A back translation of pregabalin and carbamazepine against evoked and non-evoked endpoints in the rat spared nerve injury model of neuropathic pain

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\textbf{A R T I C L E  I N F O}

\textbf{Article history:}
Received 8 March 2013
Received in revised form 20 May 2013
Accepted 22 May 2013

\textbf{Keywords:}
Pharmacokinetic
Exposure
Efficacy
Translation

\textbf{A B S T R A C T}

The purpose of the present study was twofold. First to characterize endpoints distinct to the reflexive responses to sensory stimuli typically used in neuropathic pain models. A second aim was to evaluate two clinically approved drugs carbamazepine (Tegretol\textsuperscript{\textcopyright}) and pregabalin (Lyrica\textsuperscript{\textcopyright}) against these endpoints with the purpose to backtranslate from the clinical to preclinical setting. The selected neuropathic pain model was the spared nerve injury (SNI) model and the endpoints were burrowing and measures of paw posture in Sprague Dawley rats. As previously described, SNI surgery produced a robust heightened sensitivity to tactile and thermal (cold) stimuli. SNI surgery also produced robust decreases in burrowing and affected multiple measures of paw position. There was no correlation between magnitude of change in burrowing and sensory allodynia within SNI operated rats. Pregabalin (10\textsuperscript{\texttimes}30 mg/kg IP) produced a reliable reversal of both tactile and cold allodynia and also the burrowing deficit, with minimal effect on neurological function evaluated using rotorod, beam walking and open field activity. Pregabalin did not affect any measure of paw position. Pharmacokinetic studies conducted in satellite animals identified plasma levels of pregabalin at the 10 mg/kg IP dose to be equivalent to clinically efficacious levels recorded in neuropathic patients (3–6 \mu g/ml). In contrast carbamazepine (10–60 mg/kg IP) had only a very modest effect against a reflexive (tactile) measure, and no effect against the burrowing deficit. Carbamazepine also affected various measures of neurological function, complicating interpretation of the reflexive measure. Measurement of burrowing appears to detect a behavioural deficit associated with the SNI model, that may be attenuated by pregabalin but not carbamazepine. Overall the present findings support an advantage of pregabalin over carbamazepine in terms of both efficacy and tolerability which is consistent with clinical experience. The inclusion of additional endpoints beyond traditional reflexive behaviours further supports the value of rodent neuropathic pain models, such as the SNI, as behavioural assays to detect new chemical entities to treat this pain condition.

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\textbf{1. Introduction}

Both pregabalin (Lyrica\textsuperscript{\textcopyright}) and carbamazepine (Tegretol\textsuperscript{\textcopyright}) are anticonvulsant drugs that have been approved for the treatment of conditions related to neuropathic pain (\textit{Eisenberg et al., 2007; Dworkin et al., 2010; Finnerup et al., 2010}). Pregabalin is specifically approved for diabetic peripheral neuropathy (DPN) and postherpetic neuralgia (PHN), while carbamazepine is approved for trigeminal neuralgia. Today Lyrica\textsuperscript{\textcopyright} (pregabalin) has the broadest neuropathic pain label of all marketed agents and accounts for almost 50\% of sales in the current neuropathic pain market (\textit{Nightingale, 2012}).

Preclinical models of neuropathic pain are important for an understanding of its aetiology, to guide clinical trials and to identify new treatments for human testing (\textit{Mogil et al., 2010a,b}). A variety of models based on peripheral nerve injury have been described with several involving a specific insult to the descending sciatic nerve and concomitant hypersensitivity of the partially denervated paw (see \textit{Dowdall et al., 2005; Qu and Ma, 2011} for review). The spared nerve injury model (SNI), initially described by \textit{Decosterd and Woolf (2000)}, involves a selective transaction to two (tibial and peroneal) of the three terminal branches of the sciatic nerve,
the sural nerve remaining intact. Over a period of days, the lateral portion of the affected paw, which is innervated by the sural branch, develops a hypersensitivity to a tactile or thermal stimulus. Measurement of the behavioural, i.e. flinch or withdrawal, response to an evoked sensory stimulus applied to the affected region represents the primary endpoint of this and related models.

Because clinical pain conditions are recognized to affect various aspects of daily living there is increasing awareness that the assessment of drug effects in preclinical pain models should be broadened beyond the measurement of reflexive responses, which do not reflect the chronic discomfort experienced by affected individuals and makes a minimal contribution to the measurement of clinical pain (Blackburn-Munro, 2004; Mogil, 2009; Rice et al., 2008; Burge, 2011). Recently Andrews et al. (2012) proposed burrowing as a novel means of assessing pain chronicity and an endpoint for therapeutics. Burrowing is an objective and readily quantifiable natural behaviour of rodents (Deacon, 2006, 2009) and Andrews et al. (2012) demonstrated its persistent reduction in rats following peripheral nerve injury (spinal nerve ligation (SNL) and tibial nerve transection (TNT)), and restoration by a pain relief medication (gabapentin, Neurontin®). In addition to burrowing deficits following peripheral nerve injury, the majority of animals also adopt a characteristic gait and postural change to the denervated paw which might reflect a guarding behaviour (Attal et al., 1990; Na et al., 1996; Piesla et al., 2009). The sustained nature of this change may also indicate that it too reflects a chronic discomfort and, thus serve as a useful endpoint. Consequently we decided to examine the impact of SNI surgery on both endpoints of burrowing and foot posture. We used a footprint analysis method to study paw posture with the aim to quantify specific parameters such as toe spread, print length, stride length and limb rotation angle (de Medinaceli et al., 1982; Klapdor et al., 1997; Varejão et al., 2003).

Having established experimental conditions that produced robust changes in burrowing and foot posture following SNI surgery, a second purpose of the present studies was to evaluate pregabalin and carbamazepine against these endpoints. In an initial study the effect of multiple doses of pregabalin and carbamazepine were evaluated against MES induced seizures, which served as a pharmacodynamic marker of drug action to guide dose and pretreatment time for the subsequent pain studies (Gabrielson et al., 2010). Next, the effect of both drugs was evaluated against the heightened reflexive response to tactile and cold stimuli, characteristic of the SNI model. Third, the effect of pregabalin and carbamazepine pretreatment on burrowing was examined. Fourth, using footprint analysis we characterized the postural changes following SNI surgery and assessed how doses of pregabalin effective against evoked sensory stimuli, influenced these parameters. In a further experiment, because we only found partial reversals of tactile/thermal allodynia following acute dosage, the effect of a chronic 12 day regimen of pregabalin treatment was examined to see if efficacy against an evoked tactile and thermal (cold) stimulus was enhanced by repeated treatment (see also Bauer et al., 2009). Finally, the pharmacokinetics of pregabalin were studied to establish plasma concentrations necessary for preclinical efficacy for comparison to therapeutic levels in humans (Whiteside et al., 2008; Burge, 2011). Given the benefit of extensive clinical experience with both pregabalin (Lyrica®) and carbamazepine (Tegetrol®) this also provided an opportunity to backtranslate clinical experience to the present preclinical findings.

2. Methods

2.1. Animals and housing

Male Sprague-Dawley rats were used throughout (source Charles River, St. Constant, Quebec, Canada). Animals were housed in polycarbonate cages with sawdust bedding with water and food (LabDiet, 5001) freely available. The housing room was maintained at a constant temperature of 22 ± 2 °C, under a 12 h light–dark cycle (lights on: 06:00–18:00 h). All testing was conducted under the light phase of the cycle forCHASED and animals light/dark cycle, and care was taken to randomize treatment sequences to control for possible order effects. All studies were approved by an Institutional Animal Care and Use committee and conducted in accordance with guidelines established by the Canadian Council of Animal Care (CCAC).

2.2. SNI surgery and recovery

Approximately 8 week old Sprague-Dawley rats weighed approximately 200–230 g at the time of surgery (Rode et al., 2007). Following anaesthesia with ketamine (75 mg/kg IP) and xylazine (10 mg/kg IP), the skin on the lateral surface of the thigh was incised and a section made directly through the biceps femoris muscle to expose the sciatic nerve on both sides of the tibia. The sciatic nerve was ligated with 4-0 silk sutures and sectioned distal to the ligation, removing 2–4 mm of the distal nerve stump. Extreme care was taken to avoid any contact with, or stretching of, the intact sural nerve. Sham controls involved exposure of the sciatic nerve without any lesion or further manipulation. At the completion of surgery, the muscles were sutured and the skin closed with silk sutures. For recovery, the animals were placed in a cage with fresh sawdust bedding. A minimal postoperative recovery period of 7 days was allowed before any testing and all drug studies commenced after a minimum period of 20 days post surgery. Over this period the animals developed marked sensory hypersensitivity of the hind paw ipsilateral to the nerve injury and changes to the paw posture became evident in most animals reflecting an avoidance of weight bearing on the lateral portion of the affected paw. Autotomy was never evident throughout post surgery and body weight gain was equivalent to sham controls. Prior to any experiment, a preliminary test was undertaken to assess mechanical allodynia (see below). Any non-responders (<5%) were removed from the study and these adjustments are highlighted in the relevant experimental sections.

2.3. Measurement of evoked responses to sensory stimuli

For the measurement of mechanical static allodynia, the animals were singly placed in clear elevated chambers on a Perspex grid floor and allowed 10–15 min to settle. The lateral plantar surface of the paw was stimulated with a series of ascending force Von Frey hair filaments (0.4, 1, 2, 4, 6, 8, 10, and 15 g). Application of filament to plantar surface was applied beginning with the lowest force filament (0.4 g). The threshold was taken as the lowest force that evoked a brisk withdrawal response. A filament with the next highest force was applied to confirm the threshold. The average score from three separate assessments was taken as the final measure for that animal.

The measurement of cold allodynia (acetone drop test) was conducted in the same chamber approximately 5 min following the Von Frey test. A drop of acetone solution was carefully dropped onto the lateral plantar surface of the paw, using a blunt needle connected to a syringe, without touching the skin. The magnitude of the withdrawal response was scored according to a 4 point rating scale where 0 – no visible response, 1 – response but without paw withdrawal, 2 – clear withdrawal of the paw, 3 – withdrawal combined with flinching and licking of the paw, 4 – paw withdrawal but not licking (Erichsen and Blackburn-Munro, 2002). The average score from three separate assessments was taken as the final measure for that animal.

For all experiments only the operated paw was measured, the nonsurgical baseline response being derived from the corresponding paw from sham operated animals. All evoked measurements were made with the experimenter unaware of the animals treatment identity.

2.4. Measurement of paw position and walking pattern

The effect of sciatic nerve injury on hind limb walking patterns was assessed using parameters previously reported (de Medinaceli et al., 1982; Klapdor et al., 1997; Varejão et al., 2003). The rats were trained on three consecutive days to cross strips of paper through a walkway (1 m long, 10 cm wide). The rats were motivated to cross the walkway through access to their home cages located at the end of the walkway. On the test day, the rat's hind paws were dipped in blue tempera paint. The rats subsequently crossed strips of paper through a walkway following the same procedure used for training. At least three stepping cycles were evaluated. The parameters limb rotation (angle between a virtual line through the third digit, the centre of the palm, and a virtual line parallel to the walking distance), print length (distance between back of palm to the third digit), total toe spreading (distance between first and fifth digits), intermediate toe spreading (distance between second and fourth digits), distance between feet (distance between inside of the left and right palmar), distance to opposite foot (distance between two footprints on opposite side) and stride length (distance between two footprints on each side) were analysed (see Fig. 1).
2.5. Burrowing

The burrows were hollow plastic tubes (320 mm long \( \times \) 100 mm diameter) sealed at one end with an open end raised approximately 60 mm above the ground to prevent gravel loss. Each burrow was filled with 2.5 kg aquarium gravel (5 mm grade: Aqua Culture, Walmart), for in pilot studies we found that the aquarium gravel was a preferred burrowing substrate for rats when compared to sand or 10 mm grade shingle (data not shown). The burrow was placed in a test cage of 240 mm (\( W \)), 240 mm (\( H \)), and 110 mm (\( L \)) (Fig. 1). The rats were habituated to burrowing conditions on two consecutive days. Animals were placed in pairs (with their cage-mate) into individual cages and allowed to burrow in the dark. The procedure was repeated on the second training day. If a pair of rats did not burrow on the first day, then a technique of social facilitation (see Andrews et al., 2012) was used by which one rat of this pair was swapped with a rat from a burrowing pair for the second day.

After the second day of habituation, rats that presented a tendency to burrow were placed individually into test cages with burrows on two separate days to determine their baseline activity of burrowing. Once trained, test sessions were of 1 h duration and the primary measure was the amount of shingle displaced from the burrow at the end of the test period. All burrowing measurements were made with the experimenter unaware of the animals treatment identity.

2.6. MES procedure

Approximately 4–5 week old male Sprague-Dawley rats of body weight 80–100 g were used for these studies. On the test day following a defined drug pretreatment period, rats received a maximal electroshock (150 mA, 0.2 s duration, 60 Hz) via corneal electrodes moistened with saline. Protection was determined by the Prism v.4.02 curve fitting program. ED50 values were derived from dose response curves consisting of a minimum of 5 dose levels.

2.7. Neurological test procedure

Approximately 10–13 week old male Sprague-Dawley rats of body weight 300–400 g were used for these studies. The test procedure consisted of a rectal body temperature recording made immediately before treatment and at a second pre-determined timepoint post treatment. Beam walking (1 m), rotorod (8 rpm and 16 rpm, cutoff 120 s); accelerating speed (no time cutoff); best score from three attempts at each condition, and locomotor activity (20 min test duration) were sequentially assessed. Drug pretreatment times and subject body weights were designed to match those used in the SNI model. All animals had been preexposed and trained to the rotorod and beam walking procedures 1–2 days prior to formal testing.

2.8. Drugs

Pregabalin (Neuraxxon, Toronto, Canada) and carbamazepine (Sigma, Oakville, Canada) were dispersed by sonication in 5% tween 80 in saline in a final dose volume of 5 ml/kg. Doses are expressed as that of the base.

2.9. Bioanalytical measurement of pregabalin

The concentration of pregabalin in the rat plasma samples was determined using an AB Sciex API4000 QTrap liquid chromatography/mass spectrometric system (LC-MS/MS) equipped with an Agilent 1200 series HPLC binary pump, solvent degaser, CTC autosampler and a Valco VICI divert valve. Pregabalin was monitored using selected reaction monitoring (SRM) of m/z 160–55 (collision energy 27 V) for quantification and m/z 160–142 (collision energy 17 V) for analyte confirmation. A gradient LC method using three serially connected Javelin Aquasil C18 columns (three columns of 20 \( \times \) 2.1 mm each) and mobile phase of 0.1% formic acid in water and 0.1% formic acid in methanol was developed to elute pregabalin and its internal standard (the deuterium labelled analog D4-pregabalin). Pregabalin and its internal standard eluted at 1.85 min which was well separated from two endogenous compounds (retention time of 1.11 min and 1.38 min) found in rat plasma blanks. The calibration range of pregabalin was from 10 to 10,000 ng/ml utilizing seven standards with a 1/e weighing least quadratic fitting. Pregabalin was extracted from rat plasma using protein precipitation in the ratio of 10:1 of organic (50/50 methanol/acetone) to plasma using 10 \mu/l of plasma. Internal standard was introduced in the first step of the protein precipitation procedure. The supernatant of protein precipitated mixture was subsequently dried down and the extract reconstituted in 50 \mu/l of 0.1% formic acid in water prior to LC–MS/MS quantification.

2.10. Experiment 1: effect of pregabalin and carbamazepine against MES induced seizures

Prior to commencing the studies in SN I prepared rats, the effect of pregabalin and carbamazepine against the tonic seizures induced by maximal electroshock was examined in experimentally naive rats. Both pregabalin (1–60 mg/kg SC; 2 h pretreatment, \( N = 6–8 \) per group) and carbamazepine (1–60 mg/kg IP; 0.5 h pretreatment, \( N = 4–6 \) per group) were tested using an independent groups design.

2.11. Experiment 2: effect of pregabalin and carbamazepine against evoked responses to sensory stimuli

Based on outcomes from the MES study, in an initial experiment both pregabalin (3–30 mg/kg IP; 2 h pretreatment) and carbamazepine (10–60 mg/kg IP; 30 min pretreatment) were independently tested in experimentally naive rats using a repeated measures design (pregabalin study: SNI rats, \( N = 11 \), Shams, \( N = 8 \); carbamazepine study: SNI rats, \( N = 11 \), Shams, \( N = 9 \)). Rats were between 20 and 25 days post surgery at the study start. Treatments were administered in a randomized sequence with 2–4 days between each cycle. The primary endpoints in both studies were the effect of test drug on response to a tactile (von Frey) and cold (acetone drop) stimulus.

In a second series of experiments both pregabalin (3–30 mg/kg oral; 4 h pretreatment) and carbamazepine (10–60 mg/kg IP; 90 min pretreatment) were reexamined using either a different route of administration (pregabalin) or pretreatment time (carbamazepine), otherwise test design was identical (pregabalin study: SNI rats, \( N = 12 \), Shams, \( N = 8 \); carbamazepine study: SNI rats, \( N = 8 \), Shams, \( N = 8 \)).

2.12. Experiment 3: effect of pregabalin and carbamazepine on burrowing behaviour

In experiment 3a, following familiarization to the burrowing procedure, rats were randomly divided into two groups based on equivalent overall burrowing scores, based on a test conducted 2 days before surgery. A total of 12 rats were surgically prepared with sciatic nerve lesion (SNI), and a total of 12 rats received sham surgery. At day 5, 10, 15 and 20 post surgery the animals were tested for sensitivity to a tactile stimulus, followed by a 1 h burrowing test.

On day 27 and 30 post surgery, the effect of pregabalin (10 mg/kg IP) and vehicle control was examined against a tactile stimulus followed by a burrowing test. A
cross-over design was adopted in both SNI and sham prepared rats. Von Frey testing was conducted 4 h post treatment.

In experiment 3b, a further group of rats were prepared with either SNI surgery (n = 15) or sham controls (n = 15). Two rats from the SNI surgical group were subsequently removed due to Von Frey scores that were identical to shams. On Day 27 and 31 post surgery, the effect of carbamazepine (30 mg/kg IP) and vehicle control was examined against a tactile stimulus followed by a burrowing test. A cross-over design was adopted in both SNI and sham prepared rats. Von Frey testing was conducted 0.5 h post treatment.

2.13. Experiment 4: effect of pregabalin against SNI-induced change in paw position

A group of 20 Sprague-Dawley rats were first trained to cross a walkway as previously described. Next, n = 10 received SNI surgeries, and n = 10 received sham control surgery. One SNI prepared rat was subsequently dropped from the study due to lack of hypersensitivity to a tactile stimulus. Following a 28 day incubation period, during which footprint measures were formally conducted at day 10 (data not shown) and day 20, the animals were tested with pregabalin at 10 and 30 mg/kg IP and saline control. Both Von Frey testing and footprint analysis was conducted 2 h and 4 h post treatment. A time interval of 3 days was allowed between each treatment cycle, i.e on days 28, 31 and 34 post surgery.

2.14. Experiment 5: effect of pregabalin and carbamazepine on neurological function

Unoperated, male Sprague-Dawley rats (300–400 g body weight) pretreated with either pregabalin (10–30 mg/kg IP) or carbamazepine (30–60 mg/kg IP) were assessed for neurological function following pretreatment times corresponding to the SNI experiments. Accordingly the pregabalin examination ran from 100 to 140 min post dosing and the carbamazepine examination 15–45 min and 75–105 min post dosing. A between subjects design was used with N = 8 rats per treatment group.

2.15. Experiment 6: effect of chronic pregabalin treatment against evoked response to sensory stimuli

A total of 36 Sprague-Dawley rats were used for this study, 26 of which received SNI surgeries and 10 received sham surgery. 18 and 20 days post surgery all animals were tested for response to Von Frey and acetone stimuli as previously described (baseline 1 and baseline 2). Based on equivalent outcomes, the SNI rats were divided into 2 groups, one designated to be SNI-vehicle group, the other SNI-pregabalin (10 mg/kg IP). Sham operated rats were designated vehicle.

Treatments started on day 22 post surgery, and were administered twice daily at a dose and duration as previously described. On treatment days 1 and 12 following the evoked sensory measures, the animals were also tested for neurological function on rotorod (8, 16 rpm), open field activity (20 min duration), beam walking and body temperature.

On treatment Day 13 all SNI-pregabalin rats received pregabalin (10 mg/kg IP), and 5 rats from the SNI-vehicle group also received pregabalin (10 mg/kg IP), i.e an acute dose of pregabalin. 4 h post treatment, 300 μL of whole blood was collected from the saphenous vein for subsequent determination of pregabalin concentration. Blood was placed into EDTA coated tubes, centrifuged at 2000 g at 4 °C for 10 min, the plasma decanted and stored at −80 °C until biochemical quantification of pregabalin concentration.

2.16. Experiment 7: pharmacokinetic studies of pregabalin

Separate groups of male Sprague Dawley rats were treated either with pregabalin (10 or 30 mg/kg IP, N = 3 and N = 6 respectively) or 30 mg/kg (oral, N = 4). At multiple time points (0.5, 1, 2, 4, 6, 24 h) approximately 300 μL of whole blood was collected from the saphenous vein into EDTA coated tubes, centrifuged at 2000 g at 4 °C for 10 min, the plasma decanted and stored at −80 °C until biochemical quantification of pregabalin concentration.

2.17. Data analysis

All statistical analyses for experiments 2–6 were performed using Statistica Version 11 (StatSoft, Tulsa OK). Analysis of variance with repeated measures was completed for experiments 2–6 with the grouping factor of SNI and sham treated groups for experiments 3, 4 and 6 and drug treatment (drug vs. vehicle) for experiment 5. The repeated measure of dose was included for experiments 2 and 3, treatment (vehicle vs. drug) for experiment 4 and time for experiments 5 and 6. Post hoc analyses included Dunnett’s 1-t-test, Tukey’s HSD or Fisher’s LSD for further group comparisons when warranted. Effect sizes for parametric analyses was also calculated (partial eta squared and Cohen’s d) in recognition of ARRIVE guidelines (Kilkenny et al., 2010). The Kruskall Wallis H test was used to analyse rotorod performance at both 8 and 16 rpm in experiments 5 and 6. Correlation analysis was used in experiment 4 to compare burrowing performance with Von Frey withdrawal threshold. Statistical significance was set at P < 0.05, and a trend towards significance was considered P < 0.1.

Plasma concentration data were analysed by noncompartmental methods using WinNonlin Pro (Pharsight Corp., Mountainview, CA). Areas under the plasma concentration–time curves (AUC) for each animal were calculated by the linear–log-linear trapezoidal rule and extrapolated to infinity (AUC0–inf) by the addition of Clast/k, where Clast represents the last measurable plasma concentration and k represents the terminal rate constant. Terminal half-lives (t1/2) were calculated as ln(2)/k. Mean residence times (MRT) were calculated as: AUMC/AUC where AUMC denotes the area under the first moment curve. The time (tmax) to reach the maximum concentration (Cmax) was determined from nominal values. Pharmacokinetics parameters (Cmax/Dose, AUC0–inf/Dose and t1/2) for the 2 IP doses (10 and 30 mg/kg) and between the IP and oral routes (30 mg/kg) were compared statistically using the unpaired t-test, with P = 0.05 considered as significant.

3. Results

3.1. Experiment 1: effect of pregabalin and carbamazepine against MES induced seizures

Pretreatment with both pregabalin (1–60 mg/kg IP; 2 h pretreatment) and carbamazepine (1–60 mg/kg IP; 0.5 h pretreatment) produced a dose related protection against tonic seizures induced by maximal electroshock (see Fig. 2A). Calculated ED50’s (95% confidence limits) measured from these experiments were pregabalin 6.7 (3.8–11.6) mg/kg, carbamazepine 2.0 (1.7–2.4) mg/kg.

3.2. Experiment 2: effect of pregabalin and carbamazepine against evoked responses to sensory stimuli

In all SNI experiments, there was a highly significant (P < 0.01, nφp < 0.3) effect of surgery on both tactile and cold allodynia compared to sham operated controls (see Fig. 2B and C). In experiment 2a, a main effect of pregabalin (3–30 mg/kg IP; 2 h) on measures of tactile (F3,30 = 6.2, P < 0.01, nφp = 0.38) and cold allodynia (F3,30 = 7.0, P < 0.01, nφp = 0.41) reflected a significant effect of pregabalin against the hypersensitivity in vehicle treated SNI rats at the 10 and 30 mg/kg doses (Fig. 2B and C). Neither dose restored threshold levels to sham operated controls. In experiment 2b, similar effects of pregabalin (3–30 mg/kg oral; 4 h) on tactile (F3,33 = 25.9, P < 0.01, nφp = 0.70) and cold allodynia (F3,33 = 8.9, P < 0.01, nφp = 0.45) were found, although the effect size at the 30 mg/kg dose against tactile allodynia seemed more marked than the equivalent dose delivered.

In experiment 2c, a main effect of carbamazepine (10–60 mg/kg IP; 0.5 h) on tactile allodynia (F3,27 = 4.7, P < 0.01, nφp = 0.34) reflected a small, but significant attenuation of this measure at the 60 mg/kg dose compared to vehicle controls. Lower doses were ineffective. There was no main effect of carbamazepine on cold allodynia (F3,27 = 0.7, NS, nφp = 0.07). In a subsequent experiment (experiment 2d), utilizing identical doses of carbamazepine but an extended pretreatment time of 90 min, there was no main effect against either tactile (F3,21 = 2.4, P > 0.1, nφp = 0.26) or cold allodynia (F3,21 = 1.8, NS, nφp = 0.21) (Fig. 2B and C).

3.3. Experiment 3: effect of pregabalin and carbamazepine on burrowing in SNI prepared rats

In experiment 3a, presurgical burrowing and Von Frey scores were similar between groups (see Fig. 3A and B). Post surgery, a main effect of group (F1,22 = 5.9, P = 0.02, nφp = 0.21), and day (F4,88 = 6.8, P < 0.01, nφp = 0.24) reflected that following SNI surgery, rats showed reduced burrowing compared to sham operated controls at Day 10, 15 and 20 (see Fig. 3B). As expected the tactile threshold to a Von Frey stimulus was also affected by SNI surgery (group: F1,22 = 20.3, P < 0.01, nφp = 0.48; day: F4,88 = 8.8, P < 0.01,
However comparing the degree of burrowing with tactile threshold for all SNI operated rats at Days 5, 10, 15 and 20 failed to identify any correlation ($r(10) < 0.5$, $P > 0.1$) (see Fig. 3C).

Next, on Day 27 and 30 post surgery, the effect of pregabalin (10 mg/kg IP) and vehicle control was examined against a tactile stimulus followed by a burrowing test using a crossover design. Main effect of surgery ($F_{1,22} = 4.3$, $P = 0.05$, $\eta^2_p = 0.16$) and a pregabalin $\times$ surgery interaction ($F_{1,22} = 6.3$, $P = 0.02$, $\eta^2_p = 0.22$) reflected that pregabalin reversed the burrowing deficit evident in SNI operated rats. Pregabalin had no effect on burrowing in sham operated rats (see Fig. 4B). In this same experiment, pregabalin attenuated the tactile allodynia evident in SNI prepared rats (surgery: $F_{1,22} = 10.5$, $P < 0.01$, $\eta^2_p = 0.32$; pregabalin: $F_{1,22} = 5.7$, $P = 0.03$, $\eta^2_p = 0.21$, see Fig. 4A).

In a further experiment (experiment 3b), using experimentally naïve rats, an equivalent study was conducted to examine the effect of carbamazepine (30 mg/kg IP) against the burrowing deficit and tactile allodynia in SNI prepared rats. As with experiment 3a, we first evaluated burrowing scores in sham and SNI operated rats at various times post surgery in the absence of any treatment. Although at D5 (sham: 1857 ± 92 g; SNI: 1577 ± 158 g; NS, $d = 0.58$) and D10 (sham: 1977 ± 55 g; SNI: 1746 ± 147 g; NS, $d = 0.56$) burrowing was modestly reduced, only at D20 was a significant group difference noted (sham: 1801 ± 120 g; SNI: 1197 ± 218 g).

Fig. 2. Both pregabalin (1–60 mg/kg IP; 2 h ptt) and carbamazepine (1–60 mg/kg IP; 0.5 h ptt) were tested against seizures induced by (A) maximal electroshock (MES). Based on anticonvulsant efficacy in this model, both drugs were tested against both the (B) tactile and (C) thermal (cold) allodynia in SNI prepared rats using equivalent pretreatment times and dosages (see methods for detail). Pregabalin was tested following both IP and oral administration (3–30 mg/kg) and carbamazepine was tested following IP injection at 0.5 h and 1.5 h post treatment. In each experiment a group of sham operated animals (Sham) were used to determine baseline threshold to each stimulus. *$P < 0.05$ vs. respective vehicle treated group. #P < 0.05 vs. SNI control treatment group. N = 8–12 rats per group (see methods for experimental detail).

Fig. 3. Effect of SNI surgery on (A) the threshold withdrawal response to a tactile (Von Frey) stimulus. (B) the amount of gravel displaced in a 1 h burrow test. Rats were surgically prepared on Day 1, and at Day 5, 10, 15 and 20 the rats were assessed. A presurgical baseline measure was also taken 2 days prior to surgery. (N = 12 sham, N = 12 SNI rats). (C) Lack of correlation between the threshold withdrawal response and burrowing score for individual SNI prepared rats tested over D5–D20.
P = 0.02, d = 0.92). Consistent with experiment 4a, there was no correlation between burrowing scores and tactile allodynia (e.g. Day 20: correlation 0.14, NS).

The effect of carbamazepine in these animals was evaluated on D27 and D31, again using a crossover design. A main effect of surgery ($F_{1,25} = 8.4, P < 0.01, \eta^2_p = 0.25$) but no main effect of carbamazepine or carbamazepine × surgery interaction, reflected that drug treatment failed to affect the reduced burrowing evident in SNI prepared rats. A similar null effect of carbamazepine was found on tactile allodynia, despite SNI rats showing significant tactile allodynia (see Fig. 4C and D).

In a final experiment the effect of carbamazepine (60 mg/kg IP) on burrowing in sham prepared rats was examined. Consistent with observations made from the neurological testing (see experiment 5), carbamazepine reduced burrowing (Vehicle: 1995 ± 209 g; CBZ 60 mg/kg IP: 1185 ± 345 g; $P < 0.05, d = 1.07$ paired one tailed t-test).

3.4. Experiment 4: effect of pregabalin against SNI-induced change in paw position

Measured at Day 20, SNI surgery produced highly robust changes on paw length, toe spread, paw rotation on the ipsilateral side compared to sham operated controls. Specifically, SNI surgery reduced toe spread, increased paw length and resulted in a modest rotation of the affected limb away from the long axis. There was also a marked tendency for the affected paw to be everted so that only the internal edge was in contact with the floor surface (see Fig. 1A and B), although we made no attempt to quantify this change. Contralateral paw measures seemed essentially unaffected (see Fig. 5). Stride length was also slightly reduced in SNI prepared rats compared to controls.

Given the apparent reliability of these changes, the effect of pregabalin (10–30 mg/kg IP) was investigated. Although both Von Frey and footprint measures were taken at 2 and 4 h post treatment, because the most robust effects of pregabalin on Von Frey measure were at the 4 h timepoint (see Table 1), only gait data are presented for the 4 h timepoint. Pregabalin (10–30 mg/kg) produced a main effect on withdrawal threshold ($F_{2,34} = 13.7, P < 0.01, \eta^2_p = 0.45$), with the 30 mg/kg dose reaching significance at each timepoint, yet the 10 mg/kg dose was significant at the 4 h timepoint only. On each of the 4 primary measures of toe spread, foot rotation, paw length and stride length there was a highly significant main effect of surgery ($F_{1,18} > 54.5, P < 0.01, \eta^2_p > 0.75$), however no main effect of pregabalin ($F_{2,36} < 0.6, \eta^2_p < 0.01$) or surgery × pregabalin interaction ($F_{2,36} < 1.0, \eta^2_p > 0.01$) was recorded, reflecting that pregabalin treatment had no effect on any footprint measure (see Table 1). At the 2 h timepoint, pregabalin had no effect on any paw measure (data not shown).

3.5. Experiment 5: effect of pregabalin and carbamazepine on neurological function

Pregabalin (10–30 mg/kg) was generally well tolerated following intraperitoneal administration. There was no treatment effect on general activity (distance travelled) at the 2 h and 4 h
timepoints, although a small decrease in rearing was measured at 30 mg/kg (2 h timepoint only, see Table 2). No main effects of treatment on beam walking latency or body temperature was evident at either timepoint. Rotorod testing revealed treatment effects that were related to task difficulty. At the slowest speed (8 rpm) there were trends towards significance for 2 h ($H(2, N = 24) = 5.5, P = 0.06$), and 4 h ($H(2, N = 24) = 5.0, P = 0.08$) and at 16 rpm at 2 h ($H(2, N = 24) = 5.4, P = 0.07$) at 30 mg/kg. Testing under 16 rpm at 4 h ($H(2, N = 24) = 7.5, P = 0.02$) and accelerating speed ($F(2,21) = 6.4, P < 0.01$) identified treatment effects with pregabalin reducing performance defined by fall latencies at 30 mg/kg, and in the case of the accelerating condition, at 10 mg/kg (see Table 2).

Carbamazepine (60 mg/kg IP) detrimentally affected all aspects of neurological test performance at the 0.5 h pretreatment time (see Table 3). Open field activity measured by distance travelled and rearing was reduced at the 30 mg/kg dose (see Table 3). At the 1.5 h timepoint, the intensity of these effects of carbamazepine on neurological test performance had declined (see Table 3).

### 3.6. Experiment 6: effect of chronic pregabalin treatment against evoked response to sensory stimuli induced by SNI surgery

At pre-drug baseline there was no difference between the SNI operated groups, although relative to sham operated controls both groups had a significant tactile and cold allodynia. During the treatment phase, a main effect of treatment ($F(2,33) = 84.5, P < 0.01, \eta^2_p = 0.84$), day ($F(3,99) = 3.6, P = 0.02, \eta^2_p = 0.10$) but not treatment/day interaction ($F(6,99) = 0.8, NS, \eta^2_p = 0.05$) reflected that pregabalin (10 mg/kg IP x 2 daily) increased tactile threshold compared to the vehicle/SNI group, although the magnitude of this change was similar over days (see Fig. 6). Within day post hoc tests

![Fig. 5](image-url). Effect of SNI or Sham surgery on the various static paw parameters measured 20 days post surgery. (□) Paw ipsilateral to surgery, or (■) paw contralateral to surgery. N = 10 Sham, N = 9 SNI. *P < 0.05 vs. contralateral (unoperated) paw.

### Table 1
Effect of pregabalin on various measures of static paw posture.

<table>
<thead>
<tr>
<th>Tactile alldynia</th>
<th>Gait measures (4 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>Pregabalin 10</td>
<td>Sham</td>
</tr>
<tr>
<td>Pregabalin 30</td>
<td>Sham</td>
</tr>
<tr>
<td>Vehicle</td>
<td>SNI</td>
</tr>
<tr>
<td>Pregabalin 10</td>
<td>SNI</td>
</tr>
<tr>
<td>Pregabalin 30</td>
<td>SNI</td>
</tr>
</tbody>
</table>

Effect of Pregabalin against the various static paw measures induced by SNI surgery. On Days 28–34 post surgery, the effect of PGB (10–30 mg/kg IP) was evaluated against the 4 principal paw measures. Readings were taken at both 2 h and 4 h post treatment, however only the 4 h gait data are presented which corresponded to peak effect of PGB against tactile (Von Frey) alldynia. PGB failed to influence any of the paw measures. Data presented as means ± SEM. *$P < 0.05$ for Vehicle SNI vs. Sham surgery group. # $P < 0.05$ for pregabalin/SNI treatment group compared to vehicle/SNI treatment group (see Methods for experimental detail).
showed pregabalin to significantly elevate tactile threshold on each test day. A similar finding was noted for pregabalin against the acetone drop with a main effect of treatment ($F_{2,33} = 26.4, P < 0.01$, $\eta^2_p = 0.62$), day ($F_{3,99} = 10.2$, $P < 0.01$, $\eta^2_p = 0.24$) but not treatment $\times$ day interaction ($F_{6,99} = 0.95$, NS, $\eta^2_p = 0.05$). The difference between the vehicle/SNI and pregabalin/SNI groups on cold allodynia was more subtle compared to the Von Frey measure, with pregabalin only significantly attenuating the withdrawal response on Days 1, but narrowly missing significance on Days 8 and 12. Nonetheless, based on these results there was no evidence of either tolerance or sensitization to repeated pregabalin treatment.

Neurological tests were conducted at Day 1 and Day 12 and these results are presented by Table 4. Comparisons between vehicle treated sham and SNI groups failed to detect any significant effect of SNI surgery on locomotor activity, rearing, beam walking or rotordor performance, although the latter measure showed a trend to reduced rotordor fall latency in the SNI group (16 rpm Day 1, H(2, N = 36) = 5.7, $P = 0.06$). Consistent with outcomes from the neurological tests conducted in intact rats (see Table 2), pregabalin (10 mg/kg IP) had minimal impact on locomotor activity, rearing and beam walking latency in SNI rats either 4 h post initial treatment, or after 12 days of treatment (see Table 4). The modest deficit in rotordor performance evident in SNI rats relative to shams was unaffected by pregabalin treatment.

Measurement of pregabalin plasma levels on Day 13, 4 h after either the final dose in the pregabalin-SNI group, or a single dose in the vehicle-SNI group identified blood levels to be similar between groups (chronic: $3.7 \pm 0.2$ µg/mL, acute: $3.1 \pm 0.4$ µg/mL; NS), suggesting negligible accumulation or autoinduction.

### 3.7. Experiment 7: Pharmacokinetic studies of pregabalin

The plasma concentration versus time profiles for pregabalin following administration of two doses IP (10 and 30 mg/kg) and the 30 mg/kg oral dose are depicted graphically in Fig. 7. The estimated pharmacokinetics parameters are summarized in Table 5. The dose-normalized $C_{\text{max}}$ and $AUC_{0-\infty}$ are not significantly different between the 10 and 30 mg/kg IP doses, confirming that plasma exposure of pregabalin is proportional to dose, and the elimination half-lives (1/2) are essentially identical. These parameters did also not differ significantly between the 30 mg/kg IP and oral doses.

### 4. Discussion

#### 4.1. Characterisation of endpoint measures of paw posture and burrowing in the SNI model

A significant aspect to this work was to examine endpoints alternative to the reflexive tests which it is argued do not measure pain per se but rather the hypersensitivity that may accompany pain (Blackburn-Munro, 2004; Mogil, 2009; Rice et al., 2008). Since the predominant feature of neuropathic pain conditions are spontaneous and chronic in nature, endpoints that attempt to measure this aspect are required to improve face validity. To date a variety have been examined, including affective state measured indirectly by techniques such as place conditioning and sucrose preference, motor/exploratory behaviour, paw posture/gait, facial encoding, vocalization and burrowing (Mogil, 2009, 2010a,b; Andrews et al., 2012; Munro et al., 2012). For such endpoints to have significant value, they need to demonstrate a robustness of response and reliability across multiple experiments, a specificity to pain, and a sensitivity to pain therapeutics (Mogil, 2009). The primary endpoints chosen were (a) burrowing, an ethologically relevant, relatively simple and quantifiable active behaviour displayed by rodents (Deacon, 2006, 2009; Andrews et al., 2012), and (b) changes in paw posture which typically emerge following partial damage to the sciatic nerve in rodents and maintained for weeks thereafter, and suggestive of an attempt by the animal to guard against a chronic discomfort (Attal et al., 1990; Na et al., 1996; Piesla et al., 2009). One of the primary reasons for selecting these two approaches was a perceived likelihood of reliability based on a relative simplicity to the endpoint and its measurement.

### Table 2

Summary of pregabalin on motor function in Sprague-Dawley rats.

<table>
<thead>
<tr>
<th></th>
<th>Open field</th>
<th>Rotorod</th>
<th>Beam walking</th>
<th>Core body temp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LMA</td>
<td>Rears</td>
<td>8 rpm</td>
<td>16 rpm</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2 h</td>
<td>4245 ± 237</td>
<td>152 ± 14</td>
<td>92(38–120)</td>
</tr>
<tr>
<td></td>
<td>4 h</td>
<td>1729 ± 211</td>
<td>38 ± 6</td>
<td>120(120–120)</td>
</tr>
<tr>
<td>Pregabalin</td>
<td>10 h</td>
<td>5650 ± 730</td>
<td>147 ± 17</td>
<td>120(57–120)</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>1731 ± 419</td>
<td>31 ± 7</td>
<td>111(62–120)</td>
</tr>
<tr>
<td>Pregabalin</td>
<td>2 h</td>
<td>4739 ± 930</td>
<td>97 ± 14*</td>
<td>29(17–70*)</td>
</tr>
<tr>
<td></td>
<td>3 h</td>
<td>1157 ± 269</td>
<td>21 ± 6</td>
<td>67(43–114)</td>
</tr>
</tbody>
</table>

Data presented as means ± SEM, or median and interquartile range (rotordor data). $N$ = 8 Sprague-Dawley rats per group. All rats were tested at 2 h and 4 h following vehicle or Pregabalin (10 and 30 mg/kg IP), i.e timepoints corresponding to the evoked pain testing. See methods for details. *$P < 0.05$ vs. Vehicle control tested under equivalent timepoint (see Methods for statistical detail).

### Table 3

Summary of carbamazepine on motor function in Sprague-Dawley rats.

<table>
<thead>
<tr>
<th></th>
<th>Open field</th>
<th>Rotorod</th>
<th>Beam walking</th>
<th>Core body temp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LMA</td>
<td>Rears</td>
<td>8 rpm</td>
<td>16 rpm</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.5 h</td>
<td>2413 ± 280</td>
<td>72 ± 13</td>
<td>120(113–120)</td>
</tr>
<tr>
<td></td>
<td>1.5 h</td>
<td>798 ± 186</td>
<td>22 ± 4</td>
<td>128(120–120)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>30 h</td>
<td>1458 ± 277</td>
<td>38 ± 5*</td>
<td>115(62–120)</td>
</tr>
<tr>
<td></td>
<td>30 h</td>
<td>759 ± 188</td>
<td>21 ± 4</td>
<td>120(120–120)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>60 h</td>
<td>1207 ± 393*</td>
<td>26 ± 11*</td>
<td>83(41–120)*</td>
</tr>
<tr>
<td></td>
<td>60 h</td>
<td>972 ± 276</td>
<td>25 ± 8</td>
<td>105(76–120)</td>
</tr>
</tbody>
</table>

Data presented as means ± SEM, or median and interquartile range (rotordor data). $N$ = 8 Sprague-Dawley rats per group. All rats were tested at 0.5 h and 1.5 h following vehicle or Carbamazepine (30 and 60 mg/kg IP), i.e timepoints corresponding to the evoked pain testing. See methods for details. *$P < 0.05$ vs. Vehicle control tested under equivalent timepoint (see Methods for statistical detail).
Similar to the report of Andrews et al. (2012), peripheral nerve injury produced a decline in burrowing behaviour which appeared consistent from 20 to 30 days post surgery and within animals. This was seen in two separate experiments using similar methodologies. In each study, there was no apparent correlation between the level of tactile hypersensitivity with the burrowing deficit, indeed the emergence of the burrowing deficit seemed delayed relative to the emergence of sensory allodynia post SNI surgery, suggesting an independence between both measures. Nonetheless because of the good within animal consistency across repeated burrowing test sessions, this facilitated a cross-over design to examine the effect of pregabalin or carbamazepine against vehicle control on the deficit in burrowing apparent following SNI surgery. All drug studies were conducted after a minimum of 25 days post surgery by which time in the two separate cohorts, the SNI operated rats showed a reliable decrease in burrowing relative to their sham controls.

Postural changes in the denervated paw have been previously reported in various neuropathic pain models involving sciatic nerve damage. For example, Na et al. (1996) described postural changes subsequent to spinal nerve ligation (SNL) surgery. This group described some variability amongst individual rats, as well as a post-surgical time dependence, although prominent signs included foot eversion and ventroflexed toes. Similar changes were reported by Attal et al. (1990) in rats post CCI surgery. To our knowledge this is the first report detailing the effect of SNI surgery on paw posture in the rat and one notable feature was the high interanimal reliability in these changes, at least when measured between 20 and 40 days post surgery. The characteristic features of the denervated paw were (1) increased outward rotation angle, (2) reduced toe spread reflecting ventroflexion of the digits, (3) increased print length, and (4) reduced stride length; thus as expected, there was overlap with changes described in these earlier reports using alternative surgical models. Foot eversion, although not directly measured, was also visually observed in most rats (see Fig. 1). Na et al. (1996) describe these changes as reflecting a complex mix of motor and sensory abnormalities, with at least some reflecting an attempt by the animal to guard against a heightened sensory function. Nonetheless, the transection of the large diameter motor axons carried within the peroneal and tibial branches of the sciatic nerve support the likelihood of a significant contribution of motor abnormality.

In a study primarily designed to measure the impact of chronic pregabalin treatment against evoked measures, we also included a neurological test component which enabled us to evaluate the effect of SNI surgery on a forced/skilled motor behaviour (rotorod, beam walking) and open field exploration. Similar to another recent report using the CCI model (Munro et al., 2012), there was minimal change in any of these measures when assessed 20–32 days post SNI surgery.

4.2. Characterisation of carbamazepine and pregabalin in the SNI model

Reversals of tactile (both static and dynamic) and thermal (cold) allodynia have been previously reported following acute administration of pregabalin (Field et al., 1999; Tanimoto-Mori et al., 2008; Gustafsson and Sandin, 2009; Nakazato-Imasato et al., 2009) in multiple models of rat neuropathic pain. By way of contrast, and despite clinical utility, findings for carbamazepine appear less positive with the majority suggesting at best only marginal effects against evoked responses in equivalent models (Hunter et al., 1997; Idanpaan-Heikkila and Guilbaud, 1999; Decosterd et al., 2004; Fox

Table 4
Summary of acute and chronic treatment with pregabalin on motor function.

<table>
<thead>
<tr>
<th></th>
<th>Open field</th>
<th>Rotorod</th>
<th>Beam walking</th>
<th>Core body temp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LMA Rears</td>
<td>8 rpm</td>
<td>16 rpm</td>
<td>Latency Footslip</td>
</tr>
<tr>
<td>Sham + Vehicle</td>
<td>Day 1</td>
<td>4039±293</td>
<td>169±9</td>
<td>60(60–60)</td>
</tr>
<tr>
<td></td>
<td>Day 12</td>
<td>4325±434</td>
<td>205±20</td>
<td>60(60–60)</td>
</tr>
<tr>
<td>SNI + Vehicle</td>
<td>Day 1</td>
<td>4244±411</td>
<td>220±14</td>
<td>60(47–60)</td>
</tr>
<tr>
<td></td>
<td>Day 12</td>
<td>4057±397</td>
<td>242±14</td>
<td>60(60–60)</td>
</tr>
<tr>
<td>SNI + Pregabalin</td>
<td>Day 1</td>
<td>4738±303</td>
<td>185±17</td>
<td>60(55–60)</td>
</tr>
<tr>
<td></td>
<td>Day 12</td>
<td>4048±251</td>
<td>276±31</td>
<td>60(60–60)</td>
</tr>
</tbody>
</table>

Effect of acute (i.e Day 1) or chronic (2 × daily × 12 days; i.e Day 12) treatment with pregabalin (PGB; 10 mg/kg IP) or vehicle on tests of motor function in SNI or sham operated rats. N = 10 (Sham operated group) or 13 rats (each SNI operated group). Data presented as means ± SEM, or median and interquartile range (rotorod data). NT = not tested. *P < 0.05 vs. SNI/vehicle treated group at respective timepoint (see Methods for experimental detail).
et al., 2003; De Vry et al., 2004). The present studies, while generally consistent with these findings, report an extended characterization. Firstly, to our knowledge there is only a limited characterization of pregabalin (Gustafsson and Sandin, 2009) and carbamazepine (Decosterd et al., 2004) in the SNI model. Given methodological differences and likelihood for variable contributions for inflammatory components and symptomatologies between certain peripheral nerve injury models (e.g. Cui et al., 2000; Dowdall et al., 2005; Hu et al., 2007), assessment across multiple models is generally considered necessary. Second, the present study extends the assessment of pregabalin and carbamazepine to a chronic behavioural measure of burrowing. Third, the effect of both acute and chronic pregabalin treatment in the SNI model and its relationship to plasma levels was also examined (Whiteside et al., 2008).

In an initial study we utilized the anticonvulsant properties of pregabalin and carbamazepine to establish dosage parameters suitable for testing in the SNI model (Gabrielsson et al., 2010). Pregabalin produced a robust and highly reproducible attenuation of the hypersensitivity to both a tactile and cold stimulus following both IP and oral routes. Potency against the evoked measures overlapped with its anticonvulsant property (Vartanian et al., 2006). While there was a modest effect on neurological and motor function at the 30 mg/kg dose (both following oral and IP injection), at the 10 mg/kg dose there was minimal effect, yet a significant attenuation of the evoked measures, particularly 4 h post dosing. It should also be noted that pregabalin failed to produce a complete reversal of the tactile allostynia (static) at any pretreatment schedule adopted in the current studies. This contrasts with the findings of Field et al. (1999) who reported complete reversal following an oral dose of 30 mg/kg in both the CCI and SNL (Chung) models. Whether this reflects differences between models or experimental differences such as test procedure is presently unclear.

In contrast, carbamazepine showed significantly greater potency compared to pregabalin against MES-induced seizures, yet was less effective against the evoked measures in the SNI model using a 0.5 h pretreatment (see also Hunter et al., 1997). Indeed significance against tactile allostynia was only evident at a dose of 60 mg/kg which negatively affected multiple measures of motor function thus complicating the interpretation of this result, although it is interesting to note that cold allostynia was not significantly affected.Extending the pretreatment time of carbamazepine to 1.5 h resulted in a decline in both efficacy and side-effect, likely reflecting drug clearance.

In terms of extending the characterization of pregabalin and carbamazepine into the burrowing assay, we selected doses of 10 mg/kg and 30 mg/kg of each drug respectively, given that higher doses would likely compromise burrowing scores in sham treated animals. Pregabalin produced a significant reversal of the burrowing deficit in SNI prepared rats at a dose of 10 mg/kg IP. This reinstatement of a suppressed motor behaviour in tandem with the suppression of a heightened sensory reflex, argues against a simple motor interpretation and extends efficacy to a chronic behavioural deficit associated with the SNI model. The failure of carbamazepine at a 30 mg/kg dose is consistent with its null effect against evoked responses at the same dose.

Despite activity in the burrowing assay, pregabalin clearly failed to affect any of the paw position parameters, which is consistent with the recent findings of others (Piesla et al., 2009; Mogil et al., 2010a,b) and would suggest that the postural changes evident following SNI surgery primarily reflect motor dysfunction, rather than a direct relationship to any sensory abnormality per se. For this reason, and for the generally null effect of carbamazepine against other endpoints, we did not test this drug on paw posture. Piesla et al. (2009) incorporated dynamic measures of gait into multiple nerve injury models and examined gabapentin and duloxetine in each model. While both drugs reversed the mechanical hyperalgesia evident in the SNL, PSNL and CCI models, neither affected the impact of each type of nerve injury on either the propel or brake phase of stride. Further evidence to support that these gait changes reflected motor nerve damage was the close relationship between these two variables across the different models, i.e complete sciatic nerve axotomy produced the most marked change to gait compared to the partial sciatic lesion models (Piesla et al., 2009). Thus both dynamic and static (postural) measures of gait induced by sciatic nerve surgery seem insensitive to change by current pain therapeutics. Interestingly, gait changes induced by inflammatory interventions such as Freund’s Adjuvant do seem sensitive to appropriate drug treatments (Piesla et al., 2009; Anjeyb-Möller et al., 2008), possibly reflecting absence of motor nerve damage in these models.

The gabapentinoids pregabalin and gabapentin bind to the α2δ subunit present on voltage activated calcium channels having almost identical affinity for both the α2δ1 and α2δ2 subtypes (Li et al., 2011). However clinical efficacy of both drugs is currently believed to be due to a selective interaction at the α2δ1 calcium

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Table 5

Summary of pharmacokinetic parameters for pregabalin following intraperitoneal and oral dosing in male Sprague-Dawley rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Dose (mg/kg) &amp; route</th>
<th>10 ip</th>
<th>30 ip</th>
<th>30 p.o</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>t_{max}</td>
<td>h</td>
<td></td>
<td>0.5 ± 0.0</td>
<td>0.542 ± 0.246</td>
<td>1.00 ± 0.000</td>
</tr>
<tr>
<td>C_{max}/Dose</td>
<td>kg/mg/ml/mg</td>
<td></td>
<td>1130 ± 32.1</td>
<td>1100 ± 24.4</td>
<td>860 ± 245</td>
</tr>
<tr>
<td>Apparent half-life</td>
<td>h</td>
<td></td>
<td>2.3 ± 0.03</td>
<td>2.3 ± 0.2</td>
<td>4.2 ± 2.2</td>
</tr>
<tr>
<td>AUC_{0→inf}/Dose</td>
<td>h*kg/mg/ml/mg</td>
<td></td>
<td>4260 ± 246</td>
<td>3800 ± 296</td>
<td>4480 ± 1320</td>
</tr>
<tr>
<td>MRT_{0→inf}</td>
<td>h</td>
<td></td>
<td>3.43 ± 0.051</td>
<td>3.22 ± 0.25</td>
<td>5.71 ± 2.04</td>
</tr>
</tbody>
</table>

All data presented as means ± SEM.
channel subunit (Dooley et al., 2007; Taylor, 2009). Evidence for this view is provided by the relative anatomical distribution of each subunit (Bian et al., 2006), with only the α2δ1 subunit expressed in the dorsal root ganglion (DRG) neurons and dorsal horn of the spinal cord and upregulated following peripheral nerve injury (Luo et al., 2002; Li et al., 2004; Melrose et al., 2007). Furthermore knock-in mice expressing a mutant α2δ1 subunit that does not bind gabapentin or pregabalin, develop neuropathic pain (CCI model) that is insensitive to these drugs (Field et al., 2006). While the majority of studies with pregabalin report acute dosage, Bauer et al. (2009) reported that in rats tested two days postoperatively following SNL surgery, a chronic (3 × daily, 8 days) regimen of pregabalin treatment resulted in maximal inhibition of a tactile allodynia, possibly through preventing the trafficking of α2δ1 subunits within the DRG to their presynaptic terminals located in the dorsal horn of the spinal cord. This prompted us to also evaluate the effect of a chronic pregabalin treatment regimen (10 mg/kg, 2 × daily, 12 days) in the SNI model.

We formally investigated the effect of a chronic 12 day × 2 daily treatment regimen of pregabalin on the tactile and thermal hypersensitivity in rats with an established SNI procedure. The results clearly demonstrated that when tested under such conditions, the effect of an acute 10 mg/kg dose, is equivalent to that administered twice daily for 12 days. As such the results are distinct to those of Bauer et al. (2009) although differences in the model selected (SNL vs. SNI) and stage of model development (established vs. early) may account for these disparate findings. The chronic studies did confirm the sustained nature of pregabalin efficacy against evoked hypersensitivity responses and consequently a lack of tolerance development, in contrast to other classes of pain medication particularly opioids (Morgan and Christie, 2011). Plasma levels of pregabalin were similar following either an acute, or chronic 12 day regimen (i.e. 3.0–3.7 μg/ml) consistent with no accumulation or autoinduction.

4.3. Backtranslation of carbamazepine and pregabalin

In terms of efficacy assessed using both an evoked (tactile and cold) and spontaneous behavioural measure (burrowing), and tolerability assessed using a neurological test battery, the profile of pregabalin was clearly superior to carbamazepine in the SNI model. Viewed in this context, the current preclinical data (see also Hunter et al., 1997; Fox et al., 2003; Idnapaan-Heikkila and Guibaud, 1999; Field et al., 1999; Decosterd et al., 2004; De Vry et al., 2004; Tanimoto-Mori et al., 2008; Gustafsson and Sandin, 2009; Nakazato-Imasato et al., 2009) are similarly predictive of the respective clinical utility for Tegretol® (carbamazepine) and Lyrica® (pregabalin) in the treatment of certain neuropathic pain states, although direct head to head comparisons between these drugs in the clinic seems to be lacking (Eisenberg et al., 2007; Finnerup et al., 2010; Dworkin et al., 2010). Nonetheless a recent position paper outlining current treatments for neuropathic pain, placed carbamazepine as a third-line treatment, recommended only for patients unresponsive to first and second-line treatments which included the gabapentinoids gabapentin and pregabalin (Dworkin et al., 2010).

Whiteside et al. (2008) highlighted a close correspondence between the efficacious exposure for three marketed neuropathic pain drugs (lamotrigine, gabapentin, carbamazepine) between a rat model of neuropathic pain (SNL model) and humans. The current studies extend this analysis to pregabalin and similarly show a close correspondence between the efficacious plasma exposure across both species. Evaluation of plasma levels following a 10 mg/kg IP dose of pregabalin (either administered acutely or chronically), indicates this corresponds to an exposure of ~3–4 μg/ml at 4 h post treatment, which falls within the clinically relevant exposure range for Lyrica® for treatment of neuropathic pain (2.8–8.2 μg/ml Berry and Millington, 2005; 2.4–4.8 μg/ml Dworkin et al., 2003). Furthermore, the maximum plasma concentrations observed following the 10 mg/kg IP dose (~11 μg/ml) are similar in magnitude to those measured in a multiple dose study in healthy volunteers administered a dose of 600 mg/day (Ben-Menachem, 2004). At this exposure level, pregabalin is also well tolerated and without significant neurological complication in both the rat and human (Dworkin et al., 2003; Brodie, 2004; Toth, 2012). A three-fold increase in the dose of pregabalin, and equivalent increase in Cmax and overall exposure resulted in some modest neurological side-effects, which again seems predictive of a higher incidence of certain clinical side-effects such as dizziness and sedation at supratherapeutic exposures in humans (Toth, 2012; Pregabalin NDA 21–446 Clinical Pharmacology and Biotherapeutics).

In summary, the present studies support the value of neuropathic pain models, in this case the SNI model, as a valuable resource to study novel treatments for neuropathic pain states (Kontinen and Meert, 2003; Whiteside et al., 2008; Berge, 2011). Inclusion of the additional outcome measure of burrowing adds significant value to the behavioural characterization of novel therapeutics by providing a means to assess drug against more chronic model deficits, and offering a behavioural counterpart to the more traditional evoked hypersensitivity measures. With the advantage of back translation, Pregabalin (Lyrica®), the most widely used treatment in humans with broadest label (Nightingale, 2012) was clearly superior to carbamazepine (Tegretol®) which has a much more restricted usage and lower recommendation as a therapy for neuropathic pain (Dworkin et al., 2010). Finally, the effectiveness of pregabalin against both evoked responses and burrowing with minimal adverse effect on neurological function, was at a plasma exposure that falls within the therapeutic exposure range for pregabalin (Lyrica®) in clinical neuropathic pain.

Acknowledgements

We would like to acknowledge Dr. Nick Andrews and Professor Andrew Rice for their helpful comments and advice relating to this work, and Mojgan Mohagheghi for surgical expertise. We would also like to thank Dr. Suman Rakshit for the generous supply of pregabalin. This study was funded by InterVivo Solutions Inc.

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