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2017-06-01

Amorim , D , Puga , S , Braganca , R , Braga , A , Pertovaara , A , Almeida , A & Pinto-Ribeiro , F 2017 , ' Minocycline reduces mechanical allodynia and depressive-like behaviour in type-1 diabetes mellitus in the rat ' , Behavioural Brain Research , vol. 327 , pp. 1-10 . https://doi.org/10.1016/j.bbr.2017.03.003

http://hdl.handle.net/10138/236229
https://doi.org/10.1016/j.bbr.2017.03.003

unspecified
publishedVersion

Behavioural Brain Research 327 (2017) 1–10

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Minocycline reduces mechanical allodynia and depressive-like behaviour in type-1 diabetes mellitus in the rat

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HIGHLIGHTS

- Experimental rat model of type-1 diabetes was induced by streptozotocin.
- Diabetes induced in 4 weeks hypersensitivity to cold and mechanical stimulation.
- Hypersensitivity was accompanied by depression-like behaviour.
- Minocycline treatment attenuated mechanical allodynia and depression.
- Minocycline failed to attenuate cold allodynia or mechanical hyperalgesia.

ARTICLE INFO

Article history:
Received 25 January 2017
Received in revised form 24 February 2017
Accepted 2 March 2017
Available online 8 March 2017

Keywords:
Minocycline
Type-1 diabetes animal model
Mechanical allodynia
Depressive-like behaviour

ABSTRACT

A common and devastating complication of diabetes mellitus is painful diabetic neuropathy (PDN) that can be accompanied by emotional disorders such as depression. A few studies have suggested that minocycline that inhibits microglia may attenuate pain hypersensitivity in PDN. Moreover, a recent study reported that minocycline has an acute antidepressive-like effect in diabetic animals. Here we studied whether (i) prolonged minocycline treatment suppresses pain behaviour in PDN, (ii) the minocycline effect varies with submodality of pain, and (iii) the suppression of pain behaviour by prolonged minocycline treatment is associated with antidepressive-like effect. The experiments were performed in streptozotocin-induced rat model of type-1 diabetes. Pain behaviour was evoked by innocuous (monofilaments) and noxious (paw pressure) mechanical stimulation, innocuous cold (acetone drops) and noxious heat (radiant heat). Depression-like behaviour was assessed using forced swimming test. Minocycline treatment (daily 80 mg/kg *per os*) of three-week duration started four weeks after induction of diabetes. Diabetes induced mechanical allodynia and hyperalgesia, cold allodynia, heat hypoalgesia, and depression-like behaviour. Minocycline treatment significantly attenuated mechanical allodynia and depression-like behaviour, while it failed to produce significant changes in mechanical hyperalgesia, cold allodynia or heat hypoalgesia. The results indicate that prolonged per oral treatment with minocycline has a sustained mechanical antiallodynic and antidepressive-like effect in PDN. These results support the proposal that minocycline might provide a treatment option for attenuating sensory and comorbid emotional symptoms in chronic PDN.

Abbreviations: DIAB, diabetic animals; DIAB_MINO, diabetic animals receiving minocycline; DIAB_VEH, diabetic animals receiving the vehicle (tap water); FST, forced swimming test; PDN, painful diabetic neuropathy; SHAM, control animals injected with citrate buffer; SHAM_VEH, control animals receiving the vehicle (tap water); STZ, streptozotocin.

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http://dx.doi.org/10.1016/j.bbr.2017.03.003
0166-4328/

1. Introduction

Diabetic neuropathy is one of the most common complications of diabetes affecting about 50% of patients [1]. In addition, one out of five patients with diabetic neuropathy will develop painful diabetic neuropathy (PDN), a chronic and progressive disorder described as tingling, burning, electrical or stabbing pain and hyperesthesia [2]. Importantly PDN greatly impacts the patient's quality of life causing sleep disturbances, mood and physical impairments [3]. As in other chronic diseases, some studies suggest diabetes and depression share a bidirectional relation [4] as diabetic patients are more likely to develop depression while depressive patients are more disposed to develop diabetes throughout their lifetime [5]. However, PDN management therapies are mainly aimed at preventing/retarding nerve fibre loss and alleviating painful symptoms in order to restore normal physical and psychological function [6]. The currently used PDN management therapies require prolonged administration to achieve therapeutic effect and importantly, their effectiveness is far from satisfactory [7].

In PDN, several conditions contribute to aberrant nociceptive processing. Among them are impairment of axon-glia relationship, segmental or paranodal demyelination [8], axonal injury [9] and regeneration [10]. In the central nervous system, the development of chronic pain in diabetes was attributed predominantly to neuronal dysfunction such as aberrant hyperexcitability of nociceptive spinal dorsal horn neurons and loss of function of inhibitory interneurons [11]. Recent works, however, showed that spinal glial cells, in particular microglia, are strongly activated and proliferate after diabetes as well as nerve injury [12,13]. Microglia is considered essential for surveillance, protection and restoration of the CNS [14]. Additionally, after induction of diabetes an intense microglial activation was detected in the spinal cord [15,16] and, with the progression of diabetes, evident signs of microglial activation were also present at supra-spinal level, namely in the rostroventromedial medulla (RVM), a major supraspinal modulatory centre [17,18]. This area displayed an increase in the expression of IBA-1, a marker of microglial cells, along with morphologic alterations typical of microglial activation such as retraction of glial branches [19]. Microglia activation results in the synthesis and local release of the proinflammatory cytokines IL-1β, IL-6, and TNF-α [20] that elicit robust heat hyperalgesia and mechanical allodynia [21]. Furthermore, recent studies yielded compelling evidence that releasing of proinflammatory mediators after microglial activation also play a critical role in the development of a depressive condition [22,23].

Interestingly, administration of minocycline, an FDA-approved second-generation semisynthetic derivate of tetracycline [24,25], selectively inhibited microglia activation to the *classical* proinflammatory state (M1), but not the activation to the *alternative* anti-inflammatory state (M2), which is believed to promote neuroprotection and regeneration after injury [26]. As a result, minocycline treatment attenuated hypersensitivity in several models of neuropathic pain (*e.g.*, [27–29]), including PDN [15,30–32]. Moreover, minocycline has also been proposed to exert an antidepressant effect in both humans and experimental animals [22] as a result of its neuroprotective actions including increased neurogenesis [33,34], antioxidant activity [35], anti-glutamate excitotoxicity [36–38], and regulation of pro-inflammatory molecules [39,40].

Of the several animal models of diabetic neuropathy available, the streptozotocin-induced type-1 diabetes model was shown, in addition to robust hyperglycemia and hypoinsulinemia, to display chronic pain characterized by heat allodynia and mechanical hyperalgesia [41,42]. This was associated from three weeks onwards with an increased number of spinal microglia [43] and depressive-like behaviour [44]. A recent study suggested that acute minocycline treatment might ameliorate depression-like behaviour in diabetic animals [45]. However, this study did not assess whether the minocycline-induced acute antidepressant-like effect was associated with attenuation of pain hypersensitivity. Since diabetes in the clinic is a chronic disease, it would be of potential clinical interest to assess whether prolonged treatment with minocycline in animals with painful diabetic neuropathy has an effective antidepressant effect as did the acute treatment, and whether the antidepressant effect is associated with suppression of pain behaviour. Therefore, we determined the effect of a three-week *per os* treatment with minocycline on pain sensitivity and depression-like behaviour in established PDN.

2. Materials and methods

2.1. Animals and ethical considerations

The experiments were performed using 24 eight week old male Wistar Han rats weighting between 250 and 325 g (Charles Rivers, Barcelona, Spain). Animals were randomly assigned two by two to boxes with soft bedding upon arrival; a blue line was painted in the tail of one rat and a red line in the tail of the other. Each box was numbered from 1 to 12, no indication concerning if the animals were assigned to the SHAM or DIAB group was displayed. The list discriminating the boxes corresponding to the SHAM or DIAB groups was kept by an independent party.

Food and water were available *ad libitum* and animals were maintained in a climate-controlled room, under 22 °C of temperature, 55% of humidity and a 12 h light/dark cycle with lights on at 8:00 a.m. Before the beginning of the experiments, all animals were handled daily by the experimenter for a week, and the protocols were conducted during the light phase of the cycle.

The experimental protocol followed the European Community Council Directives 2010/63/EU concerning the use of animals for scientific purposes. Moreover, all experiments were performed in accordance with the ethical guidelines for the study of pain in conscious animals [46] and were approved by the Institutional Ethical Commission (SECVS 109/2016). All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

As diabetes is a very debilitating disorder, several humane endpoints were established prior to the beginning of the experiment including loss of 20% weight per week, blindness, inability to move and urinary tract infections, which was supervised by the experimenters and the resident veterinary.

After the completion of the study, animals were euthanized with a lethal dose of pentobarbitone.

2.2. Induction of experimental type-1 diabetes

The induction of experimental type-1 diabetes mellitus was performed following an overnight fasting period and four weeks before the start of the pharmacological treatment, as described in detail elsewhere [47]. Briefly, animals received a single intraperitoneal injection of streptozotocin (STZ) (60 mg/kg body weight; Sigma–Aldrich, St. Louis, USA) dissolved in citrate buffer (0.1 M, pH 4.5) (DIAB) as described elsewhere [47,48]. Control animals were injected with citrate buffer (SHAM) alone.

In each animal, development of experimental diabetes was verified 72 h later by a third party without any specific order and without prior knowledge of the box number. Glucose concentration was measured using Accu Chek Sensor Comfort (Roche Diagnostics, Germany) in blood samples collected from the tail vein.

Only those rats that displayed glucose levels higher than 270 mg/dl were included in the DIAB group. Glycaemia levels were also determined in SHAM animals. Besides hyperglycaemia,

STZ-treated animals can also display major clinical signs of diabetes, such as polyuria, polydipsia and polyphagia, as well as cataract [41,42].

2.3. Behavioural assessment of nociception

All behavioural tests were performed during the day time, starting at 9:30 a.m. and ending at 1:30 p.m. after which the animals were returned to the animal house. All experiments were performed by trained observers and all efforts were made to ensure that the experimenters were blind to the treatment.

2.3.1. Tactile allodynia

Mechanical allodynia was measured using the up-down method with von Frey filaments [49]. Briefly, in an elevated grid the animals were placed inside an inverted transparent plastic box, to limit their movements, and each hind paw (ipsi- and contralateral) was probed with von Frey filaments (0.4, 0.6, 1.0, 2.0, 4.0, 6.0, 8.0, and 15.0 g; Stoelting, Wood Dale, IL, USA) on its plantar surface for 1–2 s. Testing was initiated with the application of the 2.0 filament, if no response was observed (nocifensive hind paw flexion reflex) a stronger stimulus was presented otherwise the next weaker stimulus was applied. The up-down process was repeated 4 times after the first change in response, and the 50% threshold for paw withdrawal was determined combining the individual response pattern and the value of the last von Frey filament used [49,50].

2.3.2. Cold allodynia

Cold hypersensitivity was assessed by applying a drop of acetone to the plantar surface of both hind limbs with the animals atop the elevated grid and confined by an inverted transparent plastic box. The pain-related behaviour evoked by the cooling of acetone was graded to the following 4-point scale: 0 – no response; 1 – quick withdrawal, flick or stamp of the paw; 2 – prolonged withdrawal or repeated flicking of the paw; 3 – repeated flicking and licking of the paw [51]. Acetone was applied three times to the hind paw (with 3 min interval between each successive application) and the responses scored categorically. For each animal, the average of the scores obtained in the 3 measurements was used for the analysis.

2.3.3. Mechanical hyperalgesia

Behavioural evaluation of mechanical hyperalgesia was performed using the paw pressure test (Randall–Selitto test, Model 37215, Ugo-Basile, Comerio, Italy) [47,52]. To perform the test each animal was carefully immobilized in the experimenter hand and increasing mechanical forces were applied to the medial portion of the dorsal surface of both hind paws (alternately) with a cone-shaped plunger. The mechanical force (in grams) that evoked hind paw withdrawal was recorded. The paw withdrawal threshold (PWT) of each animal was considered to be the average of three consecutive measurements, sampled with 5 min intervals.

2.3.4. Heat hyperalgesia

Heat hyperalgesia was evaluated using the tail-flick test [53,54]. For assessing heat hyperalgesia, a radiant heat source was placed midway in the tail of the awake animals and the time spent between the heat application and the withdrawal response (Plantar Test Instrument, Model 37360, Ugo Basile, Varese, Italy) was registered as the tail withdrawal latency (TWL). In each time point, the TWL was evaluated twice at an interval of 1 min and the mean of these values was used in further calculations. Cut-off time was 15 s.

2.4. Assessment of depressive-like behaviour

Learned helplessness using the forced-swimming test (FST) [55], (with minor modifications) was used as an index of depressive-like behaviour. Animals were submitted to a pre-habituation session (10 min) in which they were individually placed in a cylinder filled with water (25 °C; depth 30 cm). Twenty-four hours later animals were again placed in the cylinders for a period of 5 min and the testing session was recorded with a video camera. The quantification of (i) latency to immobility, time until the first bout of immobility after placing the animal in the cylinder [56], (ii) Immobility duration, when the animal was floating or making only minimal movements to keep the head above water; (iii) swimming duration, when the animal was making active swimming with movement of all four limbs with body aligned horizontally, and; (iv) climbing duration, when the animal was making active movements with its forepaws in and out of the water, usually directed against the cylinder walls [57,58]. Behavioural evaluations were performed by a blind observer that rated the videos using the Etholog software [59]. Learned helplessness behaviour was defined as an increase in time of immobility at the expense of the time spent swimming/climbing and a decrease in the latency to immobility.

2.5. Drugs

Minocycline (Minotrex, Medinfar, Portugal) was prepared in sterilized tap water, and administered once a day (80 mg/kg body weight p.o.). Control treatment was performed with sterilized tap water alone (VEH; p.o.). The route of administration and concentration were chosen based on previous work indicating microglial inhibition after chronic minocycline administration [29,30].

2.6. Experimental design

On the week preceding the STZ injection, animals were submitted to a baseline evaluation of the general health parameters, such as body weight, blood glucose levels, and water and food intake, as well as nociceptive behaviour using the von Frey, acetone, Randall-Selitto and tail-flick tests. Animals were then randomly assigned to the experimental group DIAB or SHAM, and four weeks after STZ administration all animals were again tested for nociceptive behaviour. Diabetic rats were randomly selected to receive daily minocycline (DIAB_MINO) or vehicle (DIAB_VEH) treatment for 3 weeks, whereas the SHAM non-diabetic rats received vehicle (tap water) alone (SHAM_VEH). At the end of the pharmacological treatment all animals were again tested to assess the nociceptive and comorbid depressive-like behaviour (FST) (Fig. 1).

2.7. Statistical analyses

To evaluate the effect of minocycline upon nociceptive and emotional-like behaviour, the minimum number of animals needed was determined à priori using the G power software (version 3.1.9.2, University of Kiel, Germany). Considering a two-way ANOVA test, an error probability of 0.05, power of 0.95 and an effect size of 0.25, the minimum number was n = 24.

The GraphPad Prism® 6 software (GraphPad Software Inc, La Jolla, CA, USA) was used to perform the statistical analyses. Differences in weight gain, glycaemia levels, water and food consumption and nociceptive behaviours between DIAB and SHAM groups were evaluated by applying a mixed-design two-way analysis of variance (ANOVA) followed by *t*-test with a Bonferroni correction for multiple comparisons.

The comparisons of differences between groups in the behavioural assessment were performed using a two-way and one-way ANOVA followed by *t*-test with a Bonferroni correction for multiple comparisons. The association between mechanical sensitivity in the von Frey test and depressive-like behaviour (immobility time and time spent swimming) was performed using a Pearson's correlation in order to explore if the suppression of

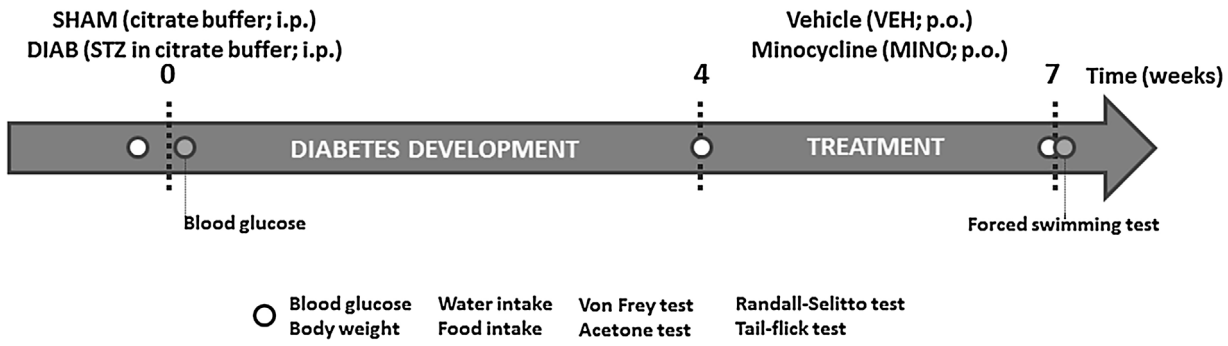

Fig. 1. Experimental design. On the week preceding the STZ injection, animals were submitted to a baseline evaluation of the general health parameters, such as body weight, blood glucose levels, and water and food intake, as well as nociceptive behaviour using the von Frey, acetone, Randall-Selitto and tail-flick tests. Animals were then randomly assigned to the experimental group DIAB (animals receiving STZ in citrate buffer, i.p) or SHAM (control animals receiving citrate buffer alone, i.p), and four weeks after STZ administration all animals were again tested for nociceptive behaviour. DIAB rats were randomly selected to receive daily *per os* (p.o.) minocycline (DIAB_MINO) or vehicle (tap water) (DIAB_VEH) treatment for three weeks, whereas the SHAM non-diabetic rats received vehicle alone (SHAM_VEH). At the end of the pharmacological treatment all animals were again tested to assess the nociceptive behaviour as well as tested for the development of comorbid depressive-like behaviour in the forced swimming test. Open circles represent the repeated evaluation of general health parameters and nociception.

pain behaviour by prolonged minocycline treatment is associated with antidepressive-like effect. Statistical significance was accepted for $P < 0.05$. Data in the results section are expressed as mean ± standard error of the mean (SEM). All statistical analyses were performed on raw data. No method of data normalization was used.

3. Results

3.1. Development of metabolic signs of diabetes

Blood glucose levels, body weight, water and food intakes were measured throughout the experimental period. All STZ-injected animals exhibited hyperglycaemia (458.16 ± 68.16 mg/dl) when measured 3 days post injection. Across the experimental period, the levels of glucose in the blood varied throughout time (main effect of time: $F_{(2,42)} = 610.20$, $P < 0.001$) and with the experimental group (interaction time x group: $F_{(2,42)} = 184.30$, $P < 0.001$). *Post-hoc* tests showed higher glucose levels in DIAB_VEH and DIAB_MINO than in SHAM_VEH animals while treatment with minocycline partly decreased the glucose levels when compared to DIAB_VEH animals (Fig. 2A).

Body weight also varied throughout time (main effect of time: $F_{(2,42)} = 31.63$, $P < 0.001$) and with the experimental group (interaction time x group: $F_{(2,42)} = 57.54$, $P < 0.001$). *Post-hoc* tests showed decreased body weight in DIAB animal, independently of minocycline treatment, when compared with SHAM_VEH animals (Fig. 2B).

Water and food intakes were significantly altered during time (Water: main effect of time: $F_{(2,18)} = 443.20$, $P < 0.001$); (Food: main effect of time: $F_{(2,18)} = 91.17$, $P < 0.001$) and varied with the experimental group (Water: interaction time x group: $F_{(2,18)} = 111.00$, $P < 0.001$); (Food: main effect of time x group: $F_{(2,18)} = 19.14$, $P < 0.001$). *Post-hoc* tests showed that DIAB animals drank and ate significantly more than SHAM animals, independent of minocycline treatment (Fig. 2C,D).

3.2. Minocycline and nociceptive behaviour in diabetic animals

Mechanical allodynia varied throughout time (main effect of time: $F_{(2,42)} = 31.63$, $P < 0.001$) and with the experimental group (interaction time x group: $F_{(2,42)} = 19.23$, $P < 0.001$). P*ost-hoc* tests indicated DIAB animals gradually increase their mechanical allodynia post STZ injection with minocycline treatment partly reversing the increase of allodynia (Fig. 3A).

Cold allodynia varied throughout time (main effect of time: $F_{(2,42)} = 10.46$, $P < 0.001$) and with the experimental group (interaction time x group: $F_{(2,42)} = 2.63$, $P = 0.048$). *Post-hoc* tests showed DIAB animals displayed increased cold sensitivity when compared to SHAM animals, an effect that was not reversed by treatment with minocycline (Fig. 3B).

Mechanical hyperalgesia varied throughout the experimental period (main effect of time: $F_{(2,42)} = 38.46$, $P < 0.001$) and with the experimental group (interaction time x group: $F_{(2,42)} = 16.57$, $P < 0.001$). *Post-hoc* tests showed DIAB animals displayed decreased paw withdrawal threshold. Diabetes-induced mechanical hyperalgesia was not significantly influenced by treatment with minocycline (Fig. 3C).

Heat sensitivity varied throughout time (main effect of time: $F_{(2,42)} = 29.47$, $P < 0.001$) and with the experimental group (interaction time x group: $F_{(2,42)} = 7.02$, $P < 0.001$). *Post-hoc* tests showed a decrease in heat sensitivity (increase tail-flick latency) in DIAB animals, independent of treatment with minocycline (Fig. 3D).

3.3. Minocycline and mood-like behaviour in diabetic animals

In the FST, DIAB animals displayed depressive-like behaviour as shown by an increased immobility time ($F_{(2,21)} = 16.65$, $P < 0.001$) (Fig. 4B) as well as decreased time spent swimming ($F_{(2,21)} = 16.39$, $P < 0.001$) (Fig. 4C) and climbing ($F_{(2,21)} = 4.21$, $P = 0.029$)(Fig. 4D), while the latency to immobility was not significantly influenced by diabetes ($F_{(2,21)} = 1.86$, $P = 0.180$)(Fig. 4A). The treatment of DIAB animals with minocycline improved the depressive-like phenotype by decreasing the immobility time and increasing the time spent swimming (Fig. 4B,C), but failed to influence latency to immobility (Fig. 4A) or climbing time (Fig. 4D). Moreover, the time animals spent immobile correlates with the mechanical sensitivity in the von Frey test ($r = -0.713$, $n = 24$, $P < 0.001$) as did the time spent swimming ($r = -0.667$, $n = 24$, $P < 0.001$).

4. Discussion

The present work investigated the antidepressant-like and antinociceptive effect of minocycline administration in the STZ-induced type-1 diabetes experimental model. Four weeks after the induction of diabetes, animals displayed sustained hyperglycaemia, polyuria, polydipsia, polyphagia, weight gain impairment, tactile and cold allodynia, mechanical hyperalgesia and heat hypoalgesia. These findings mimic some of the features observed in non-treated diabetic human patients. The administration of minocycline partly reversed the diabetes-induced tactile allodynia, but failed to have a significant effect on the diabetes-induced mechanical hyperalgesia or heat hypoalgesia. Moreover, minocycline treatment for three

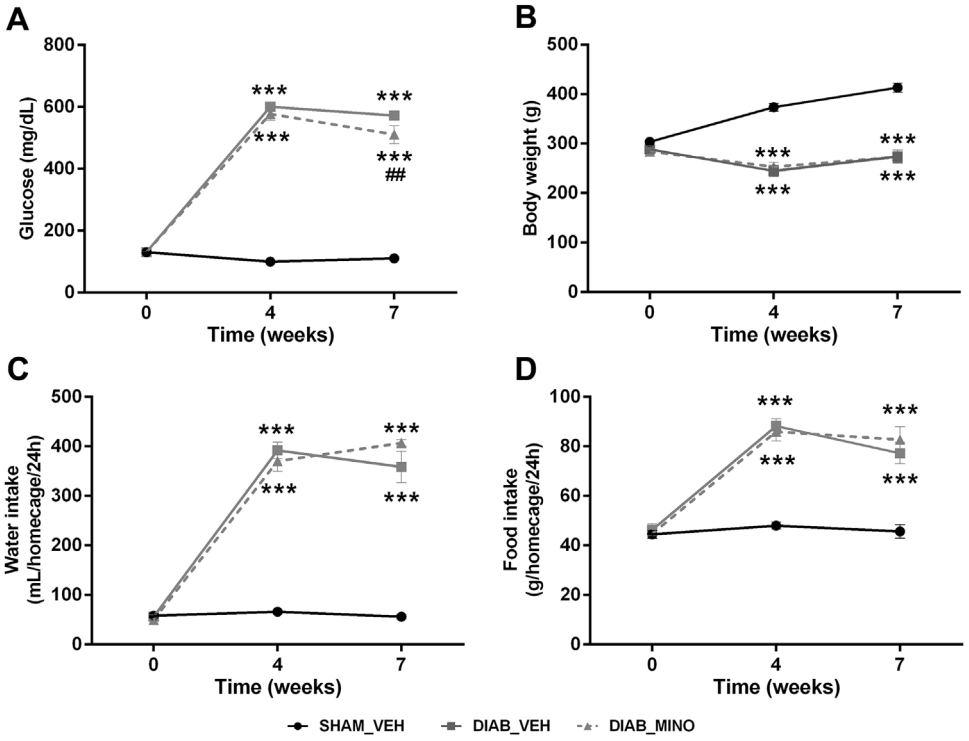

Fig. 2. General health condition of animals throughout the experimental period. Minocycline treatment partly decreased glycaemia (A) in diabetic animals although it did not improve the body weight alterations (B) induced by diabetes. Minocycline administration did not alter water (C) or food (D) intake in diabetic animals. SHAM_VEH (n = 8): sham animals receiving vehicle in the treatment period; DIAB_VEH (n = 8): diabetic animals receiving vehicle in the treatment period; DIAB_MINO (n = 8): diabetic animals receiving minocycline in the treatment period. Graphs show mean + SEM. $^{***}P < 0.001$: Comparing with the SHAM_VEH group at the same time point. $^{\#\#}P < 0.01$: Comparing with the DIAB_VEH group at the same time point (*t*-test with a Bonferroni correction for multiple comparisons).

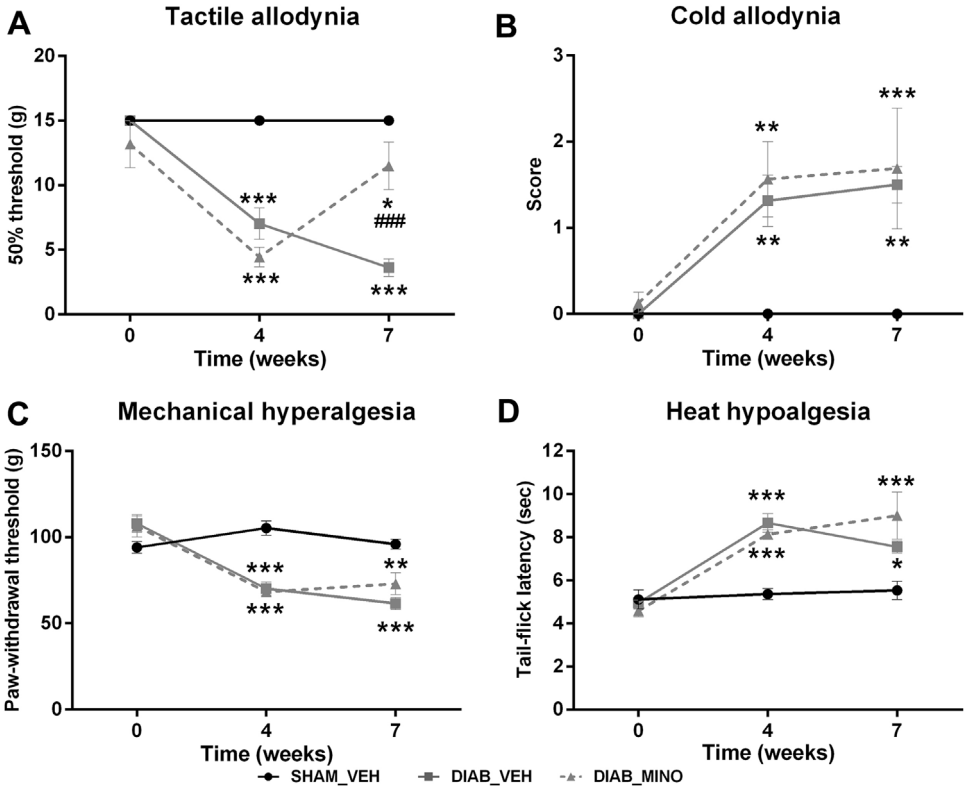

Fig. 3. Nociceptive behaviour. Minocycline treatment partially reversed tactile allodynia in diabetic animals (A) although it did not improve cold allodynia (B), mechanical hyperalgesia (C) and heat hypoalgesia (D) observed in diabetes. SHAM_VEH (n = 8): sham animals receiving vehicle in the treatment period; DIAB_VEH (n = 8): diabetic animals receiving vehicle in the treatment period; DIAB_MINO (n = 8): diabetic animals receiving minocycline in the treatment period. Graphs show mean + SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$: Comparing with the SHAM_VEH group at the same time point. $^{\#\#\#}P < 0.001$: Comparing with the DIAB_VEH group at the same time point (*t*-test with a Bonferroni correction for multiple comparisons).

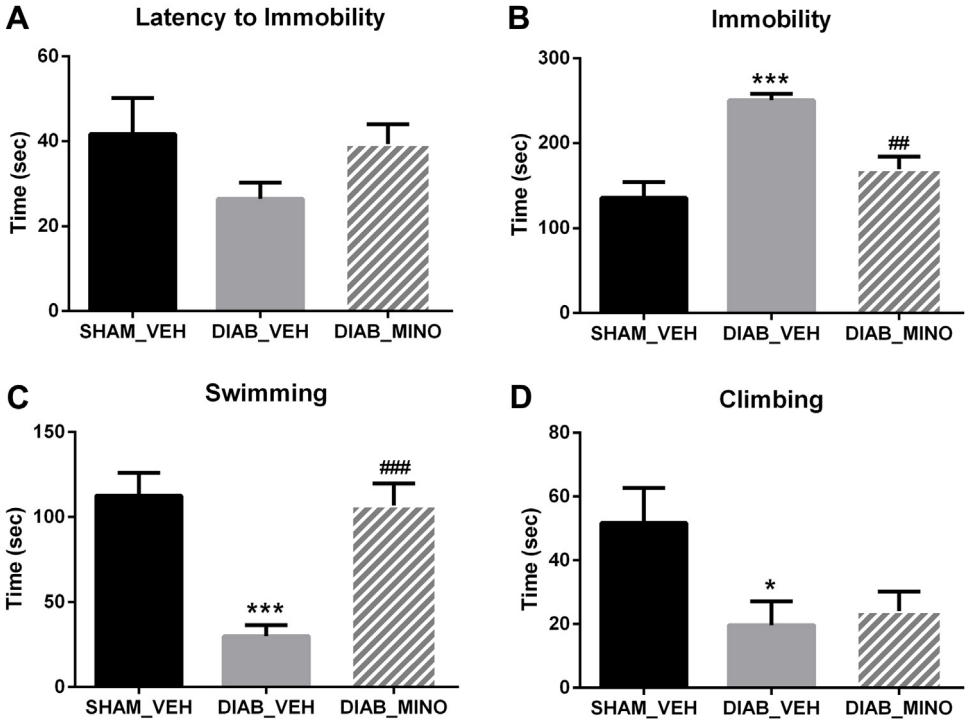

Fig. 4. Depressive-like behaviour. Minocycline treatment partly improved depressive-like behaviour displayed by diabetic animals by decreasing the time animals spent immobile (B) and increasing the time spent swimming (C). The latency to immobility (A) and the time spent climbing (D) were not altered after minocycline treatment. SHAM_VEH (n = 8): sham animals receiving vehicle in the treatment period; DIAB_VEH (n = 8): diabetic animals receiving vehicle in the treatment period; DIAB_MINO (n = 8): diabetic animals receiving minocycline in the treatment period. Graphs show mean + SEM. $^{*}P < 0.05$, $^{***}P < 0.001$: Comparing with the SHAM_VEH group. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$: Comparing with the DIAB_VEH group (*t*-test with a Bonferroni correction for multiple comparisons).

weeks rescued the diabetes-induced depressive-like phenotype by reducing immobility time and increasing swimming time. Together our results suggest that minocycline could be used as adjuvant drug in pain management therapies of patients displaying PDN and comorbid depression.

4.1. Minocycline in the control of chronic pain in experimental diabetes

The present findings on mechanically evoked pain behaviour in diabetic animals are in line with those of earlier experimental animal studies demonstrating an association between prolonged diabetes and the development of chronic pain, as suggested by mechanical allodynia and hyperalgesia [60]. Concerning heat sensitivity of diabetic animals, the results from earlier animal studies have been variable. Many studies have reported heat hyperalgesia (*e.g.*, [41]), while some studies have reported no marked change in heat sensitivity up to eight weeks of diabetes (*e.g.*, [61,62]), or heat hypoalgesia (*e.g.*, [63]) as in the present study. Among potential explanations for this variability in heat sensitivity are diabetes-induced changes in the skin temperature that is a confounding factor in heat pain assessments [63], the animal strain used, and other differences in various experimental parameters. Among these is the duration of diabetes that is likely to be an important parameter explaining variability in heat sensitivity, since peptidergic intraepidermal nerve fibres that presumably are involved in transduction of noxious heat start to express signs of damage as early as four weeks after chemical induction of diabetes [64]. Damage of nociceptive nerve fibre endings may contribute to the development of heat hypoalgesia. In human studies, the development of hyper- or hypoalgesia has been inconsistent [65,66]. It may be argued that the insensate symptoms experienced by many diabetic patients resemble heat hypoalgesia observed in diabetic animals of the present study.

Although there is a large body of pharmacological agents used in the management of diabetic neuropathy, current therapies are still unsatisfactory and the need to find new and efficient drugs remains. In the last decade, some promising data from animal models suggested that minocycline, a semi-synthetic second-generation tetracycline antibiotic able to cross the blood brain barrier, could be used to ameliorate several complications associated to diabetes, such as diabetic macular oedema [67] and neuronal death due to hypoglycaemia [68]. This drug, unlike other tetracyclines, has an absorption rate of 95–100% when orally administered. Moreover, high hydrophilic properties facilitates the crossing of the blood brain barrier, with cerebrospinal fluid presenting around 50% of the concentration found in the plasma [69,70]. Importantly, plasma concentration is almost not affected by renal impairments, a condition usually present in diabetes [69,70]. Further support was provided by a human study reporting that a six-week oral treatment with minocycline was safe and it improved peripheral and autonomic neuropathy in type-2 diabetic patients [71].

Overall, minocycline was partly able to reverse nociceptive impairments induced by four weeks of experimental type-1 diabetes, as only diabetes-induced mechanical allodynia unlike mechanical hyperalgesia, cold allodynia or heat hypoalgesia was attenuated by minocycline in the present study. Earlier studies in diabetic animals reported that minocycline attenuated mechanical hyperalgesia [15], heat hyperalgesia [30], cold hyperalgesia or [30,32,72] as well as mechanical allodynia [31,32,72]. Among potential explanations for observing only mechanical antiallodynic effect by orally administered minocycline in the present study but no significant effect on other submodalities of pain is the route of drug administration. In line with this, drug was administered intrathecally in some of the studies reporting minocycline-induced

attenuation of pain hypersensitivity to heat, cold or noxious mechanical stimulation in diabetic animals [15,31,72]. However, other studies also reported that systemic (intraperitoneal) administration of minocycline attenuated pain behaviour evoked by heat or cold as well as innocuous mechanical stimulation [30,32]. Previous works on the effect of minocycline in non-diabetic neuropathic pain hypersensitivity suggested that the effect of treatment may depend on the time at which treatment started. Minocycline has been effective when treatment started early, before or within one to two days after the induction of non-diabetic neuropathic pain both with intrathecal [73–75] and systemic [27,29,76–78] administrations. On the other hand, when minocycline treatment started after the establishment of non-diabetic neuropathic condition, its effect on pain behaviour, independent of the route of administration, has been poor [27,73,78]. These earlier findings in non-diabetic neuropathic pain raise the hypothesis that starting the minocycline treatment as late as four weeks after the induction of diabetes may explain that in the present study it attenuated pain behaviour evoked by innocuous mechanical stimulation but not by other types of nociceptive testing. In line with this hypothesis, minocycline treatment that started early after diabetes induction resulted in a significant antihypersensitivity effect against various types of nociceptive stimuli in some of the studies [32,72]. However, other earlier studies reported that minocycline treatment starting 2–4 weeks after diabetes induction effectively attenuated pain behaviour evoked by heat, cold or noxious mechanical stimulation [15,30].

Diabetes-induced hypersensitivity to innocuous mechanical stimulation (*i.e.*, tactile allodynia) was significantly suppressed by minocycline in the present study. It has been reported that tactile allodynia in animals with type-1 diabetes is associated with increased activation of dorsal horn microglia as well as enhanced activation of intracellular signalling molecules associated to microglial functions [79]. This is in line with the evidence indicating that minocycline suppresses microglial activation, thereby preventing release of proinflammatory mediators [76,80]. Another potential mechanism explaining tactile antiallodynic action of minocycline in diabetic animals is inhibition of the expression of spinal N-methyl-D-aspartic acid receptor 1 as demonstrated by Pu et al. [81] in the spinal nerve ligation model of neuropathy. It remains to be elucidated why minocycline at the dose producing a significant tactile antiallodynic effect failed to produce a significant mechanical antihyperalgesic effect in the present study. Among possible explanations is that pain behaviours evoked by innocuous versus noxious mechanical stimulation are based on at least partly different mechanisms [82,83] and therefore, the mechanisms underlying the diabetes-induced nociceptive facilitation are differentially sensitive to minocycline. Additionally or alternatively, afferent barrage evoked by noxious stimulation is likely to be stronger than that evoked by innocuous stimulation, which may also explain why mechanical hyperalgesia is not as sensitive to suppression by minocycline treatment as mechanical allodynia.

TRPV1-expressing peptidergic nociceptive nerve fibres are considered to have an important role in transduction of noxious heat [84]. In patients with diabetic neuropathy, TRPV1-expressing cutaneous and epidermal nerve fibres were decreased [85]. In experimental animals, peptidergic intraepidermal nerve fibres were decreased four weeks after induction of diabetes [64], at which time point we showed heat hypoalgesia and treatment period of three weeks started. These findings suggest that a loss of nociceptive nerve endings or their function may have contributed to the present decrease of heat sensitivity. In our work, treatment with minocycline failed to reverse heat hypoalgesia suggesting that minocycline may not rescue loss of heat nociceptors or their function in peripheral axon terminals of diabetic animals.

STZ-induced rat model of diabetic neuropathy [41] has also been associated with hypersensitivity to cold, which finding is in line with present results. In contrast to the present results, it has been reported that systemic minocycline treatment for two to three weeks suppressed cold hypersensitivity [30,32]. However, this apparent controversy may reflect differences in methods used for testing cold-evoked behaviour rather than a difference in the minocycline effect. In the present study, cold hypersensitivity was assessed using a method (paw flick evoked by acetone drops on the skin) that allows assessing cold allodynia rather than cold hyperalgesia, whereas studies reporting minocycline-induced suppression of cold hypersensitivity in PDN used methods (exposure to water or plate of noxiously cold temperature) that allow assessing cold hyperalgesia rather than cold allodynia [30,32]. Cold allodynia and cold hyperalgesia are based on at least partly different mechanisms [86]. Together these results suggest that cold hyperalgesia is more sensitive to treatment with minocycline than cold allodynia. However, in the early phase of diabetes (4th day after its induction) acute minocycline treatment had a short-lasting antiallodynic effect in cold testing as well as mechanical allodynia [72].

It should be noted that the present study focused on a model of type-1 diabetes and therefore, the results may not be applicable to type-2 diabetes. In line with this, an earlier experimental study in animals with type-2 diabetes suggested that minocycline that inhibits microglia was inefficient in attenuating pain hypersensitivity, while a drug inhibiting astrocyte activation efficiently attenuated pain hypersensitivity induced by type-2 diabetes [87].

4.2. Minocycline in the control of diabetes-associated emotional impairments

In the present study, diabetic animals displayed depressive-like behaviour in the FST. The association between chronic PDN and the development of emotional disorders is well established in patients [88,89] and rats [48,90]. In the rat, the attempt at controlling diabetes-induced depressive-like behaviour has been explored using several drugs including antidepressants such as fluoxetine [91] and imipramine [92], antioxidants such as N-acetylcysteine [93] and endocannabinoids such as anandamide [48] among others.

A recent study in fully established STZ-induced diabetes showed that one-day intraperitoneal treatment with minocycline reversed the diabetes-induced reduction in swimming in the FST and this acute antidepressive-like effect of minocycline was associated with suppression of a neuroinflammatory pathway in the hippocampus of diabetic animals [45]. The present study is in agreement with this recent finding and extend it by showing that the antidepressive-like effect of minocycline in established diabetes is maintained even when the daily minocycline treatment is prolonged at least up to three weeks. Moreover, the antidepressive-like effect of prolonged minocycline treatment was associated with tactile antiallodynic effect in PDN. Chronic pain *per se* has been associated with comorbid depression-like behaviour in various experimental pain models [94–98] and therefore, it might be argued that the present antidepressive-like effect of minocycline was indirectly caused by attenuation of diabetes-induced pain. However, the finding that the antidepressive-like effect of minocycline was associated only with mechanical antiallodynic effect but not with corresponding changes in other submodalities of pain suggests that it may be at least in part due to action on emotion-related mechanisms [45].

Even though a commission of experts in mental health and in diabetes stressed the need to fill the gap of knowledge concerning comorbid diabetes and depression [99], most works evaluating the efficiency of different types of drugs in the treatment of diabetes comorbidities, both in patients and in rodents, still mostly focus on managing pain and sleep disorders, rarely anxiety and seldom even address depression [100–104]. By demonstrating the

dual effect of minocycline upon diabetes-induced nociceptive and depressive-like impairments we highlight the potential use of this already FDA-approved drug as an alternative to the most commonly used, but still inefficient in both pain and depression, therapies such as anticonvulsants or antidepressants. Our data also provide further evidence of the importance of microglia activation in the pathophysiology of pain and depression related to diabetes. Consequently, drugs targeting microglia activation might be suitable candidates in novel pain management therapies.

The proposal that minocycline might be useful in treatment of depression is supported by the finding that minocycline suppressed depression-like symptoms also in non-diabetic conditions, such as when induced by mild foot shocks [105]. Importantly, in human subjects, minocycline treatment was able to reduce the affective dimension associated with traumatic neuropathic pain [106] further supporting the proposal that minocycline might be a treatment option in clinical conditions.

5. Conclusions

STZ-induced model of type-1 diabetes displayed, after four weeks, mechanical allodynia and hyperalgesia, cold allodynia and heat hypoalgesia indicating that the animals had developed a PDN-like condition. These sensory findings were associated with depression-like behaviour. A three-week per oral treatment with minocycline (80 mg/kg once a day) starting on the fourth week of diabetes significantly attenuated mechanical allodynia and depression-like behaviour, while the effect of minocycline on other sensory responses was not significant. The results support the hypothesis that prolonged per oral minocycline treatment may provide a treatment option against mechanical hypersensitivity and comorbid emotional disorders in chronic PDN.

Competing interest

The authors declare that they have no competing interests.

Acknowledgements

This study was supported by grants from the Portuguese Science Foundation (FCT) Project no. PTDC/SAU-643NEU/108557/2008, FEDER-COMPETE, and the Sigrid Juselius Foundation, Helsinki, Finland and co-financed by the Portuguese North Regional Operational Program (ON.2 – O Novo Norte) under the National Strategic Reference Framework (QREN), through the European Regional Development Fund (FEDER).

References

[1] M.M. Huizinga, A. Peltier, Painful diabetic neuropathy: a management-centered review, Clin. Diabetes 25 (2007) 6–15.
[2] A.M. Aring, D.E. Jones, J.M. Falko, Evaluation and prevention of diabetic neuropathy, Am. Fam. Physicianamily Phys. 71 (2005) 2123–2128.
[3] B. Galer, A. Gianas, M. Jensen, Painful diabetic polyneuropathy: epidemiology, pain description, and quality of life, Diabetes Res. Clin. Pract. 47 (2000) 123–128.
[4] B.N. Renn, L. Feliciano, D.L. Segal, The bidirectional relationship of depression and diabetes: a systematic review, Clin. Psychol. Rev. 31 (2011) 1239–1246.
[5] S.H. Golden, M. Lazo, M. Carnethon, A.G. Bertoni, P.J. Schreiner, A.V. Diez Roux, H.B. Lee, C. Lyketsos, Examining a bidirectional association between depressive symptoms and diabetes, JAMA 299 (2008) 2751–2759.
[6] S. Javed, I.N. Petropoulos, U. Alam, R.A. Malik, Treatment of painful diabetic neuropathy, Ther. Adv. Chronic Dis. 6 (2015) 15–28.
[7] M. Wong, J.W. Chung, T.K. Wong, Effects of treatments for symptoms of painful diabetic neuropathy: systematic review, BMJ 335 (2007) 87.
[8] A. Sima, V. Nathaniel, V. Bril, T. McEwen, D. Greene, Histopathological heterogeneity of neuropathy in insulin-dependent and non-insulin-dependent diabetes, and demonstration of axo-glial dysjunction in human diabetic neuropathy, J. Clin. Invest. 81 (1988) 349–364.
[9] P.J.B. Dyck, E. Lambert, P. O'Brien, Pain in peripheral neuropathy related to rate and kind of fiber degeneration, Neurology 26 (1976) 466–471.
[10] J.A. Tracy, P.J.B. Dyck, The spectrum of diabetic neuropathies, Phys. Med. Rehabil. Clin. N. Am. 19 (2008) 1–26.
[11] R. D'Mello, A.H. Dickenson, Spinal cord mechanisms of pain, Br. J. Anaesth. 101 (2008) 8–16.
[12] M.R. Suter, Y.-R. Wen, I. Decosterd, R.-R. Ji, Do glial cells control pain? Neuron Glia Biol. 3 (2007) 255–268.
[13] D. Wang, R. Couture, Y. Hong, Activated microglia in the spinal cord underlies diabetic neuropathic pain, Eur. J. Pharmacol. 728 (2014) 59–66.
[14] M.W. Salter, S. Beggs, Sublime microglia: expanding roles for the guardians of the CNS, Cell 158 (2014) 15–24.
[15] C. Morgado, P. Pereira-Terra, C. Cruz, I. Tavares, Minocycline completely reverses mechanical hyperalgesia in diabetic rats through microglia-induced changes in the expression of the potassium chloride co-transporter 2 (KCC2) at the spinal cord, Diabetes Obes. Metab. 13 (2011) 150–159.
[16] R. Wodarski, A.K. Clark, J. Grist, F. Marchand, M. Malcangio, Gabapentin reverses microglial activation in the spinal cord of streptozotocin-induced diabetic rats, Eur. J. Pain 13 (2009) 807–811.
[17] M.M. Heinricher, I. Tavares, J. Leith, B. Lumb, Descending control of nociception: specificity, recruitment and plasticity, Brain Res. Rev. 60 (2009) 214–225.
[18] A. Almeida, H. Leite-Almeida, I. Tavares, Medullary control of nociceptive transmission: reciprocal dual communication with the spinal cord, Drug Discov. Today Dis. Mech. 3 (2006) 305–312.
[19] M. Silva, J.T. Costa-Pereira, D. Martins, I. Tavares, Pain modulation from the brain during diabetic neuropathy: uncovering the role of the rostroventromedial medulla, Neurobiol. Dis. 96 (2016) 346–356.
[20] R.-R. Ji, M.R. Suter, p38 MAPK, microglial signaling, and neuropathic pain, Mol. Pain 3 (2007) 33.
[21] Y. Kawasaki, L. Zhang, J.-K. Cheng, R.-R. Ji, Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6 and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord, J. Neurosci. 28 (2008) 5189–5194.
[22] R. Yirmiya, N. Rimmerman, R. Reshef, Depression as a microglial disease, Trends Neurosci. 38 (2015) 637–658.
[23] L.R. Frick, K. Williams, C. Pittenger, Microglial dysregulation in psychiatric disease, Clin. Dev. Immunol. 2013 (2013) 608654.
[24] N. Garrido-Mesa, A. Zarzuelo, J. Gálvez, Minocycline: far beyond an antibiotic, Br. J. Pharmacol. 169 (2013) 337–352.
[25] E. Setiawati, A. Purnomo, S. Deniati, D. Yunaidi, L. Handayani, G. Harinato, I. Santoso, Bioequivalence study of two minocycline capsule formulations in healthy volunteers, Arzneimittelforschung 59 (2011) 532–536.
[26] K. Kobayashi, S. Imagama, T. Ohgomori, K. Hirano, K. Uchimura, K. Sakamoto, A. Hirakawa, H. Takeuchi, A. Suzumura, N. Ishiguro, K. Kadomatsu, Minocycline selectively inhibits M1 polarization of microglia, Cell. Death. Dis. 4 (2013) e525.
[27] V. Raghavendra, F. Tanga, J.A. DeLeo, Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy, J. Pharmacol. Exp. Ther. 306 (2003) 624–630.
[28] J. Mika, M. Osikowicz, W. Makuch, B. Przewlocka, Minocycline and pentoxifylline attenuate allodynia and hyperalgesia and potentiate the effects of morphine in rat and mouse models of neuropathic pain, Eur. J. Pharmacol. 560 (2007) 142–149.
[29] N.N. Burke, D.M. Kerr, O. Moriarty, D.P. Finn, M. Roche, Minocycline modulates neuropathic pain behaviour and cortical M1-M2 microglial gene expression in a rat model of depression, Brain. Behav. Immun. 42 (2014) 147–156.
[30] K. Pabreja, K. Dua, S. Sharma, S.S. Padi, S.K. Kulkarni, Minocycline attenuates the development of diabetic neuropathic pain: possible anti-inflammatory and anti-oxidant mechanisms, Eur. J. Pharmacol. 661 (2011) 15–21.
[31] J. Sun, Y. Yang, Y. Zhang, W. Huang, Z. Li, Y. Zhang, Minocycline attenuates pain by inhibiting spinal microglia activation in diabetic rats, Mol. Med. Rep. 12 (2015) 2677–2682.
[32] M. Zychowska, E. Rojewska, A. Piotrowska, G. Kreiner, J. Mika, Microglial inhibition influences XCL1/XCR1 expression and causes analgesic effects in a mouse model of diabetic neuropathy, Anesthesiology 125 (2016) 573–589.
[33] L. Zhang, K. Kitaichi, Y. Fujimoto, H. Nakayama, E. Shimizu, M. Iyo, K. Hashimoto, Protective effects of minocycline on behavioral changes and neurotoxicity in mice after administration of methamphetamine, Prog. Neuro Psychopharmacol. Biol. Psychiatry 30 (2006) 1381–1393.
[34] W. Noble, C.J. Garwood, D.P. Hanger, Minocycline as a potential therapeutic agent in neurodegenerative disorders characterised by protein misfolding, Prion 3 (2009) 78–83.
[35] R.L. Kraus, R. Pasieczny, K. Lariosa-Willingham, M.S. Turner, A. Jiang, J.W. Trauger, Antioxidant properties of minocycline: neuroprotection in an oxidative stress assay and direct radical-scavenging activity, J. Neurochem. 94 (2005) 819–827.
[36] R. Pi, W. Li, N.T. Lee, H.H. Chan, Y. Pu, L.N. Chan, N.J. Sucher, D.C. Chang, M. Li, Y. Han, Minocycline prevents glutamate-induced apoptosis of cerebellar granule neurons by differential regulation of p38 and Akt pathways, J. Neurochem. 91 (2004) 1219–1230.
[37] B. Goñi-Allo, M. Ramos, J. Jordán, N. Aguirre, In vivo studies on the protective role of minocycline against excitotoxicity caused by malonate or N-methyl-d-aspartate, Exp. Neurol. 191 (2005) 326–330.

[38] L.-J. Jin, F. Schlesinger, Q. Guan, Y.-P. Song, Z.-Y. Nie, The two different effects of the potential neuroprotective compound minocycline on AMPA-type glutamate receptors, Pharmacology 89 (2012) 156–162.
[39] C.-U. Pae, D.M. Marks, C. Han, A.A. Patkar, Does minocycline have antidepressant effect? Biomed. Pharmacother. 62 (2008) 308–311.
[40] N. Wang, X. Mi, B. Gao, J. Gu, W. Wang, Y. Zhang, X. Wang, Minocycline inhibits brain inflammation and attenuates spontaneous recurrent seizures following pilocarpine-induced status epilepticus, Neuroscience 287 (2015) 144–156.
[41] C. Courteix, A. Eschalier, J. Lavarenne, Streptozocin-induced diabetic rats: behavioural evidence for a model of chronic pain, Pain 53 (1993) 81–88.
[42] A. Fox, C. Eastwood, C. Gentry, D. Manning, L. Urban, Critical evaluation of the streptozotocin model of painful diabetic neuropathy in the rat, Pain 81 (1999) 307–316.
[43] S.H. Kim, J.K. Kwon, Y.B. Kwon, Pain modality and spinal glia expression by streptozotocin induced diabetic peripheral neuropathy in rats, Lab. Anim. Res. 28 (2012) 131–136.
[44] S. Haider, S. Ahmed, S. Tabassum, Z. Memon, M. Ikram, D.J. Haleem, Streptozotocin-induced insulin deficiency leads to development of behavioral deficits in rats, Acta Neurol. Belg. 113 (2013) 35–41.
[45] I.C. da Silva Dias, B. Carabelli, D.K. Ishii, H. de Morais, M.C. de Carvalho, L.E. Rizzo de Souza, S.M. Zanata, M.L. Brandão, T.M. Cunha, A.C. Ferraz, J.M. Cunha, J.M. Zanoveli, Indoleamine-2,3-dioxygenase/kynurenine pathway as a potential pharmacological target to treat depression associated with diabetes, Mol. Neurobiol. 53 (2016) 6997–7009.
[46] M. Zimmermann, Ethical guidelines for investigations of experimental pain in conscious animals, Pain 16 (1983) 109–110.
[47] M. Silva, D. Amorim, A. Almeida, I. Tavares, F. Pinto-Ribeiro, C. Morgado, Pronociceptive changes in the activity of rostroventromedial medulla (RVM) pain modulatory cells in the streptozotocin-diabetic rat, Brain Res. Bull. 96 (2013) 39–44.
[48] H. de Morais, C.P. de Souza, L.M. da Silva, D.M. Ferreira, C.H. Baggio, A.C. Vanvossen, M. Cristina de Carvalho, J.E. da Silva-Santos, L.J. Bertoglio, J.M. Cunha, J.M. Zanoveli, Anandamide reverses depressive-like behavior, neurochemical abnormalities and oxidative-stress parameters in streptozotocin-diabetic rats: role of CB1 receptors, Eur. Neuropsychopharmacol. 26 (2016) 1590–1600.
[49] S. Chaplan, F.W. Bach, J. Pogrel, J. Chung, T. Yaksh, Quantitative assessment of tactile allodynia in the rat paw, J. Neurosci. Methods 53 (1994) 55–63.
[50] H. Leite-Almeida, L. Almeida-Torres, A.R. Mesquita, A. Pertovaara, N. Sousa, J.J. Cerqueira, A. Almeida, The impact of age on emotional and cognitive behaviours triggered by experimental neuropathy in rats, Pain 144 (2009) 57–65.
[51] S.J. Flatters, G.J. Bennett, Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy, Pain 109 (2004) 150–161.
[52] L. Randall, J. Selitto, A method for measurement of analgesic activity on inflamed tissue, Arch. Int. Pharmacodyn. Thér. 111 (1957) 409–419.
[53] F.E. D'Amour, D.L. Smith, A method for determining loss of pain sensation, J. Pharmacol. Exp. Ther. 72 (1941) 74–79.
[54] F. Pinto-Ribeiro, D. Amorim, A. David-Pereira, A.M. Monteiro, P. Costa, A. Pertovaara, A. Almeida, Pronociception from the dorsomedial nucleus of the hypothalamus is mediated by the rostral ventromedial medulla in healthy controls but is absent in arthritic animals, Brain Res. Bull. 99 (2013) 100–108.
[55] R. Porsolt, A. Bertin, M. Jalfre, Behavioral despair in mice: a primary screening test for antidepressants, Arch. Int. Pharmacodyn. Ther. 229 (1977) 327–336.
[56] V. Castagné, R.D. Porsolt, P. Moser, Use of latency to immobility improves detection of antidepressant-like activity in the behavioral despair test in the mouse, Eur. J. Pharmacol. 616 (2009) 128–133.
[57] M. Detke, M. Rickels, I. Lucki, Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants, Psychopharmacology (Berl.) 121 (1995) 66–72.
[58] H. Hashimoto, R. Hashimoto, N. Shintani, K. Tanaka, A. Yamamoto, M. Hatanaka, X. Guo, Y. Morita, M. Tanida, K. Nagai, M. Takeda, A. Baba, Depression-like behavior in the forced swimming test in PACAP-deficient mice: amelioration by the atypical antipsychotic risperidone, J. Neurochem. 110 (2009) 595–602.
[59] E. Ottoni, EthoLog 2.2: a tool for the transcription and timing of behavior observation sessions, Behav. Res. Methods Instrum. Comput. 32 (2000) 446–449.
[60] C.A. Lee-Kubli, N.A. Calcutt, Painful neuropathy: mechanisms, Handb. Clin. Neurol. 126 (2014) 533–557.
[61] M. Malcangio, D.R. Tomlinson, A pharmacologic analysis of mechanical hyperalgesia in streptozotocin/diabetic rats, Pain 76 (1998) 151–157.
[62] G. Khan, S.-R. Chen, H.-L. Pan, Role of primary afferent nerves in allodynia caused by diabetic neuropathy in rats, Neuroscience 114 (2002) 291–299.
[63] A. Pertovaara, H. Wei, J. Kalmari, M. Ruotsalainen, Pain behavior and response properties of spinal dorsal horn neurons following experimental diabetic neuropathy in the rat: modulation by nitecapone a COMT inhibitor with antioxidant properties, Exp. Neurol. 167 (2001) 425–434.
[64] M.S. Johnson, J.M. Ryals, D.E. Wright, Early loss of peptidergic intraepidermal nerve fibers in an STZ-induced mouse model of insensate diabetic neuropathy, Pain 140 (2008) 35–47.
[65] P. Dyck, T. Larson, P. O'Brien, J. Velosa, Patterns of quantitative sensation testing of hypoesthesia and hyperalgesia are predictive of diabetic polyneuropathy: a study of three cohorts. Nerve growth factor study group, Diabetes Care 23 (2000) 510–517.
[66] K. Sugimoto, Y. Murakawa, A. Sima, Diabetic neuropathy – continuing enigma, Diabetes Metab. Res. Rev. 16 (2000) 408–433.
[67] C.A. Cukras, P. Petrou, E.Y. Chew, C.B. Meyerle, W.T. Wong, Oral minocycline for the treatment of diabetic macular edema (DME): results of a phase I/II clinical study, Investig. Ophthalmol. Vis. Sci. 53 (2012) 3865–3874.
[68] S.J. Won, J.H. Kim, B.H. Yoo, M. Sohn, T.M. Kauppinen, M.-S. Park, H.-J. Kwon, J. Liu, S.W. Suh, Prevention of hypoglycemia-induced neuronal death by minocycline, J. Neuroinflammation 9 (2012) 225.
[69] R. Brogden, T. Speight, G. Avery, Minocycline: a review of its antibacterial and pharmacokinetic properties and therapeutic use, Drugs 9 (1975) 251–291.
[70] K. Agwuh, A. MacGowan, Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines, J. Antimicrob. Chemother. 58 (2006) 256–265.
[71] A. Syngle, I. Verma, P. Krishan, N. Garg, V. Syngle, Minocycline improves peripheral and autonomic neuropathy in type 2 diabetes: MIND study, Neurol. Sci. 35 (2014) 1067–1073.
[72] S. Talbot, E. Chahmi, J.P. Dias, R. Couture, Key role for spinal dorsal horn microglial kinin B1 receptor in early diabetic pain neuropathy, J. Neuroinflammation 7 (2010) 36.
[73] X.-P. Mei, H. Xu, C. Xie, J. Ren, Y. Zhou, H. Zhang, L.-X. Xu, Post-injury administration of minocycline: an effective treatment for nerve-injury induced neuropathic pain, Neurosci. Res. 70 (2011) 305–312.
[74] X.-P. Mei, Y. Sakuma, C. Xie, D. Wu, I. Ho, J. Kotani, L.-X. Xu, Depressing interleukin-1β contributed to the synergistic effects of tramadol and minocycline on spinal nerve ligation-induced neuropathic pain, Neurosignals 22 (2014) 30–42.
[75] C. Lin, M. Tsaur, C. Chen, T. Wang, C. Lin, Y. Lai, T. Hsu, Y. Pan, C. Yang, J. Cheng, Chronic intrathecal infusion of minocycline prevents the development of spinal-nerve ligation–induced pain in rats, Reg. Anesth. Pain Med. 32 (2007) 209–216.
[76] A. Ledeboer, E.M. Sloane, E.D. Milligan, M.G. Frank, J.H. Mahony, S.F. Maier, L.R. Watkins, Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation, Pain 115 (2005) 71–83.
[77] T.M. Zanjani, M. Sabetkasaei, N. Mosaffa, H. Manaheji, F. Labibi, B. Farokhi, Suppression of interleukin-6 by minocycline in a rat model of neuropathic pain, Eur. J. Pharmacol. 538 (2006) 66–72.
[78] S.S. Padi, S.K. Kulkarni, Minocycline prevents the development of neuropathic pain, but not acute pain: possible anti-inflammatory and antioxidant mechanisms, Eur. J. Pharmacol. 601 (2008) 79–87.
[79] M. Tsuda, H. Ueno, A. Kataoka, H. Tozaki-Saitoh, K. Inoue, Activation of dorsal horn microglia contributes to diabetes-induced tactile allodynia via extracellular signal-regulated protein kinase signaling, Glia 56 (2008) 378–386.
[80] T. Kielian, N. Esen, S. Liu, N.K. Phulwani, M.M. Syed, N. Phillips, K. Nishina, A.L. Cheung, J.D. Schwartzman, J.J. Ruhe, Minocycline modulates neuroinflammation independently of its antimicrobial activity in staphylococcus aureus-induced brain abscess, Am. J. Pathol. 171 (2007) 1199–1214.
[81] S. Pu, Y. Xu, D. Du, M. Yang, X. Zhang, J. Wu, W. Jiang, Minocycline attenuates mechanical allodynia and expression of spinal NMDA receptor 1 subunit in rat neuropathic pain model, J. Physiol. Biochem. 69 (2013) 349–357.
[82] R.-D. Treede, R.A. Meyer, S.N. Raja, J.N. Campbell, Peripheral and central mechanisms of cutaneous hyperalgesia, Prog. Neurobiol. 38 (1992) 397–421.
[83] D. Le Bars, M. Gozariu, S. Cadden, Animal models of nociception, Pharmacol. Rev. 53 (2001) 597–652.
[84] S. Bevan, T. Quallo, D.A. Andersson, TRPV1, in: Handb. Exp. Pharmacol., 2014, pp. 207–245.
[85] P. Facer, M.A. Casula, G.D. Smith, C.D. Benham, I.P. Chessell, C. Bountra, M. Sinisi, R. Birch, P. Anand, Differential expression of the capsaicin receptor TRPV1 and related novel receptors TRPV3 TRPV4 and TRPM8 in normal human tissues and changes in traumatic and diabetic neuropathy, BMC Neurol. 7 (2007) 11.
[86] T.S. Jensen, N.B. Finnerup, Allodynia and hyperalgesia in neuropathic pain: clinical manifestations and mechanisms, Lancet Neurol. 13 (2014) 924–935.
[87] Y.-H. Liao, G.-H. Zhang, D. Jia, P. Wang, N.-S. Qian, F. He, X.-T. Zeng, Y. He, Y.-L. Yang, D.-Y. Cao, Y. Zhang, D.-S. Wang, K.-S. Tao, C.-J. Gao, K.-F. Dou, Spinal astrocytic activation contributes to mechanical allodynia in a mouse model of type 2 diabetes, Brain Res. 1368 (2011) 324–335.
[88] M. Gore, N.A. Brandenburg, E. Dukes, D.L. Hoffman, K.-S. Tai, B. Stacey, Pain severity in diabetic peripheral neuropathy is associated with patient functioning symptom levels of anxiety and depression, and sleep, J. Pain Symptom Manage. 30 (2005) 374–385.
[89] C. D'Amato, R. Morganti, C. Greco, F. Di Gennaro, L. Cacciotti, S. Longo, G. Mataluni, D. Lauro, G. Marfia, V. Spallone, Diabetic peripheral neuropathic pain is a stronger predictor of depression than other diabetic complications and comorbidities, Diabetes Vasc. Dis. Res. 13 (2016) 418–428.
[90] H. de Morais, C.P. de Souza, L.M. da Silva, D.M. Ferreira, M.F. Werner, R. Andreatini, J.M. da Cunha, J.M. Zanoveli, Increased oxidative stress in prefrontal cortex and hippocampus is related to depressive-like behavior in streptozotocin-diabetic rats, Behav. Brain Res. 258 (2014) 52–64.

[91] M. Habib, S. Shaker, N. El-Gayar, S. Aboul-Fotouh, The effects of antidepressants fluoxetine and imipramine on vascular abnormalities and Toll like receptor-4 expression in diabetic and non-diabetic rats exposed to chronic stress, PLoS One 10 (2015) e0120559.
[92] L.B. Ceretta, G.Z. Réus, R.B. Stringari, K.F. Ribeiro, G. Zappellini, B.W. Aguiar, B. Pfaffenseller, C. Lersh, F. Kapczinski, J. Quevedo, Imipramine treatment reverses depressive-like behavior in alloxan-diabetic rats, Diabetes Metab. Res. Rev. 28 (2012) 139–144.
[93] G.Z. Réus, M.A.B. dos Santos, H.M. Abelaira, S.E. Titus, A.S. Carlessi, B.I. Matias, L. Bruchchen, D. Florentino, A. Vieira, F. Petronilho, L.B. Ceretta, A.I. Zugno, J. Quevedo, Antioxidant treatment ameliorates experimental diabetes-induced depressive-like behaviour and reduces oxidative stress in brain and pancreas, Diabetes Metab. Res. Rev. 32 (2016) 278–288.
[94] H. Leite-Almeida, F. Pinto-Ribeiro, A. Almeida, Animal models for the study of comorbid pain and psychiatric disorders, Mod. Trends Pharmacopsychiatry (2015) 1–21.
[95] B. Hu, H. Doods, R.D. Treede, A. Ceci, Depression-like behaviour in rats with mononeuropathy is reduced by the CB2-selective agonist GW405833, Pain 143 (2009) 206–212.
[96] L. Bravo, J.A. Mico, R. Rey-Brea, B. Pérez-Nievas, J.C. Leza, E. Berrocoso, Depressive-like states heighten the aversion to painful stimuli in a rat model of comorbid chronic pain and depression, Anesthesiology 117 (2012) 613–625.
[97] G. Borges, F.L. Neto, J.A. Mico, E. Berrocoso, Reversal of monoarthritis-induced affective disorders by diclofenac in rats, Anesthesiology 120 (2014) 1476–1490.
[98] L. Gonçalves, R. Silva, F. Pinto-Ribeiro, J.M. Pêgo, J.M. Bessa, A. Pertovaara, N. Sousa, A. Almeida, Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat, Exp. Neurol. 213 (2008) 48–56.
[99] R.I. Holt, M. de Groot, I. Lucki, C.M. Hunter, N. Sartorius, S.H. Golden, NIDDK international conference report on diabetes and depression: current understanding and future directions, Diabetes Care 37 (2014) 2067–2077.
[100] J. Rosenstock, M. Tuchman, L. LaMoreaux, U. Sharma, Pregabalin for the treatment of painful diabetic peripheral neuropathy: a double-blind placebo-controlled trial, Pain 110 (2004) 628–638.
[101] D.E. Moon, D.I. Lee, S.C. Lee, S.O. Song, D.M. Yoon, M.H. Yoon, H.K. Kim, Y.W. Lee, C. Kim, P.B. Lee, Efficacy and tolerability of pregabalin using a flexible, optimized dose schedule in Korean patients with peripheral neuropathic pain: a 10-week randomized, double-blind, placebo-controlled, multicenter study, Clin. Ther. 32 (2010) 2370–2385.
[102] Y. Guan, X. Ding, Y. Cheng, D. Fan, L. Tan, Y. Wang, Z. Zhao, Z. Hong, D. Zhou, X. Pan, S. Chen, A. Martin, H. Tang, L. Cui, Efficacy of pregabalin for peripheral neuropathic pain: results of an 8-week flexible-dose, double-blind, placebo-controlled study conducted in China, Clin. Ther. 33 (2011) 159–166.
[103] S. Ogawa, A. Arakawa, K. Hayakawa, T. Yoshiyama, Pregabalin for neuropathic pain: why benefits could be expected for multiple pain conditions, Clin. Drug Investig. 36 (2016) 877–888.
[104] B. Parsons, C.E. Argoff, A. Clair, B. Emir, Improvement in pain severity category in clinical trials of pregabalin, J. Pain Res. 9 (2016) 779–785.
[105] S. Arakawa, Y. Shirayama, Y. Fujita, T. Ishima, M. Horio, K. Muneoka, M. Iyo, K. Hashimoto, Minocycline produced antidepressant-like effects on the learned helplessness rats with alterations in levels of monoamine in the amygdala and no changes in BDNF levels in the hippocampus at baseline, Pharmacol. Biochem. Behav. 100 (2012) 601–606.
[106] M. Sumitani, H. Ueda, J. Hozumi, R. Inoue, T. Kogure, T. Ogata, Y. Yamada, Minocycline does not decrease intensity of neuropathic pain, but improves its affective dimension, J. Pain Palliat. Care Pharmacother. 30 (2015) 31–35.