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Title	Chronic administration of amitriptyline differentially alters neuropathic pain-related behaviour in the presence and absence of a depressive-like phenotype
Author(s)	Burke, Nikita N.; Finn, David P.; Roche, Michelle
Publication Date	2015-02
Publication Information	Burke, NN,Finn, DP,Roche, M (2015) 'Chronic administration of amitriptyline differentially alters neuropathic pain-related behaviour in the presence and absence of a depressive-like phenotype'. Behavioural Brain Research, 278 :193-201.
Publisher	Elsevier
Link to publisher's version	http://www.sciencedirect.com/science/article/pii/S0166432814006421
Item record	http://hdl.handle.net/10379/5767
DOI	http://dx.doi.org/10.1016/j.bbr.2014.09.044

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Chronic administration of amitriptyline differentially alters neuropathic pain-related behaviour in the presence and absence of a depressive-like phenotype

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Abstract

Chronic pain and depression share a complex, reciprocal relationship. Furthermore, in addition to treating depression, antidepressants such as amitriptyline are a first-line treatment for chronic pain conditions, indicating possible common neural substrates underlying both depression and pain. However, there is a paucity of studies examining the effect of antidepressant treatment on nociceptive and neuropathic-pain responding in the presence of a depressive phenotype. The current study aimed to examine the effect of chronic amitriptyline administration on neuropathic pain-related behaviour and associated neuroinflammatory processes in the olfactory bulbectomised (OB) rat model of depression. Nociceptive responding to mechanical, innocuous cold or noxious heat stimuli in sham or OB rats was not altered by chronic amitriptyline administration. The induction of neuropathic pain following L5-L6 spinal nerve ligation (SNL) resulted in robust mechanical and cold allodynia and heat hyperalgesia in both sham and OB vehicle-treated animals. Chronic amitriptyline administration attenuated SNL-induced mechanical allodynia in both sham and OB rats at day 7 post-SNL, an effect which was enhanced and prolonged in OB rats. In comparison, chronic amitriptyline administration attenuated SNL-induced cold allodynia and heat hyperalgesia in sham, but not OB, rats. Evaluating the affective/motivational aspect of pain using the place escape avoidance paradigm revealed that OB-SNL rats exhibit reduced noxious avoidance behaviour when compared with sham-counterparts, an effect not altered by chronic amitriptyline administration. Chronic amitriptyline administration prevented the increased expression of GFAP, IL-10 and CCL5, and enhanced the expression of TNFα, in the prefrontal cortex of OB-SNL rats. In conclusion, these data demonstrate that chronic amitriptyline differentially alters somatic nociceptive responding following peripheral nerve-injury, depending on stimulus modality and the presence or absence of a depressive-like phenotype, an effect which may involve modulation of neuroinflammatory processes.

Keywords: spinal nerve ligation; olfactory bulbectomy; antidepressant; mechanical allodynia; von Frey test; thermal hyperalgesia; Hargreaves test; PEAP, cytokines, neuroinflammation, prefrontal cortex.

Abbreviations

AMI amitriptyline; CCL chemokines CC motif ligand; CD11b cluster of differentiation molecule 11b; GFAP Glial fibrillary acidic protein; IL interleukin; OB: Olfactory bulbectomy; SNL spinal nerve ligation; TNFα Tumour necrosis factor alpha.

1. Introduction

Depression is associated with an increased risk of developing chronic pain [1,2]. Over 60% of patients with major depressive disorder suffer from chronic pain [3,4], and this comorbidity increases the socioeconomic cost, with direct medical care costs over twice as high in depressed patients with pain compared to those without [5]. Thus, the combination of depression and pain is more disabling and costlier to patients and society than either alone, highlighting the need for adequate treatment options. Indeed, effective treatment of pain symptoms has been shown to result in higher remission rates in depressed patients [6,7]. It has been suggested that depression and chronic pain are caused and maintained by dysfunction of shared neurobiological processes, such as monoaminergic pathways. Further support for this arises from the efficacy of monoaminergic-based antidepressants in the treatment of depression and as a first-line treatment for chronic pain [7-10]. Amitriptyline (AMI) is a tricyclic antidepressant which inhibits the re-uptake of noradrenaline and serotonin and remains a gold-standard treatment for neuropathic pain [11].

Preclinical research has also provided evidence in support of a relationship between depression and chronic pain, with a number of rodent models of depression exhibiting altered nociceptive responding and vice versa (for review see [12]). For example, the well-characterised olfactory bulbectomised (OB) rodent model of depression has been shown to exhibit mechanical allodynia and enhanced inflammatory nociceptive responding [13,14], altered thermal nociceptive responding [13,15] and increased nociceptive responding to electrical stimulation [16]. Furthermore, recent studies from our group have demonstrated altered L5-L6 spinal nerve ligation (SNL)-induced neuropathic pain-related behaviour in the OB rat [17,18], indicating that a prior depressive phenotype alters the development and expression of neuropathic pain. Chronic administration of the selective-serotonin reuptake inhibitor fluoxetine [15] and the tricyclic antidepressant AMI [16] has been shown to

attenuate enhanced nociceptive responding to thermal and electrical stimuli respectively, in the OB rat. In addition, chronic AMI treatment is known to reverse OB-induced hyperactivity, passive avoidance deficits and anhedonia [19-22], indicating antidepressant-like activity in the OB model. AMI also attenuates thermal hyperalgesia in the spinal nerve ligation (SNL) model of neuropathic pain [23-26], indicating an analgesic effects on neuropathic pain behaviour. However, the effect of antidepressant treatment on the development and expression of neuropathic pain in the presence of a depressive-like phenotype has not yet been examined. Therefore, the aim of this study was to investigate the effect of chronic AMI administration, at a regime that elicits antidepressant-like effects, on mechanical and thermal nociceptive responding in the OB model of depression and on the development and expression of neuropathic pain behaviour following SNL.

The place escape avoidance paradigm (PEAP) is a novel behavioural test based on the avoidance of a chamber associated with noxious stimulation and has been use to assess the affective/motivational aspects of pain in several preclinical animal models including SNL [27,28]. Affective pain behaviour associated with chronic neuropathic/inflammatory pain has been shown to be attenuated by analgesics, anti-inflammatory agents and anti-convulsants [28-30]. The effect of antidepressants on affective pain behaviour has revealed that the selective serotonin/noradrenaline reuptake inhibitor duloxetine, but not fluoxetine, reduced affective pain behaviour in the complete Freund's adjuvant model of inflammatory pain and chronic constriction injury model of neuropathic pain [30,31]. However, no study to date has investigated the effect of chronic antidepressant administration on affective pain responding following SNL or if affective pain responding in SNL animals is modified in the presence of a depressive phenotype. Thus a further aim of this study was to evaluate the effect of chronic AMI administration on affective pain responding following SNL, and if this effect is altered in the OB rat.

In addition to modulation of monoaminergic neurotransmission, antidepressants are known to possess immunomodulatory properties [32-35]. AMI has been found to decrease levels of pro-inflammatory cytokines both *in vitro* and *in vivo* [36,37]. The tricyclic antidepressant desipramine attenuates stimulus-induced increases in plasma TNFα and IL-1β in OB rats [38], while imipramine normalises T-cell ratio [39]. In chronic pain models, AMI has been shown to inhibit inducible nitric oxide expression in the cerebellum and hippocampus [40], and attenuates astrocyte activation in the spinal cord [23]. Furthermore, in animal models of depression, the expression of pro-inflammatory cytokines is increased in brain regions responsible for processing emotion and pain, with concomitant inflammatory or neuropathic pain, [41-46]. Recent research from our group has demonstrated that the chronic administration of the microglial inhibitor minocycline attenuates SNL-induced mechanical allodynia earlier and more profoundly in OB rats compared with sham counterparts [17]. However, it is not known whether chronic AMI treatment alters neuroinflammatory processes in supraspinal regions differentially in sham and OB rats following SNL. Therefore, a further aim was to examine if AMI-induced changes in nociceptive responding following SNL in sham and OB rats are accompanied by alterations in the expression of inflammatory mediators in the prefrontal cortex, a key region responsible for the modulation of nociception and/or affect.

2. Materials and methods

2.1 Animal husbandry

Male Sprague-Dawley rats (180-220g: Charles River, UK) were housed singly in plastic bottomed cages containing wood shavings as bedding, in a temperature-controlled room (20 ± 2ºC), relative humidity of 40-60%, with a 12:12h light-dark cycle (lights on at 0700h). Rats were fed a standard laboratory diet of rat chow pellets; food and water were available *ad libitum*. The experimental protocol was carried out in accordance with the guidelines and approval of the Animal Care and Research Ethics Committee, National University of Ireland, Galway, under licence from the Irish Department of Health and Children and in compliance with the European Communities Council directive 86/609.

2.3 Drug administration

Animals were administered either amitriptyline (AMI; Amitriptyline hydrochloride (Sigma-Aldrich, Ireland; 10mg/kg i.p.) or vehicle (sterile saline) once daily, at an injection volume of 1 ml/kg, beginning on the day of OB/sham surgery. The choice of dose was based on the antidepressant effects of chronic AMI in OB rats [20,22,47], and the anti-nociceptive effects following L5-L6 SNL [24,26]. Behavioural testing was conducted at least 15 hours after administration of AMI or vehicle to avoid any potential confound associated with the acute effects of the drug.

2.4. Experimental design

Rats were tested in the open field (locomotor activity), von Frey (mechanical sensitivity) and acetone-drop (cold sensitivity) tests at baseline to ensure no differences between groups assigned to sham or OB surgery (see Figure 1 for experimental timeline). Animals were then assigned to either Sham-Vehicle (Sham-Veh, n=12), Sham-AMI (n=12), olfactory

bulbectomy-Veh (OB-Veh, n=13) and OB-AMI (n=12). AMI or vehicle was administered daily, beginning on the day of OB/sham surgery. Animals were allowed two weeks to recover following surgery before behavioural testing for OB-related hyperactivity in the open field and the antidepressant-like activity of AMI. Nociceptive responding to mechanical and thermal stimuli was re-evaluated 3 weeks following treatment. Twenty-four hours after the last behavioural test, all animals underwent L5-L6 Spinal Nerve Ligation (SNL) surgery. Mechanical withdrawal thresholds were examined on days 3, 7, 10 and 14 post-SNL. Animals were also assessed for cold allodynia (acetone-drop test; Day 14 post SNL), heat hyperalgesia (Hargreaves test; Day 17 post SNL) and the affective/emotional pain behaviour (place-escape avoidance paradigm (PEAP; Day 18). Animals were scarified approximately 15hr following the last vehicle/AMI administration (Day 20), brain removed and prefrontal cortex isolated and stored at -80^{O}C until assessment of neuroinflammatory gene expression.

2.3 Bilateral Olfactory Bulbectomy (OB) surgery

Bilateral olfactory bulbectomy (OB) was performed on rats anaesthetised with isoflurane (Abbot Laboratories, UK [3% induction, 1.5% maintenance in 0.5 l/min O2]) as described previously [14,17,18,48]. In brief, two burr holes of 2mm diameter were drilled into the skull, 5mm rostral to bregma and 2mm lateral to the midline and the olfactory bulbs removed by gentle aspiration. The burr holes were plugged with a haemostatic sponge to control bleeding. Sham-operated animals were treated in the same manner but the bulbs were left intact. Animals were eliminated from the analysis if the bulbs were not completely removed or if damage extended to the frontal cortex, which resulted in one rat from the OB-vehicle group being eliminated from the analysis.

2.4 L5-L6 Spinal nerve ligation (SNL) surgery

SNL was carried out on all rats as described previously [17,18,49-51]. Briefly, the rats were anaesthetised with isoflurane (2.5% in 0.6 l/min O2), the fur was clipped, an incision made between the spinal column and the left iliac crest and the paraspinal muscles removed. The L6 transverse process was removed and the L5 and L6 nerves were tightly ligated using 6-0 silk suture (Interfocus, UK).

2.5 Open Field Test

Two weeks following sham/OB surgery, animals were placed singly into a brightly-lit (lux 200) novel open field arena (diameter 75cm) with a white floor (plastic-covered wood flooring) and reflective walls for a 5 minute period. Locomotor activity (distance moved, cm) and time in the centre of the arena (diameter 50cm) was assessed using a computerised video tracking system (EthoVision®, Version 7 Noldus, Netherlands) as previously described [17,18,48].

2.6 von Frey test for assessing mechanical allodynia

Rats were habituated to the testing arena (11cm × 20cm × 15cm) for 20 minutes prior to testing. von Frey filaments (Touch-Test® Sensory Evaluators, North Coast Medical, Inc., CA, USA) of different forces (0.07g – 100g) were used to determine the lowest filament force to elicit a response and 50% withdrawal thresholds [17,18,50,52]. Briefly, filaments were applied to the plantar surface of the hind-paw, with sufficient force to cause buckling of the filament, for up to a maximum of 5 seconds or until flinching, licking or withdrawal of the paw occurred. Filaments of increasing force were applied five times (alternating between paws) until a 100% positive response (five positive responses to five applications) was observed. The filament force eliciting a 50% response was calculated by plotting a non-linear regression curve of the percentage response versus filament force for each rat.

2.7 Acetone drop test for assessing cold allodynia

The acetone drop test was carried out 20 min following von Frey testing as previously described [17,18,50]. A drop of acetone (100%: Sigma–Aldrich, Ireland) was applied to the plantar surface of the hind-paw and latency to first response and withdrawal frequency within the first 60 s after acetone application were recorded for each trial. A positive response was considered as a flinch, lick or withdrawal of the hind-paw. Each animal received 8 trials in total, four per paw, alternating between left and right with at least a 3 min interval between testing the other paw. The average of the 4 trials was calculated for each hind-paw.

2.8 Hargreaves' test for assessing thermal hyperalgesia

The Hargreaves' test was conducted as previously described [18]. The apparatus (IITC Life Science Inc., CA) consisted of a six-chamber Perspex arena placed on top of a heated glass plate (30 ± 1 °C). Rats were placed in individual chambers (15 × 20 × 11cm) and allowed to habituate for 20 minutes. A moveable radiant heat source was positioned underneath the glass and was used to focus the beam on the plantar surface of the hind-paw. A focused beam of radiant light (active intensity of 30%) was used to heat the plantar surface of the hind-paw (between the 3rd and 4th toes) and the latency to flinch, lick or withdraw the hind-paw was recorded or until a cut-off time of 20 s was reached (to prevent tissue damage). Right and left hind-paws were tested 4 times, alternating between paws, and the average withdrawal latency for each paw was calculated.

2.9 Place Escape Avoidance Paradigm (PEAP)

The PEAP test was conducted as previously described [28]. Essentially, this paradigm creates a conflict between the aversive light compartment and aversive noxious mechanical stimulation in the dark compartment, thus giving a choice to move to the light side (lux 200) in order to escape and/or avoid stimulation of the allodynic hindpaw in the dark side. In brief,

rats were placed individually into the light side of the PEAP arena (30cm x 30cm x 30cm Perspex box) which was placed on an elevated mesh floor. A 60g von Frey filament was applied to the plantar surface of the ipsilateral hind-paw while in the dark side and to the contralateral hind-paw while in the light side, every 15 sec over a period of 30 minutes. The test was recorded onto DVD and time spent (s) in the light chamber over the total trial period was assessed using EthoVision® video tracking system.

2.10 Expression of inflammatory mediators using quantitative real-time PCR

RNA was extracted from prefrontal cortical tissue using NucleoSpin RNA II total RNA isolation kit (Macherey-Nagel, Germany). RNA was reverse transcribed into cDNA using a High Capacity cDNA Archive kit (Applied Biosystems, UK). Taqman gene expression assays (Applied Biosystems, UK) were used to quantify the gene of interest and real-time PCR was performed using an ABI Prism 7500 instrument (Applied Biosystems, UK), as previously described [18,46,53-55]. Assay IDs for the genes were cluster of differentiation molecule 11b (CD11b; Rn00709342_m1), Glial fibrillary acidic protein (GFAP; Rn00566603_m1), markers of microglial and astrocyte activation respectively; the cytokines, interleukin-1 beta (IL-1β; Rn00580432_m1), Tumour necrosis factor alpha (TNFα; Rn99999017_m1) and IL-10 (Rn00563409_m1); and the chemokines CC motif ligand 2 (CCL2: Rn00580555_m1) and CC motif ligand 5 (CCL5: Rn00579590_m1). PCR was performed using Taqman Universal PCR Master Mix and samples were run in duplicate. The cycling conditions were 90^{o}C for 10 min and 40 cycles of 90^{o}C for 15 min followed by 60^{o}C for 1 min. β-actin was used as an endogenous control to normalise gene expression data. Relative gene expression was calculated using the $2^{\Delta\Delta CT}$ method and data were expressed as % Sham-SNL vehicle-treated controls.

2.11 Statistical analysis

Parametric data were analysed using t-tests or two-way analysis of variance (ANOVA), using factors of OB and AMI treatment, or repeated measures ANOVA with time as a within-subjects factor, followed by Fisher's least significant difference (LSD) post-hoc testing where appropriate. Non-parametric data were assessed using Kruskal-Wallis test to determine overall differences between groups, followed by Mann-Whitney U post-hoc tests where appropriate. The level of significance was set at $P < 0.05$. All data are presented as the mean + SEM.

3. Results

3.1 Chronic AMI treatment attenuates OB-related increases in locomotor activity in the open field

Two weeks following bulbectomy, OB animals exhibited a characteristic increase in locomotor activity in the open field test when compared to sham counterparts ($F_{(1,43)}$ = 4.77, P=0.035, Fig. 2A). Chronic AMI administration did not alter locomotor activity of sham animals but significantly attenuated the OB-related increase in locomotor activity in the open field ($F_{(1,43)}$ = 12.39, P=0.001, Fig. 2A), indicative of an antidepressant-like effect in the OB-model. Furthermore, OB animals spent less time in the centre of the open field when compared to sham-operated counterparts (4.69 ±1.06 s vs 11.98 ± 3.6 s; $F_{(1,43)}$ = 8.32, P=0.006) indicative of an anxiety-related phenotype in OB rats. Chronic AMI did not alter the time in the centre of the open field for either sham or OB rats (data not shown).

3.2 OB rats exhibit mechanical, but not thermal, allodynia, an effect not altered by chronic AMI administration

Assessment of mechanical withdrawal responses in the von Frey test revealed that OB-vehicle-treated animals responded at lower von Frey filaments when compared to sham-vehicle-treated counterparts ($\chi^2_{(3)}$ = 10.13 P=0.037, Fig. 2B), indicative of mechanical allodynia in OB rats. Chronic AMI administration did not alter mechanical withdrawal responses of sham or OB rats in the von Frey test. In addition, neither bulbectomy nor chronic AMI administration altered the latency to withdraw from an innocuous cold (acetone drop test; Fig 2C) or noxious heat (Hargreaves test; Fig 2D) stimulus.

3.3 Chronic AMI administration differentially affects mechanical and thermal nociceptive responding in sham and OB rats following SNL

As OB animals exhibited mechanical allodynia prior to SNL, mechanical thresholds post-SNL were expressed as a percentage of pre-SNL values. SNL induced a pronounced reduction in mechanical thresholds of the ipsilateral hind-paw of both sham and OB animals at all time-points examined ($F_{(3,160)}$ = 10.91, P<0.001, Fig 3A), indicating SNL-induced mechanical allodynia. Chronic AMI administration attenuated SNL-induced reductions in mechanical withdrawal threshold in both sham and OB rats on day 7, an effect sustained over the following testing period in OB but not sham animals ($F_{(1,40)}$ = 6.51, P=0.015, Fig 2B). Further analysis of area under the curve revealed that OB-SNL AMI-treated animals exhibit higher mechanical withdrawal thresholds when compared to OB-SNL vehicle controls ($F_{(1,40)}$ = 9.54, P=0.004), an effect not observed in sham-operated counterparts. Thus chronic AMI administration attenuates SNL-induced mechanical allodynia in OB rats to a greater extent than in sham-operated counterparts.

The effect of bulbectomy and chronic AMI administration was also assessed on SNL-induced cold allodynia and heat hyperalgesia. Analysis revealed that chronic AMI administration resulted in an increased latency to respond to an innocuous cold ($F_{(1,40)}$ = 7.65, P = 0.009; Fig 3C) and noxious heat ($F_{(1,39)}$ = 4.146 P = 0.049; Fig 3D) stimulus following SNL, an effect not observed in OB rats.

3.4 OB-SNL animals spend less time in the light compartment of the PEAP arena, an effect not altered by chronic AMI administration

Assessment of the amount of time spent in the light-side of the PEAP arena revealed that OB-SNL rats spend significantly less time in the light side of the arena over the 30 minute test period when compared to sham-SNL counterparts ($F_{(1,40)}$ = 47.81, P<0.001, Fig. 4). Chronic

AMI did not alter the amount of time spent in the light side of the arena of either sham-SNL or OB-SNL rats.

3.5 Effect of OB and/or chronic AMI administration on the expression of neuroinflammatory genes in the PFC of SNL rats

OB-SNL vehicle-treated animals exhibited increased expression of markers of microglial (CD11b $F_{(1,26)}$ = 15.95, P<0.001, CD40 $F_{(1,26)}$ = 15.84, P<0.001) and astrocyte (GFAP $F_{(1,26)}$ = 8.19, P=0.008) activation, increased expression of the anti-inflammatory cytokine IL-10 ($F_{(1,24)}$ =4.724, P=0.04) and the chemokines CCL2 ($F_{(1,27)}$ = 11.31, P=0.002) and CCL5 ($F_{(1,26)}$ = 6.67, P=0.016), when compared to sham-SNL counterparts (Fig. 5 A-H). The expression of CD11b, CD40 and CCL2 in the PFC remained elevated in OB-SNL animals that received chronic AMI administration, when compared to sham-counterparts. However, chronic administration of AMI prevented the increase in GFAP, IL-10 and CCL5 expression in the PFC of OB-SNL rats. Furthermore, OB-SNL AMI-treated animals exhibited an increase in TNFα expression when compared to sham-SNL AMI-treated counterparts ($F_{(1,25)}$ = 5.238, P=0.031, Fig. 5F). In addition, chronic AMI reduced the expression of IL-1β in the prefrontal cortex of sham- and OB-SNL rats ($F_{(1,23)}$ = 4.942, P=0.036).

4. Discussion

The present data demonstrate that chronic AMI treatment does not modulate evoked nociceptive responding to mechanical or thermal stimuli in the absence of nerve injury, in either sham or OB rats. However following SNL, chronic administration of AMI elicits differential effects on nociceptive responding in sham and OB rats. In particular, chronic AMI administration in sham rats resulted in an attenuation of SNL-induced cold allodynia and heat hyperalgesia, as well as mechanical allodynia, although this latter effect was not sustained. In comparison, in OB animals, chronic administration of AMI elicited an antidepressant-like effect and attenuated SNL-induced mechanical allodynia, without modulating nociceptive responding to either of the thermal stimuli. Furthermore, AMI prevented the increased expression of some, but not all, inflammatory genes in the prefrontal cortex of OB rats, effects which may underlie some of its behavioural effects. Taken together, these data demonstrate that chronic AMI differentially alters neuropathic-like pain-related, depending on stimulus modality and the presence or absence of a depressive-like phenotype.

Findings from several research groups, including our own, have demonstrated that OB rats exhibit enhanced responding to mechanical stimuli applied to the hind-paw, indicative of OB-related mechanical allodynia [14,17,18,56], an effect again confirmed in the present study and highlighting the robustness of this behavioural alteration. Previous studies have shown that OB rats exhibit cold allodynia in the absence of nerve injury [17,18], however this effect was not observed in the current study. It is possible that the repeated handling combined with daily i.p. injections may have impacted on or prevented the development of this behavioural change, but not more robust alterations such as hyperactivity and mechanical allodynia in the model. Although increased paw withdrawal latency to a radiant heat source and transient thermal heat hyperalgesia in the hotplate, but not tail flick, test have been reported in OB rats

[13,14,57], the results of the present study confirm our earlier finding [18] that OB rats do not exhibit any alteration in nociceptive responding in the Hargreaves test. Thus, OB animals appear to exhibit alterations in nociceptive responding to thermal stimuli in a manner which is dependent on the test and experimental conditions employed.

Chronic AMI administration did not alter nociceptive responding to mechanical or thermal stimuli in either sham or OB animals. Similarly, chronic AMI administration does not alter nociceptive thresholds to thermal or pressure stimuli applied to the tail or following electrical foot-shock [58]. The attenuation of OB-induced hyperactivity following chronic AMI administration confirms reports on the antidepressant-like effect of this tricyclic antidepressant in the model [19-22]. Although enhanced nociceptive responding to electrical stimulation of the dura matter in the OB rat has been shown to be attenuated by chronic AMI administration [16], this is the first study to examine the effect of AMI on withdrawal responses to somatic nociceptive stimuli in the model. Recent data from our group have demonstrated that although OB-related hyperactivity can be attenuated by chronic administration of the microglial inhibitor and putative antidepressant minocycline, this treatment regime also does not modify OB-related mechanical allodynia [17]. Thus although it is possible that a longer treatment regime is required, these data indicate that chronic administration with the tricyclic antidepressant AMI does not alter basal somatic nociceptive thresholds in the presence or absence of a depressive phenotype.

Given that AMI is one of the most widely prescribed pharmacological agents for the treatment of neuropathic pain, it is not surprising that several preclinical studies have examined the effect of this agent in models of neuropathy. Both acute and repeated (beginning on the day of surgery) administration of AMI has been shown to decrease thermal hyperalgesia, but not mechanical allodynia, following both SNL and chronic constriction

injury [23,24,26,59-62], indicating modality-specific antinociceptive effects of AMI in neuropathic pain models. Intrathecal administration of AMI prior to SNL surgery has been shown to enhance and prolong the anti-allodynia effects of systemic repeated AMI and prevent the development of SNL-induced mechanical allodynia until after day 7 post surgery [23]. Thus, pre-emptive administration of antidepressants may enhance the analgesic effects of their subsequent administration. The present study is the first to show that repeated administration of AMI prior to SNL surgery results in an attenuation of cold allodynia, heat hyperalgesia and elicits a transient effect on mechanical allodynia (in sham animals). AMI may act to modulate nociceptive processing via various mechanisms, including increasing monoamine levels, enhancing opioid, adenosine, histamine and acetylcholine tone and/or binding at peripheral sodium, potassium and calcium channels. The attenuation of spinal nerve injury-induced thermal hyperalgesia by chronic AMI has been shown to be mediated by inhibition of noradrenaline reuptake and increasing glial- and brain-derived neurotrophic factors [61] and adenosine receptor activation [24] at the level of the spinal cord. Furthermore, recent data have demonstrated that the decrease in SNL-induced thermal allodynia/hyperalgesia by repeated AMI is associated with a reduction in Nav1.8 sodium channels and microglial activation in the dorsal horn of the spinal cord [23]. In addition, the data presented in this study indicate that chronic AMI is associated with a reduction in the expression of the pro-inflammatory cytokine IL-1β in the PFC of sham-SNL rats. Thus, multiple spinal and supraspinal mechanisms may underlie the effect of repeated AMI on neuropathic pain responding.

In comparison to the effects of AMI on SNL-induced nociceptive responding in sham/normal animals, the present study demonstrated for the first time that chronic AMI elicits a differential effect on neuropathic pain behaviour in an animal model of depression. Specifically, repeated administration of AMI attenuated SNL-induced mechanical allodynia

in OB rats without altering thermal allodynia/hyperalgesia. Although the majority of studies have indicated that AMI elicits little to no effect on mechanical responding following nerve injury, two recent studies have demonstrated that chronic AMI administration attenuates mechanical allodynia and associated depressive-like behaviour following chronic constriction injury or SNL [63,64]. Thus when administered at sufficient concentrations to elicit an antidepressant-like effect, AMI may also modulate nociceptive responding to mechanical stimuli. Chronic AMI has been shown previously to attenuate enhanced nociceptive behaviours following electrical stimulation in OB animals [16]. In addition, repeated administration of clomipramine, another tricyclic antidepressant, normalised mechanical hypoalgesia in learned helplessness model of depression [65]. Recent studies form our group have demonstrated that repeated minocycline administration attenuates SNL-induced mechanical and cold allodynic responding in OB rats [17]. Thus, alterations in neurobiological pathways underlying the depressed state may impede the antinociceptive effects of antidepressant drugs for certain modalities, while facilitating their effects on others.

In addition to enhanced sensory processing, chronic pain also modifies affective/motivational responding which can be assessed pre-clinically in the PEAP test. Analgesics, anti-inflammatory drugs and anti-convulsants increase time in the side associated with stimulation of the injured paw (and reduce the time spent in the light side of the arena) in neuropathic and chronic inflammatory pain models [28-30]. Furthermore, the antidepressant and analgesic, duloxetine, attenuates affective pain responding following peripheral nerve injury [31]. The present study demonstrated that chronic AMI does not alter the duration of time spend by sham- or OB-SNL rats in the light side of the PEAP arena, indicating that this treatment regime does not alter affective pain responding in the SNL model in the presence or absence of a depressive phenotype. However, both vehicle -and AMI-treated OB-SNL rats spend less time in the brightly lit side of the PEAP arena, which would traditionally indicate an anti-

nociceptive response of these animals. It should be noted that OB rats exhibit bilateral mechanical allodynia both prior to and following SNL [18] and the induction of bilateral pain does not elicit avoidance behaviour in this paradigm [27]. Thus, while the PEAP test would be useful in assessing the effects of chronic AMI on affective pain responding in sham-SNL rats, due to bilateral pain responding this assay acts to assess emotional reactivity or anxiety-related behaviour (as in the light dark box) in OB rats. Therefore, given that OB rats spend less time in the centre arena of the open field prior to SNL and less time in the brightly lit side of the PEAP chamber following SNL, the data indicate that OB rats maintain an anxiety-related phenotype following SNL, an effect which is not modulated by chronic AMI administration.

In order to evaluate if neuroinflammatory processes in a key brain region involved in modulating emotional and nociceptive processes may accompany the differential effects of AMI on neuropathic pain responding in the presence or absence of depression, we examined the expression of inflammatory mediators in the PFC of sham- and OB-SNL rats. OB-SNL vehicle-treated animals exhibited increased microglial (CD11b and CD40) and astrocyte (GFAP) activation, and enhanced expression of the anti-inflammatory cytokine IL-10 and chemokines CCL2 and CCL5 in the PFC, when compared to sham-SNL counterparts. Previous studies from our group have demonstrated that the expression of the M1 microglial marker CD68 was significantly increased in the PFC of OB-SNL rats [17], which, when taken together with the data presented in this study, demonstrate that OB-SNL animals exhibit an altered neuroinflammatory profile in the PFC. Chronic AMI administration reduced expression of the pro-inflammatory cytokine IL-1β in the PFC of both sham and OB rats, however this treatment regime also prevented the OB-SNL associated increase in GFAP, IL-10 and CCL5 expression while concurrently enhancing TNFα expression in OB-SNL rats.

Thus, chronic AMI profoundly alters the neuroinflammatory profile of OB-SNL rats, an effect which may underlie some of its behavioural effects.

Conclusions

The current data indicate that chronic AMI administration elicits differential effects on neuropathic pain responding in the presence and absence of a depressive phenotype, effects which may in part be mediated by neuro-immune processes in key supraspinal sites such as the PFC. Thus, in patients without co-morbid depression, AMI may be effective in alleviating neuropathic pain associated with thermal allodynia/hyperalgesia while in comparison in the depressed patient, AMI may elicit an anti-depressant effect and alleviate mechanical allodynia/hypersensitivity. Ultimately, the data arising from this study may inform the use of AMI in the treatment of neuropathic pain conditions in patients with and without co-morbid depression.

Acknowledgements

The authors would like to gratefully acknowledge technical support from Dr Daniel M Kerr, Ms Rebecca Henry and Mr Stephan Chiu. Funding received from the Discipline of Physiology and the Millennium Fund, National University of Ireland Galway. N.B. received a College of Medicine, Nursing and Health Sciences, National University of Ireland Galway, Doctoral Fellowship. The authors declare no conflict of interest.

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Figure Legends

Fig. 1 Schematic representing the experimental timeline. AMI Amitriptyline, AT Acetone-drop test, HG Hargreaves test, OB Olfactory Bulbectomy, OF Open Field, PEAP Place escape/avoidance paradigm, SNL Spinal nerve ligation, VF von Frey test

Fig. 2 The effect of OB and chronic AMI administration on (A) locomotor activity in the open field. Nociceptive responding to (B) mechanical stimuli in the von Frey test, (C) innocuous cold stimulus in the acetone drop test and (D) noxious heat stimulus in the Hargreaves test. **P<0.01 vs. Sham-Veh; $^{\#\#}$P<0.01 vs. OB-Veh. Data expressed as mean + SEM, n=12/group

Fig. 3 Effect of OB and chronic AMI administration on SNL-induced (A-B) mechanical allodynia, (C) cold allodynia and (D) heat hyperalgesia. (A) normalised mechanical withdrawal responses over 2 weeks. (B) Area under the curve for mechanical withdrawal responses. *P<0.05 vs. Sham-SNL-Veh; $^{\#}$P<0.05 vs. OB-SNL-Veh. (C-D) dotted line indicates pre-SNL levels. Data expressed as mean + SEM, n=12/group

Fig. 4 Time spent in the light chamber of the PEAP test over 30 minute test period. **P<0.01 vs. Sham-SNL-Veh, ++P<0.01 vs. Sham-SNL-AMI. Data expressed as mean ± SEM, n=12/group.

Fig. 5 Expression of inflammatory mediators in the PFC. (A) CD11b (B) CD40 (C) GFAP (D) IL-10 (E) IL-1β (F) TNFα (G) CCL2 (H) CCL5 *P<0.05, **P<0.01 vs. Sham-SNL-Veh, $^{+}$P<0.05, $^{++}$P<0.01 vs. Sham-SNL-AMI, $^{\#\#}$P<0.01 vs. OB-SNL-Veh. $^{\alpha}$ P<0.05 effect of AMI (ANOVA). Data expressed as mean ± SEM, n=12/group.

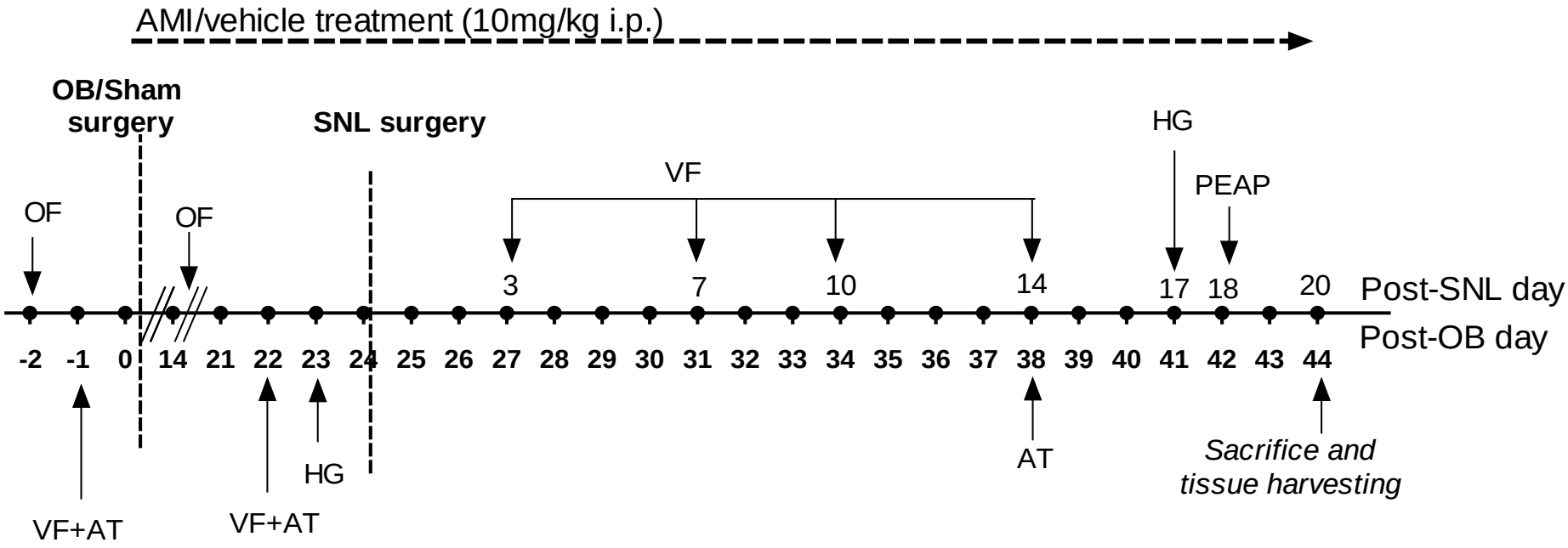
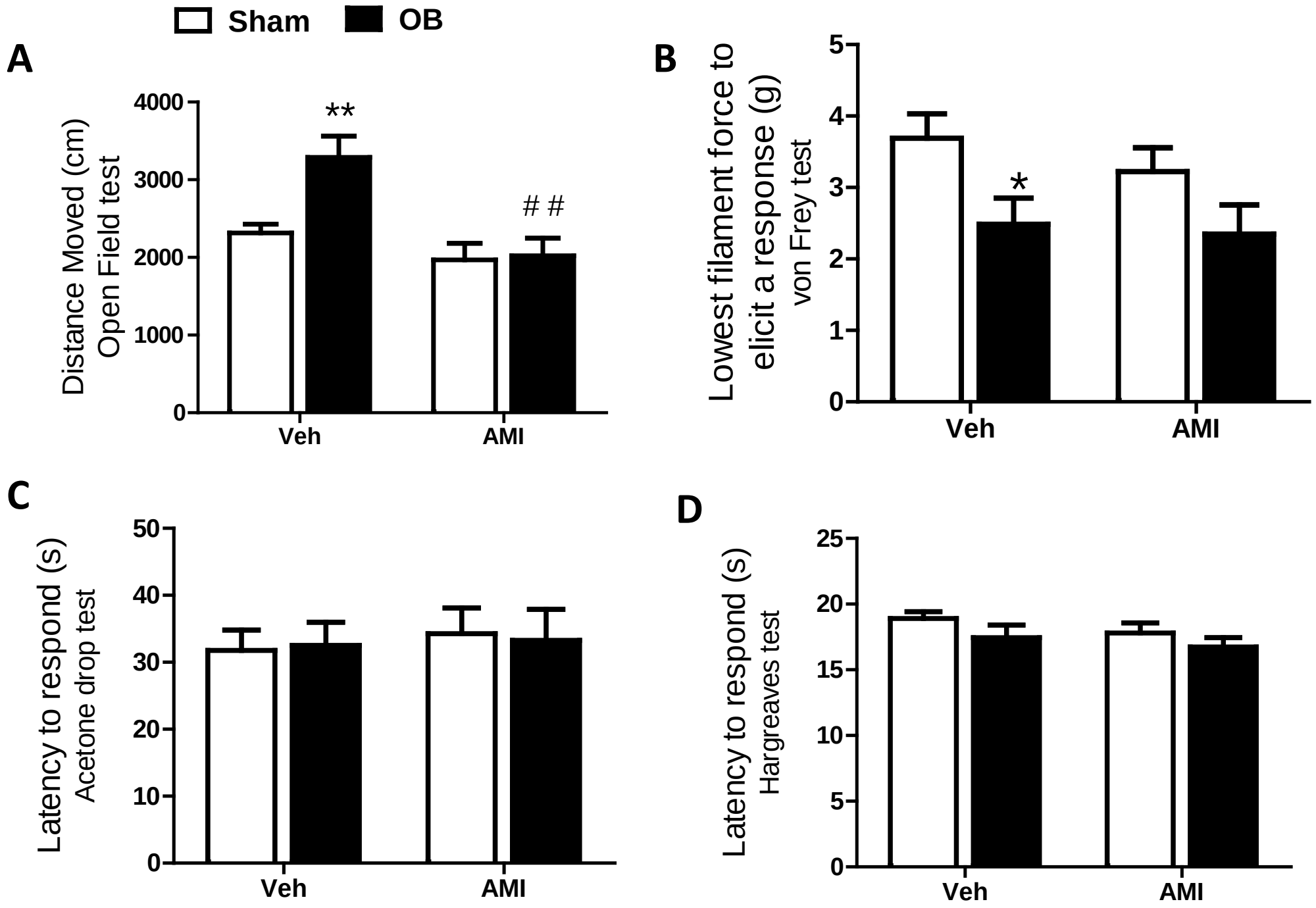

AMI/vehicle treatment (10mg/kg i.p.)
OB/Sham surgery
SNL surgery
OF
OF
VF
HG
PEAP
3
7
10
14
17
18
20
Post-SNL day
Post-OB day
-2 -1 0 14 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
VF+AT
VF+AT
HG
AT
Sacrifice and tissue harvesting

Figure 1


Sham
OB
A
Distance Moved (cm)
Open Field test
4000
3000
2000
1000
0
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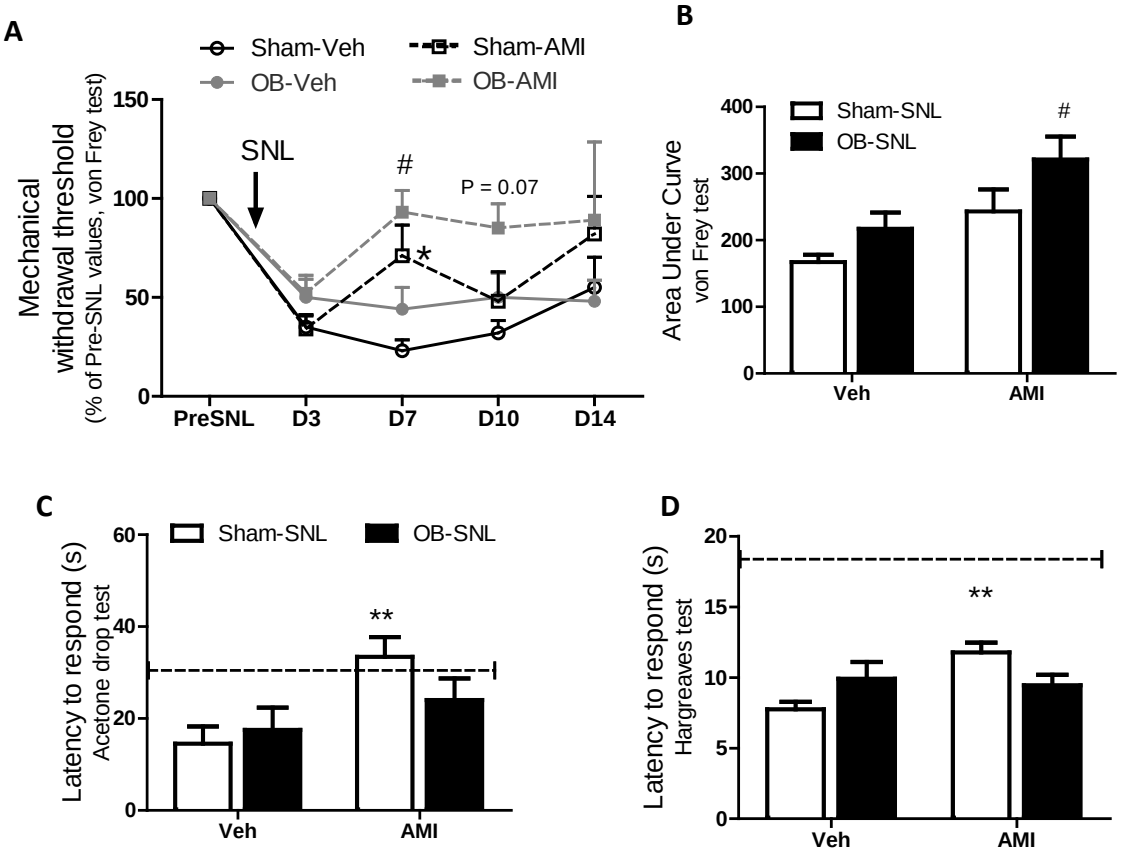
# #
Veh
AMI
B
Lowest filament force to
elicit a response (g)
von Frey test
5
4
3
2
1
0
*
Veh
AMI
C
Latency to respond (s)
Acetone drop test
50
40
30
20
10
0
Veh
AMI
D
Latency to respond (s)
Hargreaves test
25
20
15
10
5
0
Veh
AMI

Figure 3


A
Sham-Veh
Sham-AMI
OB-Veh
OB-AMI
Mechanical withdrawal threshold
(% of Pre-SNL values, von Frey test)
150
100
50
0
SNL
#
*
P = 0.07
PreSNL
D3
D7
D10
D14
B
Sham-SNL
OB-SNL
Area Under Curve
von Frey test
400
300
200
100
0
#
Veh
AMI
C
Sham-SNL
OB-SNL
Latency to respond (s)
Acetone drop test
60
40
20
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D
Latency to respond (s)
Hargreaves test
20
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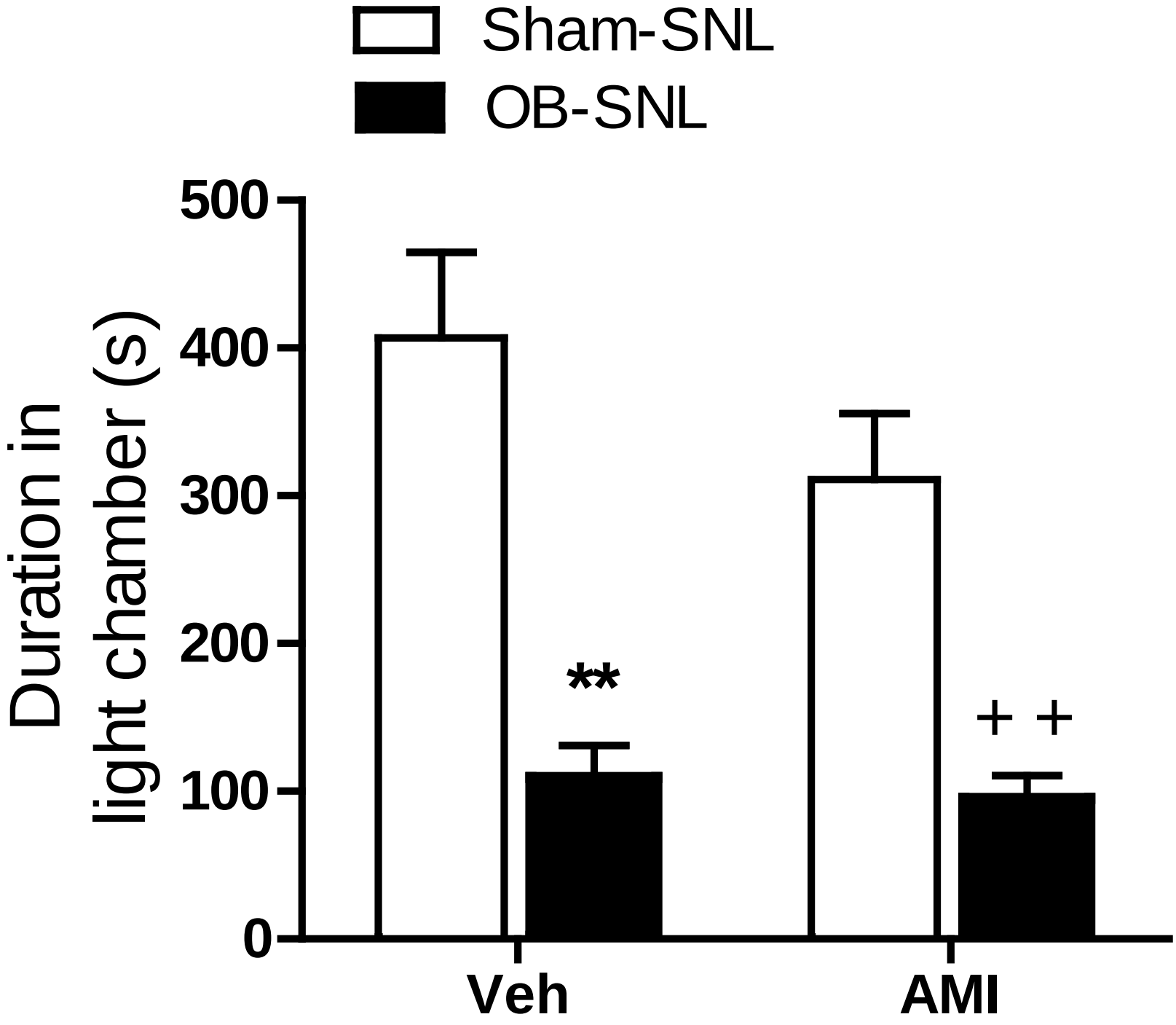
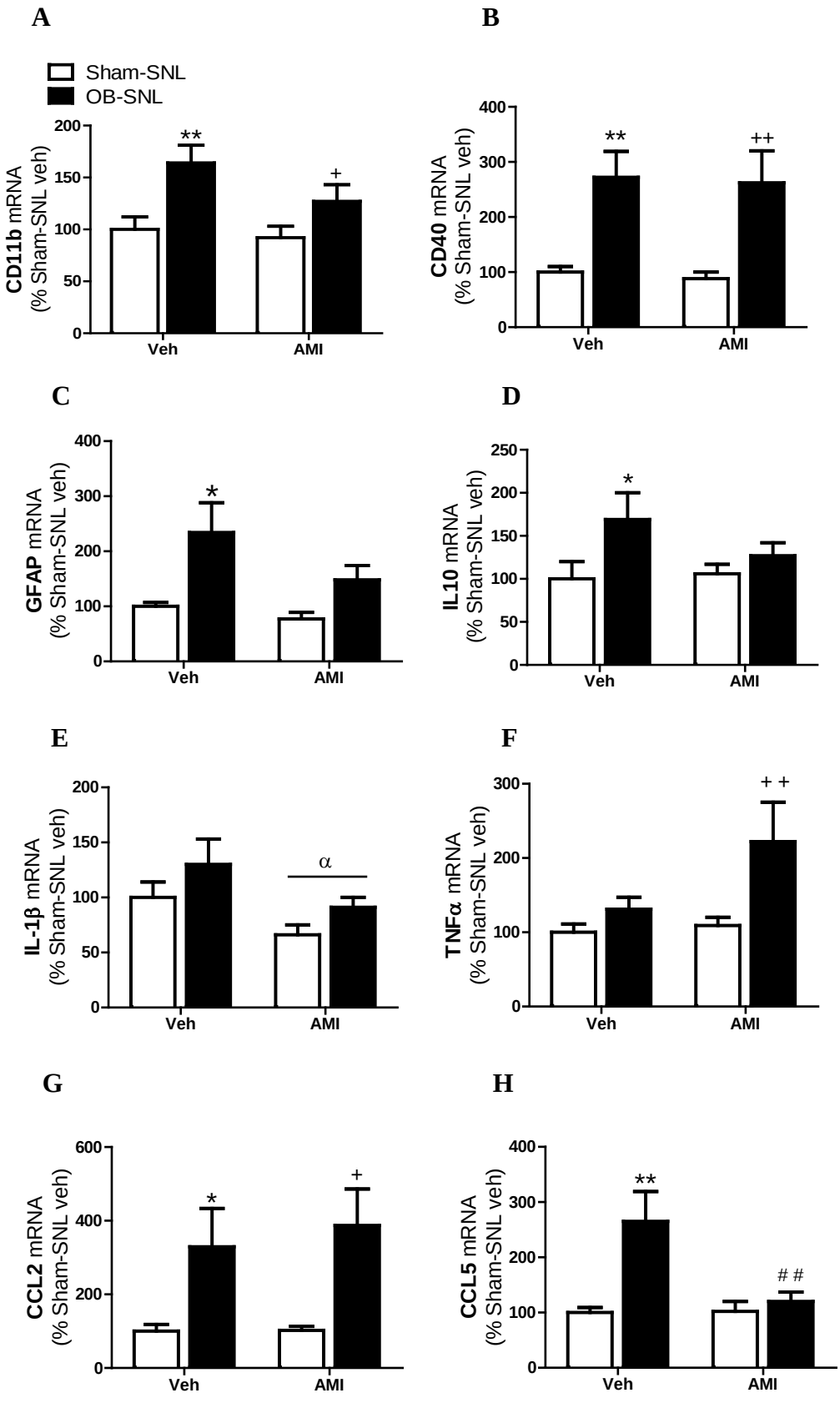

Sham-SNL
OB-SNL
Duration in light chamber (s)
500
400
300
200
100
0
**
+ +
Veh
AMI


A
Sham-SNL
OB-SNL
CD11b mRNA
(% Sham-SNL veh)
200
150
100
50
0
**
+
Veh
AMI
B
CD40 mRNA
(% Sham-SNL veh)
400
300
200
100
0
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++
Veh
AMI
C
GFAP mRNA
(% Sham-SNL veh)
400
300
200
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0
*
Veh
AMI
D
IL10 mRNA
(% Sham-SNL veh)
250
200
150
100
50
0
*
Veh
AMI
E
IL-1β mRNA
(% Sham-SNL veh)
200
150
100
50
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α
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AMI
F
TNFα mRNA
(% Sham-SNL veh)
300
200
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AMI
G
CCL2 mRNA
(% Sham-SNL veh)
600
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200
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*
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Veh
AMI
H
CCL5 mRNA
(% Sham-SNL veh)
400
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# #
Veh
AMI