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Depression-like behaviour in rats with mononeuropathy is reduced by the CB2-selective agonist GW405833

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ABSTRACT

The current study assessed whether the chronic constriction injury (CCI) model of neuropathic pain causes depression-like behaviour in animals, and if this depression-like behaviour can be reversed by anti-nociceptive and/or antidepressant drugs. CCI of the sciatic nerve in rats was selected as a neuropathic pain model, mechanical hypersensitivity was assessed by punctuate mechanical stimuli, and depression-like behaviour was evaluated in the forced swimming test (FST) measuring the time of immobility, climbing and swimming. The CCI rats displayed a significant mechanical hypersensitivity (sham 27 ± 2 g, CCI 12 ± 2 g; $P < 0.001$) and a significant increase in time of immobility (sham 133 ± 14 s, CCI 201 ± 9 s; $P < 0.001$). As time of swimming was unchanged, immobility was increased at the expense of climbing behaviour (sham 105 ± 17 s, CCI 63 ± 9 s; $P < 0.05$). There was no difference in ambulation between sham and CCI animals. In sham and CCI animals, desipramine (20 mg/kg) significantly reduced immobility (sham + vehicle 134 ± 19 s, sham + desipramine 79 ± 13 s; $P < 0.01$, CCI + vehicle 195 ± 8 s, CCI + desipramine 140 ± 11 s; $P < 0.05$) and increased climbing behaviour (sham + vehicle 118 ± 21 s, sham + desipramine 182 ± 16 s; $P < 0.05$, CCI + vehicle 59 ± 8 s, CCI + desipramine 112 ± 14 s; $P < 0.05$) with little effect on mechanical hypersensitivity. In contrast in CCI animals the cannabinoid CB2-selective agonist GW405833 (2,3-dichloro-phenyl)-[5-methoxy-2-methyl-3-(2-morpholin-4-yl-ethyl)-indol-1-yl]-methanone) (30 mg/kg) significantly attenuated immobility (CCI + vehicle 191 ± 7 s, GW405833 145 ± 14 s; $P < 0.01$) and mechanical hypersensitivity (CCI + vehicle 15 ± 1 g, CCI + GW405833 24 ± 1 g; $P < 0.001$). Moreover, differently from desipramine, GW405833 did not change the climbing behaviour. These data suggest that rats subjected to the CCI model of neuropathic pain develop depression-like behaviour, which can be reversed by appropriate anti-nociceptive treatment.

1. Introduction

A relationship between chronic pain and depression has clearly been recognized [2,10,12]. Several controlled randomized clinical trials have been published supporting the use of antidepressants for the treatment of chronic pain [11,26,28,34,35], and also as co-analgesics for the treatment of severe pain [13,15,17,25]. Although specific neuropharmacological mechanisms (in particular boosting of endogenous pain control systems via norepinephrine reuptake inhibition) are currently thought to explain clinical efficacy, interactions between chronic pain and depression may also contribute. Epidemiological studies have reported high prevalence of depression in chronic pain states [19,37], depression can intensify the experience of pain by reducing pain threshold and tolerance [8], and the presence of a depressive disorder significantly increases the risk of developing pain [18,22,23].

Preclinical studies have been performed to investigate the possible relationship between chronic pain and depression-like behaviour. For example, in animals with conventional models of traumatic peripheral nerve injury, a depression-like behaviour evaluated in the model of forced swimming test (FST) measuring the time of immobility, was observed in some studies [14,36,41], but not in others [16,41]. We have further characterized the association between peripheral nerve injury and depression-like behaviour in animals. For this purpose, the FST has been used in the present study due to its ease of use and reliability, and it is the most widely used approach to detect antidepressant activity [6,7,21]. Since FST evaluates motivated behaviour, it provides possible correlates of the affective-motivational component of ongoing pain in animals. In addition to the time of immobility measured in the previous studies [14,16,36,41], we have also evaluated in the FST the time of climbing and swimming as an

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endpoint to assess the depression-like behaviour. The CCI model has been selected because, compared with other peripheral nerve injury models, a higher mechanical hypersensitivity has been reported [9,31]. In addition, we have previously reported that CCI animals did not show motor impairments or signs of distress, and that the body weight developed normally [31]. However, to exclude any motor impairment-related components involved in our FST, the capability of swimming of CCI animals was also measured in the present study. To test whether nociceptive drive contributes to depression-like behaviour in CCI animals, we have compared the effects of the antidepressant drug desipramine, which is reported to be devoid of anti-nociceptive effects at a dose that is effective in the FST [7,20], and the effects of the selective cannabinoid CB2 receptor agonist GW405833 [40]. GW405833 has been evaluated in the present study because in pilot experiments it was found that GW405833 has anti-nociceptive effects at a dosage ineffective in the FST.

This study may help us to better understand the relationship between chronic pain and depression and the effect of analgesic drugs on those parameters.

2. Materials and methods

2.1. Animal care

Male Wistar rats (HsdCpb: Wu. Harlan Laboratories, The Netherlands), weighing 210–230 g at the beginning of the experiment were housed in groups of 4–5 per cage with chip bedding and free access to standard rodent food and water. Animals were maintained under the standard light cycle (6.00 a.m.–18.00 p.m.) and under a temperature-controlled environment (23 ± 1 °C). Animals were habituated in the animal room for 2–3 weeks after delivery. Housing, handling and testing of the animals were conducted according to the Guidelines on Ethical Standards for investigation of Experimental Pain in Animals [42]. The experiments were approved by the Local Committee for animal care and use.

2.2. Animal surgery

Under pentobarbital sodium (60 mg/kg intraperitoneally), chronic constriction injury was performed based on the original description by Bennett and Xie [3]. Briefly, the left sciatic nerve was exposed after the incision of skin and blunt separation of the muscle. The sciatic nerve was freed of the adhering tissue gently for about 7 mm and four ligatures (4/0 Ethicon GmbH, Norderstedt, Germany) were made loosely with 1.0–1.5 mm interval between each. Great care was taken to tie the ligatures so that the diameter of the nerve was just barely constricted. Sham operation was performed by exposing sciatic nerve except for nerve ligation. Animals were inspected every day and were tested 21–28 days after surgery.

2.3. Measurement of mechanical hypersensitivity

Mechanical hypersensitivity was measured by using an automated algometer (Somedic, Hörby, Sweden), as previously reported [31]. The animals were placed in a plexiglass cage (16 × 24 × 14 cm) with a grid bottom and adapted for at least 30 min. Mechanical stimuli were generated by touching the plantar region of the left and right hind paws of the rats with a continuous increasing pressure (5 g/s). The paw withdrawal threshold represents the mean of three independent measurements. The observer was blinded to pharmacological treatments.

In the present study, the mechanical hypersensitivity to punctate mechanical stimulation was measured using an electronic von Frey algometer [9,31] instead of the classical von Frey flexible filaments. This algometer leads to rapid threshold estimates by ascending ramped stimuli; values are reported as threshold crossing (method of limits). Decreasing ramped stimuli cannot be applied, since the animals would withdraw immediately at stimulus onset. Thus, in contrast to methods used in humans [32], we only averaged estimates of the lowest stimulus above threshold and had no data on the highest stimulus intensity below threshold. This leads to higher threshold estimates than 50% threshold using the method of levels with classical von Frey filaments The electronic von Frey algometer is easy to use, reliable, and provides direct measurements with improved objectivity of the withdrawal response [27,39].

2.4. Ambulation activity

Spontaneous locomotor activity and the total number of rearings were evaluated with an automatic measurement system (Coulbourn Instruments, USA). The rats were placed into a transparent box (40 × 40 × 40 cm) with two series of photocells located 3 and 13 cm above the floor. Horizontal movement of the animals was detected with the lower series of photocells, while rearing was assessed with the upper set. The total distance travelled was measured in centimeters and the total number of rearings was measured for 30-min period in darkness, by generating a data set every 5 min. A decrease in spontaneous locomotion and number of rearings in the dark field should reflect sedation or motor dysfunction.

2.5. Depression-like behaviour: forced swimming test

The rats were placed individually into plexiglass cylinders (height 40 cm, diameter 18 cm) filled with 25 ± 1 °C water. We increased the water depth to 30 cm from the traditional depths of 15–18 cm. Two different sessions were performed with a 15-min pre-medication test followed by a 5-min post-medication test 24 h later. Test sessions were videotaped. The time of immobility was determined when no additional activity was observed other than the movements necessary to keep the rats' head above the water. The time of climbing was measured when the rats made the upward movements vigorously with their forepaw in and out of the water. Swimming was considered when the rats showed active swimming movement, e.g., moving around in the cylinder. Drug treatments were administered 23, 5 and 1 h prior to the post-medication test.

2.6. Statistics and drugs

The experiments were performed in a randomized and blind manner between 9:00 and 16:00. Analysis of the data was performed using GraphPad Prism 5.01 for Windows. The results are expressed as means ± SEM. The assessment of pain, ambulation activity and depression-like behaviour in sham and CCI animals was done by analysing the absolute values using Student's *t*-test (Fig. 1). The experiments (Figs. 2 and 3), in which drugs were investigated, were analysed by a two-way ANOVA test, including the factors surgery (sham and CCI), treatment (desipramine and GW405833) and interaction, and followed by Bonferroni multiple comparison test. A P value of <0.05 was considered to be significant.

Desipramine was purchased from Sigma–Aldrich (Sigma, Germany). GW405833 was synthesized at Boehringer Ingelheim Pharma, USA.

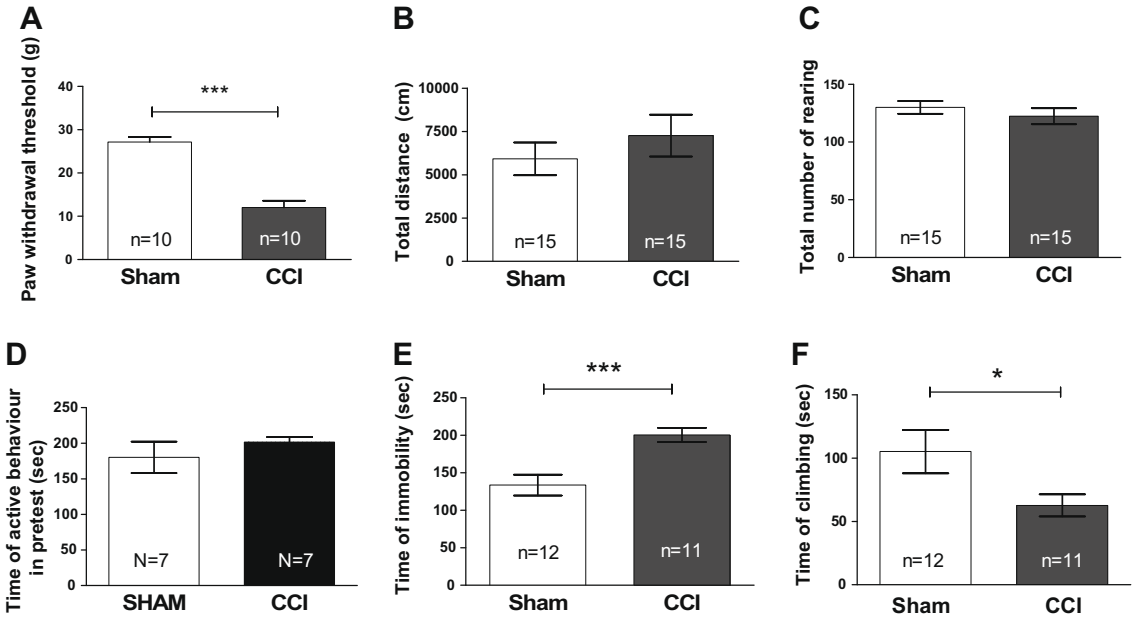

Fig. 1. Changes in (A) mechanical paw withdrawal threshold (electronic von Frey), (B) total distance travelled (activity box), (C) total number of rearings (activity box), (D) time of active behaviours (swimming + climbing) in pre-treatment test in sham and CCI animals (forced swimming test in 30 cm of water) (E) time of immobility and (F) time of climbing in sham and CCI animals (forced swimming test in 30 cm of water). Each column represents means ± SEM of 7–15 animals. Asterisks ($^{*}P < 0.05$, $^{***}P < 0.001$) indicate the values that differed significantly from sham using Student's *t*-test.

2.7. Dropouts

Animals that did not develop an enhanced response to mechanical stimuli (behavioural hyperalgesia) following nerve injury or that showed self-mutilation at the paw were excluded from the study for measuring depression-like behaviour or pain behaviour. Lack of behavioural hyperalgesia is defined as a decrease in PWT by less than 20% of the average value of the control group. Dropouts were not replaced and their numbers are given in the results section. Additional technical details are listed out in Table 2, using the suggested Extended Methods Form for experimental design and/or reporting [29].

3. Results

3.1. Mechanical hypersensitivity, ambulation and depression-like behaviour in CCI and sham rats

A significant reduction in paw withdrawal thresholds (PWTs) to the mechanical stimulation of the nerve-ligated left paw of CCI rats was observed than that observed in the sham group (sham 27 ± 2 g, CCI 12 ± 2 g, $N = 10$; $P < 0.001$, Fig. 1A). There was no decrease in PWT in the non-operated right paw of CCI and sham rats (data not shown).

In order to exclude the possibility that the lesion of the sciatic nerve affects the ambulation of the animals and their capability of swimming, the total distance travelled and the total number of rearings in a 30-min interval (activity box), and the total time of swimming (forced swimming test) were evaluated. There was no difference between sham and CCI animals in the total distance travelled (sham 5917 ± 945 cm, CCI 7263 ± 1208 cm, $N = 15$; $P > 0.05$, Fig. 1B), in the total number of rearings (sham 130 ± 6, CCI 122 ± 7, $N = 15$; $P > 0.05$, Fig. 1C) and in the active behaviours (swimming + climbing) in the pre-treatment test session (sham 180 ± 22, CCI 202 ± 7, $N = 7$; $P > 0.05$, Fig. 1D). The animals showed common health and normal behaviour. Signs of disability and distress were absent and development of body weight was regular and identical in both the groups.

After showing that ambulation and the capability of swimming were not affected in CCI animals, the depression-like behaviour was evaluated. The forced swimming test (FST) is currently used to evaluate the efficacy of antidepressant drugs. In the present study, however, the aim was to evaluate depression-like behaviour in CCI animals and not in the control group. Therefore, differently from the other studies where a high level of depression-like behaviour is needed, we have performed our experiments with 30 cm deep water where the time of immobility is quite low. In fact, a significant reduction in immobility time was observed with a deep water level of 35 cm (148 ± 19 s) or 30 cm (109 ± 10 s) versus 18 cm (269 ± 7 s) in the FST.

The immobility time of CCI animals was increased significantly compared with that of the sham group (sham 133 ± 14 s, $N = 12$, CCI 201 ± 9 s, $N = 11$; $P < 0.001$, Fig. 1E), which indicates that CCI animals displayed depression-like behaviour. As the time of swimming was unchanged, immobility was increased at the expense of climbing behaviour (sham 105 ± 17 s, $N = 12$, CCI 63 ± 9 s, $N = 11$; $P < 0.05$, Fig. 1F).

3.2. Effect of desipramine on the mechanical hypersensitivity and depression-like behaviour

The effects of desipramine (20 mg/kg) that was administered intraperitoneally are illustrated in Fig. 2.

The administration of the antidepressant desipramine did not show anti-nociceptive effects on the PWT in CCI animals (Fig. 2A). ANOVA two-way comparison revealed a statistically significant main effect for treatment ($P < 0.05$) and for surgery ($P < 0.001$), but not for interaction (Table 1). PWTs of the CCI animals were significantly decreased compared to those of the sham-operated animals (sham + vehicle 32 ± 2 g, CCI + vehicle 16 ± 2 g, $N = 10$; $P < 0.001$). Desipramine did not reverse mechanical hypersensitivity in CCI (CCI + vehicle 16 ± 2 g, CCI + desipramine 21 ± 2 g, $N = 10$; $P > 0.05$) and sham groups (sham + vehicle

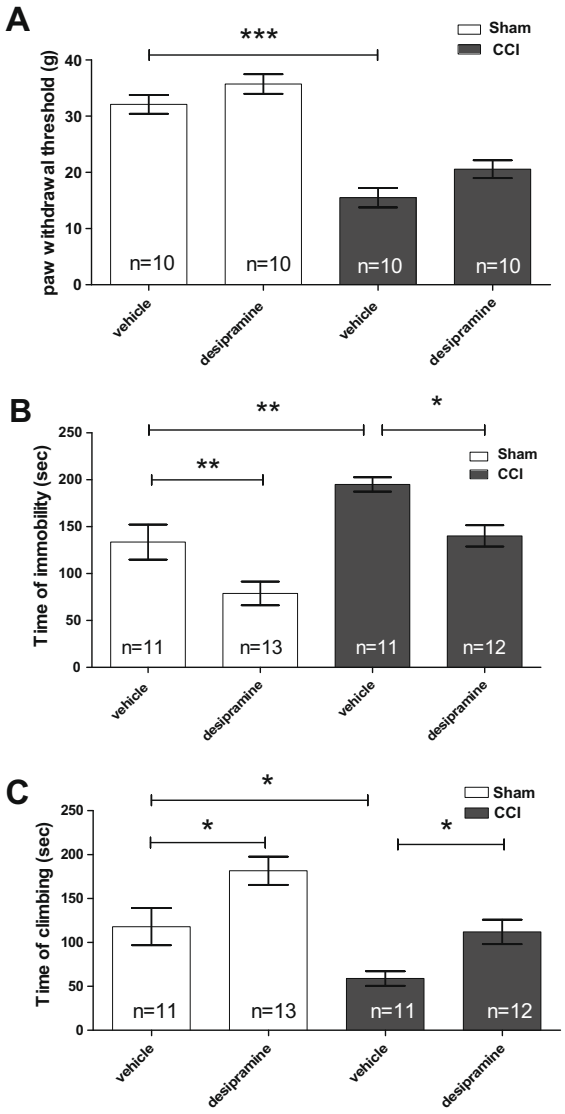

Fig. 2. Changes in (A) mechanical paw withdrawal threshold (electronic von Frey), (B) time of immobility and (C) time of climbing in sham and CCI animals. Desipramine (20 mg/kg ip) significantly reduced the time of immobility and increased the time of climbing in sham and CCI animals. Each column represents the mean ± SEM of 10–12 animals. Asterisks ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) indicate the values that differed significantly using Bonferroni multiple comparison test.

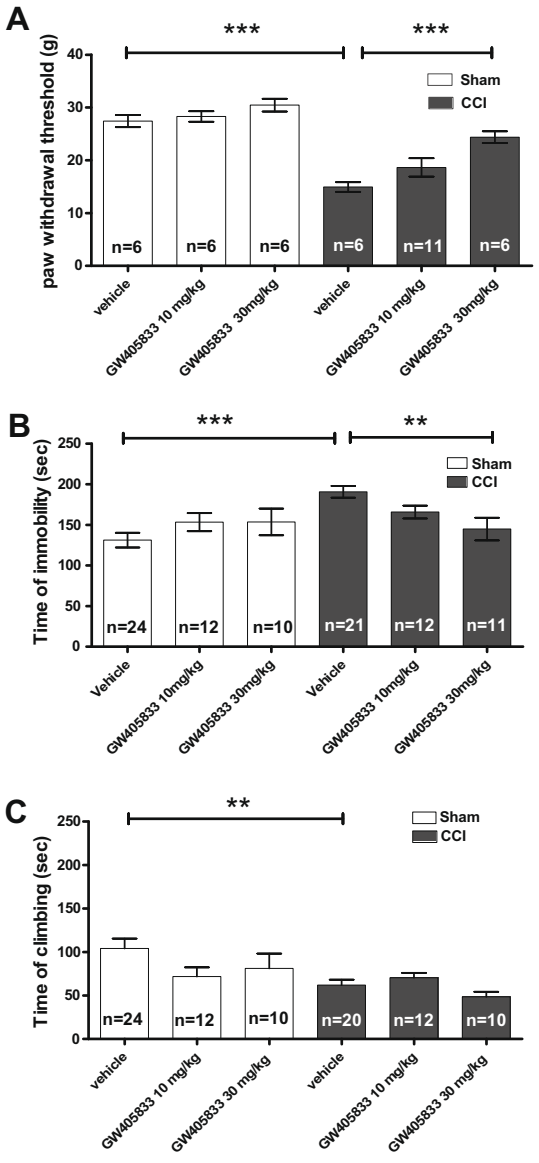

Fig. 3. Changes in (A) mechanical paw withdrawal threshold (electronic von Frey), (B) time of immobility and (C) time of climbing in sham and CCI animals. In CCI animals, GW405833 (30 mg/kg ip) had anti-nociceptive effect and significantly reduced the time of immobility. Each column represents means ± SEM of 6–11 (paw withdrawal threshold) or 11–24 (forced swimming test) animals. Asterisks ($^{**}P < 0.01$, $^{***}P < 0.001$) indicate the values that differed significantly using Bonferroni multiple comparison test.

32 ± 2 g, sham + desipramine 36 ± 2 g, $N = 10$; $P > 0.05$). There was no difference in the non-injured right paw in all the groups (data not shown).

After showing that desipramine had only a minor anti-nociceptive effect at the dosage used (significant in ANOVA but not in post hoc tests), the effect of desipramine (20 mg/kg) on FST was measured (Fig. 2B and C). Two-way ANOVA revealed a highly significant main effect in the time of immobility for treatment ($P < 0.001$) and for surgery ($P < 0.001$), but not for interaction (Table 1).

The immobility time is increased in CCI animals than in the sham-operated animals (sham + vehicle 134 ± 19 s, CCI + vehicle 195 ± 8 s, $N = 11$; $P < 0.01$). Desipramine produced a reduction in immobility time in both the groups (sham + vehicle 134 ± 19 s, $N = 11$, sham + desipramine 79 ± 13 s, $N = 13$; $P < 0.01$, CCI + vehicle 195 ± 8 s, $N = 11$; CCI + desipramine 140 ± 11 s, $N = 12$; $P < 0.05$).

Likewise, two-way ANOVA revealed a significant main effect in the time of climbing for treatment ($P < 0.001$) and for surgery ($P < 0.001$), but not for interaction (Table 1). The time of climbing is decreased in CCI animals than in the sham-operated animals (sham + vehicle 118 ± 21 s, CCI + vehicle 59 ± 8 s, $N = 11$; $P < 0.05$). Desipramine produced an increase in the time of climbing in both the groups (sham + vehicle 118 ± 21 s, $N = 11$, sham + desipramine 182 ± 16 s, $N = 13$; $P < 0.05$, CCI + vehicle 59 ± 8 s, $N = 11$, CCI + desipramine 112 ± 14, $N = 12$; $P < 0.05$).

These results demonstrate that desipramine is able to reduce the depression like behaviour of CCI rats without affecting mechanical hypersensitivity.

3.3. Effect of selective CB2-selective agonist GW405833 on the mechanical hypersensitivity and depression-like behaviour

We assumed that depression-like behaviour in CCI animals could also be attenuated by analgesic drugs that alleviate pain. In

Table 1
Effect of desipramine and GW405833 on nociception and depression-like behaviour induced by pain. *P* values and *F* values of two-way ANOVA test.

Drug		Paw withdrawal	Immobility	Climbing
Desipramine	Treatment	$F_{1,36} = 6.34$; $P < 0.05$	$F_{1,43} = 17.38$; $P < 0.001$	$F_{1,43} = 16.92$; $P < 0.001$
	Surgery	$F_{1,36} = 86.02$; $P < 0.001$	$F_{1,43} = 21.78$; $P < 0.001$	$F_{1,43} = 13.96$; $P < 0.001$
	Interaction	$F_{1,36} = 0.097$; $P = 0.757$	$F_{1,43} = 0.0001$; $P = 0.99$	$F_{1,43} = 0.11$; $P = 0.74$
GW405833	Treatment	$F_{2,35} = 7.91$; $P < 0.001$	$F_{2,84} = 0.635$; $P = 0.534$	$F_{2,84} = 1.614$; $P = 0.205$
	Surgery	$F_{1,35} = 58{,}62$; $P < 0.001$	$F_{1,84} = 5.483$; $P < 0.05$	$F_{1,84} = 7.725$; $P < 0.01$
	Interaction	$F_{1,35} = 2.19$; $P = 0.127$	$F_{1,84} = 5.953$; $P < 0.01$	$F_{1,84} = 2.001$; $P = 0.142$

order to verify this assumption, the CB2-selective agonist GW405833 was selected because it is devoid of any effect in FST in normal animals. The effects of GW405833 (10 and 30 mg/kg) on mechanical hypersensitivity and on FST in sham and CCI animals are shown in Fig. 3.

GW405833 30 mg/kg significantly attenuated mechanical hypersensitivity (Fig. 3A). ANOVA two-way comparison revealed a significant main effect for treatment ($P < 0.001$) and for surgery ($P < 0.001$), but not for interaction (Table 1). PWTs of the CCI animals are significantly decreased than those of the sham-operated animals (sham + vehicle 27 ± 1 g, CCI + vehicle 15 ± 1 g, $N = 6$; $P < 0.001$. Fig. 3A). The administration of GW405833 30 mg/kg attenuated mechanical hypersensitivity (CCI + vehicle 15 ± 1 g, CCI + GW405833 24 ± 1 g, $N = 6$; $P < 0.001$. Fig. 3A). There was no difference in the non-injured right paw in all the groups (data not shown).

After showing that GW405833 has an anti-nociceptive effect, the effect of GW405833 (10 and 30 mg/kg) on FST was evaluated (Fig. 3B and C). ANOVA two-way comparison revealed a significant main effect in the time of immobility for surgery ($P < 0.05$), and for interaction ($P < 0.01$), but not for treatment (Table 1). The time of immobility is increased in CCI animals than in the sham-operated animals (sham + vehicle 131 ± 9 s, $N = 24$, CCI + vehicle 191 ± 7 s, $N = 21$; $P < 0.001$. Fig. 3B). GW405833 30 mg/kg attenuated the immobility time in CCI animals (CCI + vehicle 191 ± 7 s, $N = 21$, CCI + GW405833 145 ± 14 s, $N = 11$; $P < 0.01$. Fig. 3B). ANOVA two-way comparison revealed a significant main effect in the time of climbing for surgery ($P < 0.01$), but not for interaction nor for treatment (Table 1). The time of climbing is decreased in CCI animals than in the sham-operated animals (sham + vehicle 104 ± 11 s, $N = 24$, CCI + vehicle 62 ± 6 s, $N = 21$; $P < 0.01$, Fig. 3C).

3.4. Dropouts

In the desipramine group, two CCI animals were excluded due to mutilations of the paw and two CCI animals were excluded because they did not develop an enhanced response to mechanical stimuli. In the GW405833 group, three animals were excluded due to mutilations of the paw and one animal was excluded due to technical problems. Dropouts were not replaced and are not included in the numbers mentioned in the figures.

4. Discussion

The present study demonstrated that rats with chronic constriction injury (CCI) of the sciatic nerve develop depression-like behaviour, reflected in an increase in the time of immobility in the forced swimming test (FST). We hypothesize that this particular behaviour is a consequence of persistent pain present in these lesioned animals, suggesting that the CCI model of neuropathic pain may have an ongoing-pain component in addition to the well-known evoked-pain component. This is based on the observation that the CB2-selective agonist GW405833 reversed the depression-like behaviour and mechanical hypersensitivity in CCI animals without having an effect on the sham-operated rats. In contrast, the antidepressant desipramine strongly reduced depression-like behaviour in both sham and CCI animals with hardly any anti-nociceptive properties in the paw-withdrawal test.

4.1. Behavioural signs of depression-like behaviour in CCI animals

The CCI model according to Bennett and Xie [3] was selected due to a more significant mechanical hypersensitivity in comparison with other animal neuropathic pain models [9,31], and FST has been chosen due to its ease of use, reliability and specificity [6,7,21].

From our observation, CCI animals showed an enhanced time of immobility that is interpreted as depression-like behaviour. There were no motor impairments and a normal swimming behaviour, suggesting that the depression-like behaviour is related to the pain status of the animals, not to motor impairment. In humans, the emotional component of pain is called "affective-motivational" [24] reflecting the fact that emotions have an expressive motor component as well as a receptive sensory component. Thus,

Table 2
Animal models of pain standard reporting form [according to ref. 29]

Environment			
(a) Housing		(b) Testing (electronic von Frey)	
Diet	Food pellets. Diet 3438 PM.BB1. Provimi (CH)	# Of testers	1
Bedding	Sawdust. Lingocel (FS14)-Rettenmaier (D)	Experience of tester(s)	High
Cage rack ventilation	No	Lighting intensity (lux)	325
Home cage enrichment	Yes	Time testing ends	4 p.m.
Habituation time before experiments (in days)	Yes. 14–21 days	Test environment noise	Radio
Handling frequency before testing	No	Recent calibration of testing equipment	Yes
Cage cleaning frequency	3 times per week	Cleaning of test equipment between subjects	No
#Housed per cage	3–4	Habituation time before testing (in minutes)	60–120
House experimental/control groups together or separately	Separately	Oestrus stage of females	———————
		Number of animals present in testing room	24
Different species housed in same room	No	Distance separated	~1–2 m
Lights on time	6:00 p.m.	In visual contact	No
Lights off time	6:00 a.m.	Arousal state	Low
Procedural anesthetic	Yes		
Post-operative analgesia	No		

immobility in the FST may be a sign of reduced motor drive related to the "affective-motivational component of pain" in animals.

These results confirm previous evidence that animals with peripheral nerve injury display depression-like behaviour [14,41]. In contrast, in two other studies, depression-like behaviour was not observed in animals with peripheral nerve injury [16,41]. These discrepancies could be due to the different methodological conditions used. Indeed, one of the negative trials [16] employed a training period run for the FST and used less deep water [16]; thus animals may have responded to previous experience with the FST rather than to their current situation. Moreover, spinal nerve injured rats cannot swim properly ([16, p. 346]). In the second and only partly negative study, a depression-like behaviour was observed in Wistar-Kyoto, but not in Wistar, rats after chronic constriction injury of the sciatic nerve [41]. This second study suggested that there are differences in neuropathic pain-related depression-like behaviour between rat strains, as previously reported for mechanical allodynia and hyperalgesia [33].

In the FST, climbing and swimming active behaviours are selectively altered by the noradrenergic and serotonergic systems, respectively [6,7,21]. In the present study, we have observed for the first time that only the climbing behaviour is decreased in CCI animals, suggesting that the noradrenergic, but not the serotonergic, system plays a major role in chronic pain depression-like behaviour. The results reported here are also consistent with the clinical evidence that the efficacy of selective noradrenergic uptake inhibitor in the treatment of chronic pain is superior to that of the selective serotonergic uptake inhibitors [1,11,26,28,34,35].

These findings that animals with neuropathic pain also exhibit depression-like behaviour are in agreement with the clinical evidence reporting a relationship between chronic pain and depression. In several clinical studies neuropathic pain-associated depressive symptoms are described [1,11,26,28,34,35]. In contrast, only few studies investigated associated symptoms, such as depression, in animals with neuropathic pain [14,16,36,41]. In pre-clinical studies, anti-nociceptive effects of analgesic drugs are mostly determined by measuring changes in pain thresholds after evoked mechanical, chemical or thermal stimuli. Measurements of ongoing pain and of associated symptoms are not part of the standard neuropathic pain models in animals. This consideration of pain in very narrow terms is one of the drawbacks of basic pain research. In contrast, clinical trials include subjective assessment of pain-associated symptoms, such as "quality of life-parameters" and emotional states. The measurement of associated symptoms in animals, such as depression, would narrow the gap between pre-clinical and clinical drug testing.

A recent study associated depression-like behaviour following neuropathic pain induced by peripheral nerve injury with structural neuroplasticity of the limbic system measured by morphological stereological techniques. This study provides evidence of an increased volume of the amygdala due to neurogenesis in the rats [14]. Since the amygdala participates in receiving and processing of the pain information [4,30,38] and is a central component of the limbic system [5], it has been suggested that the depression-like behaviour could be due to neuroplasticity changes specifically in the amygdala [14].

4.2. Pharmacological modulation of behavioural signs of depression

An additional intriguing question was whether drugs are able to affect this depression- like behaviour in CCI animals. Therefore, in the second part of the study the effects of the antidepressant desipramine 20 mg/kg and the anti-nociceptive CB2-selective agonist GW405633 10 and 30 mg/kg were evaluated.

In this study we show that the depression-like behaviour is diminished by desipramine in sham animals as well as in CCI animals at a dose that had only marginal anti-nociceptive effects. Similar results of desipramine were described in FST at the dose of 20 mg/kg [7] and in mechanical hypersensitivity in the dose range of 30–56 mg/kg in spinal nerve ligation rats [20]. These data further support the interpretation that increased time of immobility of CCI rats measured in the FST reflects a depression-like behaviour.

Additionally, in the present study, we investigated if pain-relief could also result in an attenuation of depression-like behaviour. To this aim, we selected the anti-nociceptive CB2-selective agonist GW405833 [40]. GW405833 (30 mg/kg) attenuated mechanical hypersensitivity without reducing motor activity, as previously reported [40]. Intriguingly, GW405833 dose-dependently attenuated depression-like behaviour in CCI animals without showing an antidepressive effect in sham animals. These data indicate that depression-like behaviour (immobility), as an affective-motivational component of nociception, has been attenuated by anti-nociceptive treatment. In this study the focus was mainly set on the effects of CB2-selective agonist GW405833. At the dosage used, GW405833 significantly penetrated the central nervous system [40], therefore a role of central CB2 receptors cannot be excluded. It will be very interesting to analyse whether other analgesic drugs e.g. with a purely peripheral mode of action have the same efficacy in reversing depression-like behaviour. In contrast to desipramine, GW405833 did not increase the time of climbing in CCI animals suggesting that the antidepressant effect of GW405833 should not be mediated through the noradrenergic system. Which neuronal systems are involved in the antidepressant effect of GW405833 in CCI animals remains to be elucidated.

5. Conclusions

In conclusion, animals presumably suffering from ongoing neuropathic pain after a partial peripheral nerve lesion (CCI) display depression-like behaviour (increased immobility). By using specific drugs, it was demonstrated that it is possible to differentiate between anti-nociceptive and anti-depressive effects. In the clinical situation both aspects are of importance, so investigating drugs in this model might offer the opportunity to assess novel analgesic drugs for treatment of neuropathic pain and its related symptoms. The main result of these experiments was that the CB2-selective agonist GW405833 was able to reduce mechanical hypersensitivity as well as depression-like behaviour in rats with neuropathic pain, but not in sham-treated animals. It remains to be shown whether this is a general feature of analgesic drugs, or specific for CB2 agonist.

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References

[1] Attal N, Cruccu G, Haanpää M, Hansson P, Jensen TS, Nurmikko T, Sampaio C, Sindrup S, Wiffen S. EFNS guidelines on pharmacological treatment of neuropathic pain. Eur J Neurol 2006;13:1153–69.

[2] Bair MJ, Robinson RL, Katon W, Kroenke K. Depression and pain comorbidity: a literature review. Arch Intern Med 2003;163:2433–45.
[3] Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988;33:87–107.
[4] Bernard JF, Huang GF, Besson JM. Effect of noxious somesthetic stimulation on the activity of neurons of the nucleus centralis of the amygdala. Brain Res 1990;523:347–50.
[5] Bernard JF, Bester H, Besson JM. Involvement of the spino-parabrachio-amydaloid and hypothalamic pathways in the autonomic and affective emotional aspects of pain. Prog Brain Res 1996;107:243–55.
[6] Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol Sci 2002;23:238–45.
[7] Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology 1995;121:66–72.
[8] Dickens C, McGowan L, Dale S. Impact of depression on experimental pain perception: a systematic review of the literature with meta-analysis. Psychosom Med 2003;65:369–75.
[9] Dowdall T, Robinson I, Meert TF. Comparison of five different rat models of peripheral nerve injury. Pharmacol Biochem Behav 2005;80:93–108.
[10] Dworkin RH, Gitlin MJ. Clinical aspects of depression in chronic pain patients. Clin J Pain 1991;17:79–94.
[11] Dworkin RH, O'Connor AB, Backonja M, Farrar JT, Finnerup NB, Jensen TS, Kalso EA, Loeser JD, Miaskowski C, Nurmikko TJ, Portenoy RK, Rice AS, Stacey BR, Treede RD, Turk DC, Wallace MS. Pharmacologic management of neuropathic pain: evidence-based recommendations. Pain 2007;132:237–51.
[12] Fishbain DA. Approaches to treatment decisions for psychiatric comorbidity in the management of the chronic pain patient. Med Clin North Am 1999;83:737–60.
[13] Forde G. Adjuvant analgesics for the treatment of neuropathic pain: evaluation efficacy and safety profile. J Fam Pract 2007:3–12.
[14] Gonçalves L, Silva R, Pinto-Riberio F, Pêgo F, Bessa JM, Pertovaara A, Sousa N, Almeida A. Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amigdala of the rat. Exp Neurol 2008;213:48–56.
[15] Gordon DB. Nonopioid and adjuvant analgesics in chronic pain management: strategies for effective use. Nun Clin North Am 2003;38:447–64.
[16] Kontinen VK, Kauppila T, Paananen S, Pertovaara A, Kalso E. Behavioural measures of depression and anxiety in rats with spinal nerve ligation-induced neuropathy. Pain 1999;80:341–6.
[17] Knotkova H, Pappagallo M. Adjuvant analgesics. Anesthesiol Clin 2007;25:775–86.
[18] Leino P, Magni G. Depressive and distress symptoms as predictors of low back pain, neck–shoulder pain, and other musculoskeletal morbidity: a 10-year follow-up of metal industry employees. Pain 1993;53:89–94.
[19] Lépine JP, Briley M. The epidemiology of pain in depression. Hum Psychopharmacol 2004;19:S3–7.
[20] Leventhal L, Smith V, Hornby G, Andree TH, Brandt MR, Rogers KE. Differential and synergistic effects of selective norepinephrine and serotonin reuptake inhibitors in rodent models of pain. J Pharmacol Exp Ther 2007;320:1178–85.
[21] Lucki I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav Pharmacol 1997;8:523–32.
[22] Magni G, Marchetti M, Moreschi C, Merskey H, Luchini SR. Chronic musculoskeletal pain and depressive symptoms in the National Health and Nutrition Examination. Epidemiologic Follow-up Study. Pain 1993;53:163–8.
[23] Magni G, Moreschi C, Rigatti-Luchini S, Merskey H. Prospective Study on the relationship between depressive symptoms and chronic musculoskeletal pain. Pain 1994;56:289–97.
[24] Melzack R. From the gate to the neuromatrix. Pain 1999:S121–6.
[25] McGeeney BE. Adjuvant agents in cancer pain. Clin J Pain 2008;24:S14–20.
[26] Micó JA, Ardid D, Berrocoso E, Eschalier A. Antidepressant and pain. Trends Pharmacol Sci 2006;22:348–54.
[27] Möller KÄ, Johansoon B, Berge OG. Assessing mechanical allodynia in the rat paw with a new electronic algometer. J Neurosci 1998;84:41–7.
[28] Rice ASC, Hill RG. New treatment of neuropathic pain. Annu Rev Med 2006;57:535–41.
[29] Rice ASC, Cimino-Brown D, Eisenach JC, Kontinen VS, Lacroix-Fralish ML, Machin I, Mogil JS, Stöhr T. Animal model and the prediction of efficacy in clinical trials of analgesics drugs: a critical appraisal and call for uniform reporting standards. Pain 2008;139:243–7.
[30] Rizvi TA, Ennis M, Behbehmi MM, Shipley MT. Connections between the central nucleus of the amygdala and the midbrain periaqueductal gray: topography and reciprocity. J Comp Neurol 1991;303:121–31.
[31] Roeska K, Doods H, Arndt K, Treede RD, Ceci A. Anxiety-like behaviour in rats with mononeuropathy is reduced by the analgesic drugs morphine and gabapentin. Pain 2008;139:349–57.
[32] Rolke R, Magel W, Campbell KA, Schalber C, Caspari S, Birklein F, Treede RD. Quantitative sensory testing: a comprehensive protocol for clinical trials. Eur J Pain 2006;10:77–88.
[33] Shir Y, Zeltser R, Vatine JJ, Carmi G, Belfer I, Zangen A, Overstreet D, Raber P, Seltzer Z. Correlation of intact sensibility and neuropathic pain-related behaviors in eight inbred and outbred rat strains and selection lines. Pain 2001;90:75–82.
[34] Sindrup SH, Jensen TS. Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. Pain 1999;83:389–400.
[35] Sindrup SH, Jensen TS. Pharmacology treatment of pain in polyneuropathy. Neurology 2000;55:915–20.
[36] Suzuki T, Amata M, Sakaue G, Nishmura S, Inoue T, Shibata M, Mashimo T. Experimental neuropathy in mice is associated with delayed behavioural changes related to anxiety and depression. Anesth Analg 2007;104:1570–7.
[37] Tunks ER, Crook J, Weir R. Epidemiology of chronic pain with psychological comorbidity: prevalence, risk, course, and prognosis. Can J Psychiatry 2008;53:224–34.
[38] Van Bockstaele EJ, Chan J, Pickel VM. Input from central nucleus of the amygdala efferents to pericoerulear dendrites, some of which contain tyrosine hydroxylase immunoreactivity. J Neurosci Res 1996;45:289–302.
[39] Vivancos GC, Verri WA, Cunha TM, Schivo IRS, Parada CA, Cunha FQ, Ferreira SH. An electronic pressure-meter nociceptive paw test for rats. Braz J Med Biol Res 2004;37:391–9.
[40] Whiteside GT, Gottshall SL, Boulet JM, Chaffer SM, Harrison JE, Pearson MS, Turchain PI, Mark L, Garrison AE, Valenzano KJ. A role for cannabinoid receptors, but not endogenous opioids, in the antinociceptive activity of the CB2-selective agonist, GW405833. Eur J Pharmacol 2005;528:65–72.
[41] Zeng Q, Wang S, Lim G, Yang L, Mao J, Sung B, Chang Y, Lim JA, Guo G, Mao J. Exacerbated mechanical allodynia in rats with depression-like behaviour]. Brain Res 2008;1200:27–38.
[42] Zimmerman M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10.