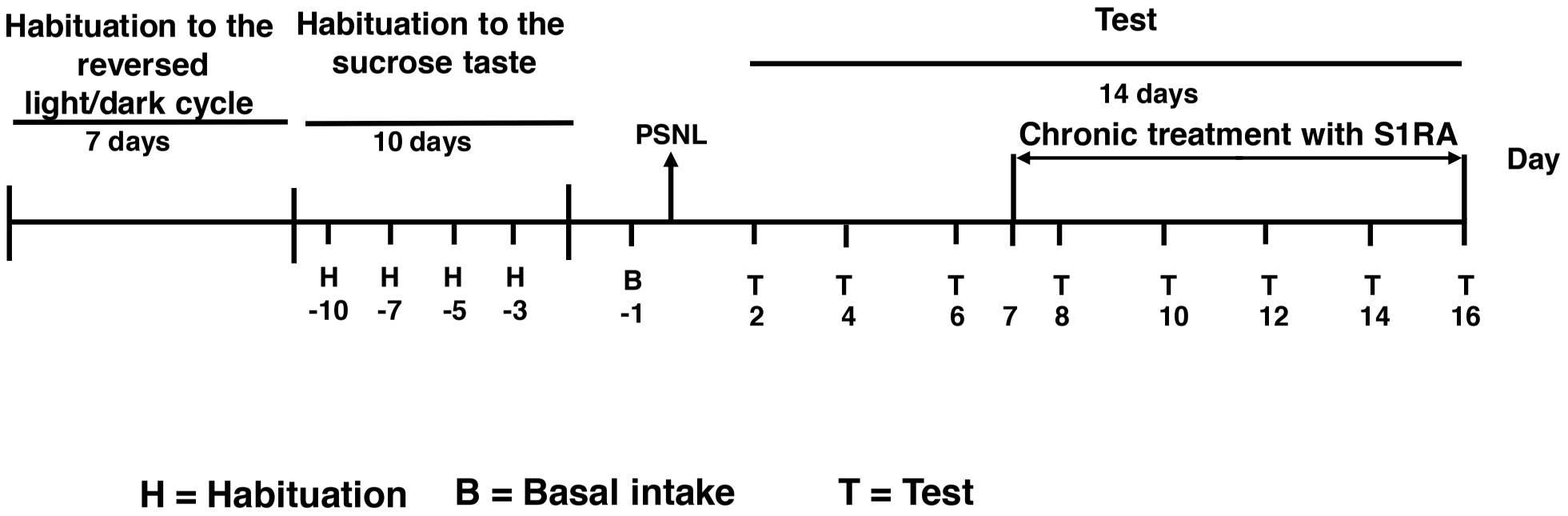
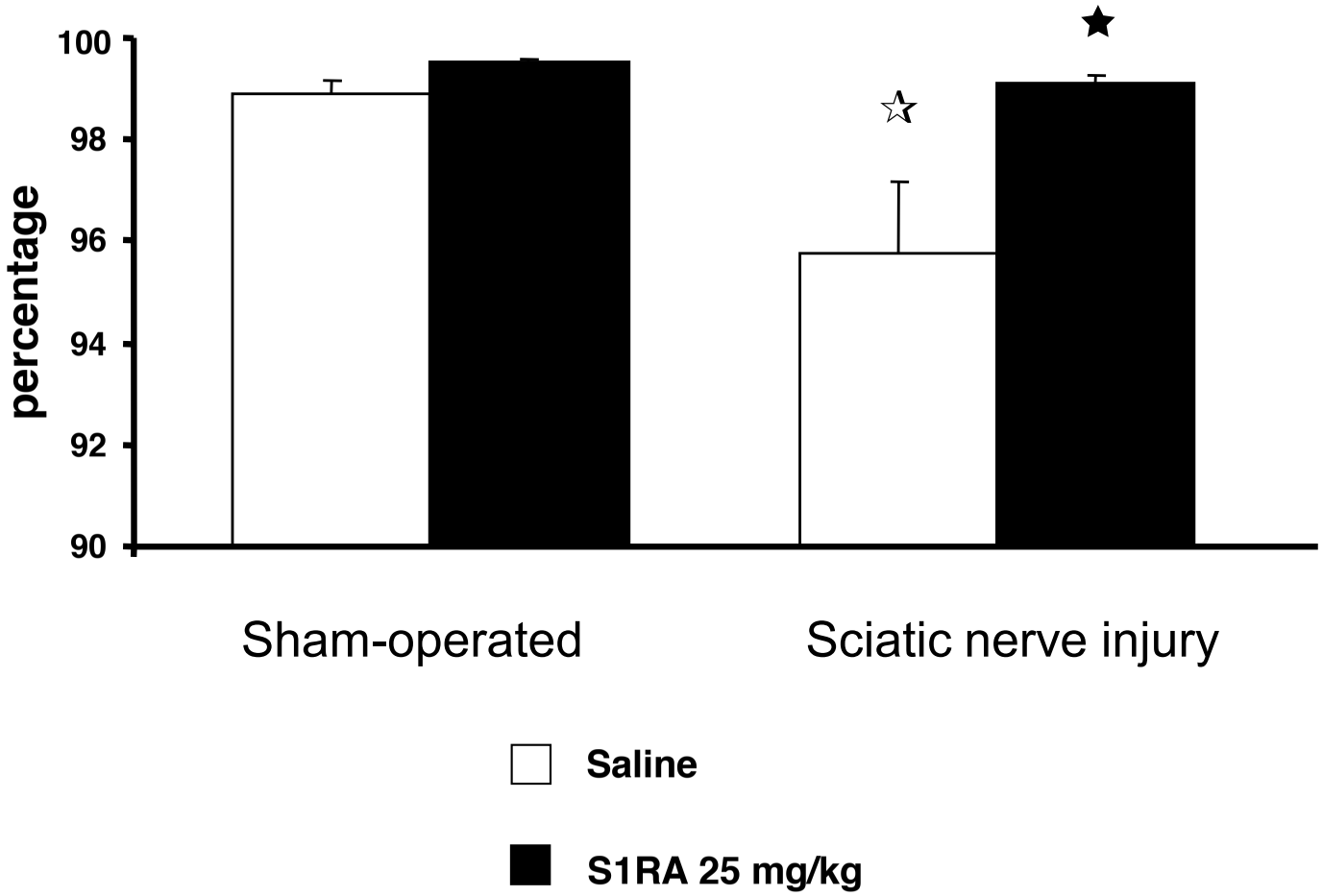

Habituation to the reversed light/dark cycle
7 days
Habituation to the sucrose taste
10 days
Test
14 days
PSNL
Chronic treatment with S1RA
Day
H -10
H -7
H -5
H -3
B -1
T 2
T 4
T 6
7
T 8
T 10
T 12
T 14
T 16
H = Habituation
B = Basal intake
T = Test

Figure 7


100
98
96
94
92
90
percentage
Sham-operated
Sciatic nerve injury
Saline
S1RA 25 mg/kg