# Characterisation of Pregabalin (Lyrica®) on multiple behavioural endpoints in the rat spared nerve injury model

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## Introduction

Both pregabalin (PGB, Lyrica®) and carbamazepine (CBZ, Tegretol®) are anticonvulsant drugs that have been approved for the treatment of conditions related to neuropathic pain (Shneker and McAuley, 2005; Dworkin et al, 2009). Pregabalin is specifically approved for the treatment of diabetic peripheral neuropathy and postherpetic neuralgia, while carbamazepine is approved for trigeminal neuralgia.

The purpose of the present studies was to expand on the preclinical profile of PGB and CBZ, focusing on the rat spared nerve injury (SNI) model of neuropathic pain. Specifically 5 studies were conducted.

(1) Investigate multiple doses of PGB (3-30 mg/kg IP and oral) and carbamazepine (10-60 mg/kg IP) against heightened sensory reactivity (tactile and cold allodynia) characteristic of the SNI model. Compare with potency in the MES test and effect on neurological function.
(2) Following SNI surgery the majority of animals adopt a characteristic gait and paw position which might reflect guarding behaviour. We therefore assessed doses of PGB effective in (1) against various gait parameters affected by SNI surgery.
(3) Andrews et al. (2012) have recently described burrowing as a plausible means of assessing pain therapeutics against an affective component of pain. Accordingly, the effect of SNI surgery on burrowing behaviour and its potential reversal by PGB and CBZ was examined.
(4) Investigate the effect of chronic (2x daily x 12 days) PGB treatment to see if efficacy against an evoked tactile and thermal (cold) stimulus was enhanced by repeated treatment (see Bauer et al, 2009).
(5) The pharmacokinetics of PGB were studied to establish plasma concentrations necessary for preclinical efficacy for comparison to therapeutic levels in humans (Whiteside et al, 2008).

Given the extensive clinical experience with both pregabalin (Lyrica®) and carbamazepine (Tegretol®) in neuropathic pain therapy, this provides opportunity to translate clinical experience back to the preclinical setting.

## Methods

*Test subjects:* Adult, male, Sprague-Dawley (SD) rats were used throughout.

*SNI surgery:* Rats were surgically prepared according to Decosterd and Woolf (2000). Briefly the common peroneal and tibial branches of the sciatic nerve were exposed, ligated and cut, leaving the sural branch intact. In sham operated animals the sciatic nerve was only exposed. Following surgery, the overlying muscle and skin was then sutured. With the exception of expt. (3), all testing was conducted at least 20 days post surgery by which time the majority of rats (>95%) displayed robust hypersensitivity to tactile and thermal stimuli.

*Measurement of evoked responses:* 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 minutes 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 15g). The threshold was taken as the lowest force that evokes a brisk withdrawal response. 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 immediately after 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 = prolonged withdrawal (5-30sec) combined with flinching and licking of the paw. Again, the average score from three separate assessments was taken as the final measure for that animal.

*Measurement of gait:* Gait was measured using footprint analysis with the animals trained to cross a runway with their hindpaws dipped in ink. Dependent measures were total toe spreading (TS), stride length (SL), limb rotation (LR) and paw length (PL) (see Figure 2).

*Measurement of burrowing*: Burrowing was essentially measured as described by Andrews et al. (2012) with 5mm aquarium shingle used as the digging medium. All animals were familiarised to the proceedure prior to formal experimentation. Burrowing tests were of 1h duration.

*MES test:* Male SD rats of body weight 80-100g were used. On the test day following a defined drug pretreatment period, rats received a maximal electroshock (150mA, 0.2s duration, 60Hz) via corneal electrodes moistened with saline. Protection was defined as absence of a full tonic seizure within 10s of stimulus delivery. $ED_{50}$ values were derived from dose response curves consisting of a minimum of 3 dose levels.

*Neurological test:* Male SD rats of body weight 325-375g were used for these studies. The test procedure consisted of a rectal body temperature recording made immediately before treatment and at a second predetermined timepoint post treatment which coincided with testing in the SNI model. Rotorod (8 rpm and 16 rpm; accelerating speed; best score from three attempts at each speed), 1m beam walking and locomotor activity (30min test duration) were sequentially assessed.

*Drugs:* PGB and CBZ were sonicated in 5% tween80 in saline and administered in a dose volume of 5ml/kg. Doses expressed as base.

*Experiment 1:* Prior to commencing the studies in SNI prepared rats, the effect of PGB and CBZ against the tonic seizures induced by maximal electroshock was examined in experimentally naïve rats. Both PGB (3-60 mg/kg IP; 2h pretreatment) and CBZ (1-60 mg/kg IP; 0.5h pretreatment) were tested using an independent groups design. The dose regimes for PGB and CBZ in the initial SNI experiment was based on outcomes from the MES study. PGB (Expt 1a: 3-30 mg/kg IP; 2h ptt.; Expt 1b: 3-30 mg/kg oral; 4h ptt.) and CBZ (Expt 1c: 10-60 mg/kg IP; 30min ptt.; Expt 1d: 10-60 mg/kg IP; 90min ptt.) were independently tested in experimentally naïve rats using a repeated measures design (sample size: SNI rats, N=10-12, Shams, N=8-10). Rats were between 20-25 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 was the effect of test drug on response to a tactile (Von Frey) and cold (acetone drop) stimulus. In separate unoperated rats, neurological tests were conducted for PGB and CBZ at doses and pretreatment times corresponding to the pain studies.

*Experiment 2*: A group of 20 SD 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 and 20, the animals were tested with PGB at 10 and 30 mg/kg IP and saline control. Both Von Frey testing and footprint analysis was conducted 2h and 4h post treatment. A time interval of 3 days was allowed between each treatment cycle, i.e on days 28, 31 and 34 post surgery.

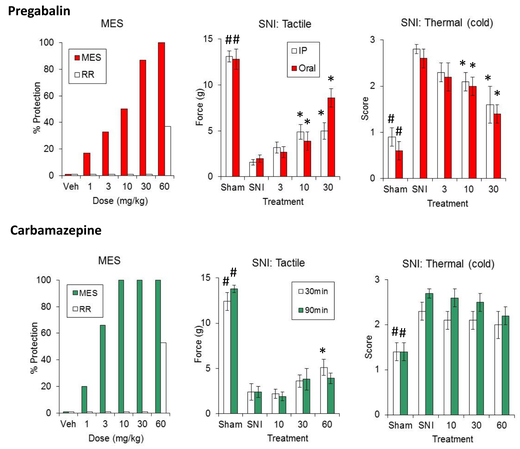
*Experiment 3*: In expt 3a, following familiarization to the burrowing procedure, rats were randomly divided into two groups based on equivalent overall burrowing scores, based on a test conducted 2 days before surgery. A total of 12 rats were surgically prepared with sciatic nerve lesion (SNI), and 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 1h burrowing test. On day 27 and 30 post surgery, the effect of PGB (10 mg/kg IP) and vehicle control was examined against a tactile stimulus followed by a 1h burrowing test. A cross-over design was adopted in both SNI and sham prepared rats. Von Frey testing was conducted 4h post treatment (based on outcome from Expt. 2). In expt 3b, a further group of rats were prepared with either SNI surgery (n=15) or sham controls (n=15). On day 27 and 31 post surgery, the effect of CBZ (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 0.5h post treatment.

*Experiment 4:* A total of 36 SD 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 outcomes, the SNI rats were divided into 2 groups, one designated to be SNI-vehicle group, the other SNI-pregabalin (10mg/kg IP). Sham operated rats were designated vehicle. Treatments started on day 22 post surgery, and were administered twice daily at approximately the same time each day (9:00h and 17:00h). On treatment days 1, 4, 8 and 12 all rats were tested 4h post morning treatment for response to Von Frey and acetone 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 r.p.m), open field activity (20min 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 PGB (10 mg/kg IP), i.e an acute dose of PGB. 4h post treatment, blood was collected for subsequent determination of PGB concentration.

*Experiment 5*: Separate groups of male SD rats were treated with PGB, either 10 or 30 mg/kg IP or 30 mg/kg PO. At multiple time points (0.5, 1, 2, 4, 6, 24 h) ~300 µL of whole blood was collected from the saphenous vein into EDTA coated tubes, centrifuged at 2000xg at 4˚C for 10 min, the plasma decanted and stored at -80˚C until bioanalytical quantification of PGB concentration (all Bioanalytical measures conducted by NoAb BioDiscoveries).

## Results

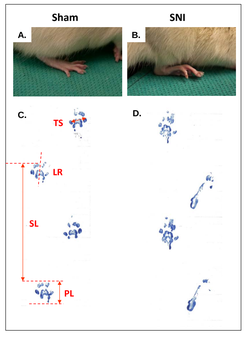
### A. Effect of PGB and CBZ on responses to evoked sensory stimuli in SNI prepared rats.

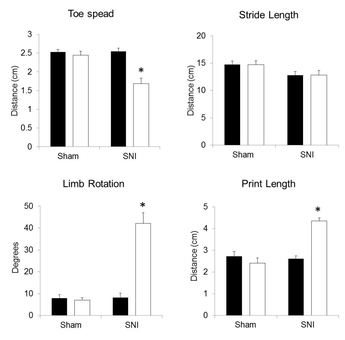



| Treatment | | PGB 2h/CBZ 0.5h LMA | Rears | 8rpm RR | 16rpm RR | Accel. RR | Beam walk | BT (°C) | PGB 4h LMA | Rears | 8rpm RR | 16rpm RR | Accel. RR | Beam walk | BT (°C) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vehicle | | 4245±237 | 152±14 | 81±15 | 73±18 | 129±18 | 4.7±0.2 | 0.1±0.2 | 1729±211 | 38±6 | 107±13 | 96±16 | 123±24 | 5.5±0.5 | -0.5±0.3 |
| Pregabalin | 10 | 5650±730 | 147±16 | 91±15 | 64±13 | **79±15 *** | 6.4±0.7 | 0.3±0.2 | 1731±419 | 31±7 | 94±11 | 67±16 | 79±10 | 5.5±0.4 | 0±0.3 |
| | 30 | 4739±930 | **97±14 *** | 46±14 | **27±11 *** | **58±10 *** | 5.0±0.4 | -0.1±0.3 | 1157±269 | 21±6 | 72±15 | **27±5 *** | **53±14 *** | 5.7±0.6 | -0.1±0.3 |
| Vehicle | | 1300 ± 171 | 57±13 | 60 | 60 | NT | NT | -0.1±0.2 | NT | NT | NT | NT | NT | NT | NT |
| Carbamazepine | 30 | 1099 ± 173 | **24±5 *** | 60 | 56±4 | NT | NT | -0.5±0.1 | NT | NT | NT | NT | NT | NT | NT |
| | 60 | **578 ± 159 *** | **5±1 *** | 43±8 | **46±8 *** | NT | NT | **-1.4±0.1 *** | NT | NT | NT | NT | NT | NT | NT |

***Figure 1.*** PGB (1-60 mg/kg IP) produced a dose-related attenuation of tonic seizures in the MES model (ED50 ~7 mg/kg IP). PGB (3-30 mg/kg) also produced a dose-related attenuation of the tactile and cold hypersensitivity following IP and oral injection. CBZ (1-60 mg/kg IP) was more potent than PGB at preventing MES seizures, (ED50 ~2.5 mg/kg IP). However CBZ was less effective than PGB at attenuating the tactile and cold hypersensitivity in the SNI model. CBZ testing was conducted at a timepoint equivalent to the MES test (30min) and also a slightly longer pretreatment time (90 min) * P<0.05 vs. respective vehicle treated group. # P<0.05 vs. SNI control treatment group. Neurological testing showed PGB to be well tolerated up to 30 mg/kg, albeit with some effect on rotorod. CBZ in contrast affected multiple measures.

### B. Characterisation of SNI surgery on gait – effect of pregabalin on gait changes




| | | | Tactile allodynia 2h | Tactile allodynia 4h | Gait measures (4h) Toe Spread | Stride Length | Limb Rotation | Print Length |
|---|---|---|---|---|---|---|---|---|
| Vehicle | - | Sham | 14.5 ± 0.4 | 14.7 ± 0.2 | 2.5 ± 0.1 | 15.4 ± 0.8 | 8.6 ± 1.9 | 2.9 ± 0.2 |
| Pregabalin | 10 | Sham | 14.5 ± 0.3 | 14.3 ± 0.6 | 2.2 ± 0.1 | 15.0 ± 1.1 | 8.2 ± 1.7 | 2.8 ± 0.2 |
| Pregabalin | 30 | Sham | 14.2 ± 0.3 | 14.8 ± 0.2 | 2.5 ± 0.2 | 16.5 ± 1.2 | 8.5 ± 2.1 | 2.8 ± 0.1 |
| Vehicle | - | SNI | **3.5 ± 1.3 #** | **3.4 ± 1.0 #** | **1.5 ± 0.1 #** | **11.2 ± 1.0 #** | **37.2 ± 2.3 #** | **4.7 ± 0.1 #** |
| Pregabalin | 10 | SNI | 3.9 ± 1.0 | **7.4 ± 1.1 *** | 1.6 ± 0.1 | 12.5 ± 1.1 | 39.1 ± 1.7 | 4.7 ± 0.1 |
| Pregabalin | 30 | SNI | **8.3 ± 1.6 *** | **10.7 ± 1.2 *** | 1.5 ± 0.1 | 11.4 ± 1.1 | 34.6 ± 2.8 | 4.7 ± 0.1 |

***Figure 2.*** Photograph of hindpaw of **(A)** a sham operated rat, and **(B)** SNI operated rat, taken 10 days post surgery. Note the elevation to the lateral portion of the SNI operated paw. Static paw measures of toe spread (TS), limb rotation (LR), stride length (SL) and print length (PL) were taken for each animal using footprint analysis. Representative footprints from **(C)** a sham operated rat, and **(D)** SNI operated rat are shown.
Effect of SNI or Sham surgery on the various static paw measures. (□) Paw ipsilateral to surgery, or (■) paw contralateral to surgery. These measures were taken from rats 20 days post surgery. * P<0.05 vs. contralateral (unoperated) paw.
On days 28 to 34 post surgery, the effect of PGB (10-30 mg/kg IP) was evaluated against the 4 principal paw measures. Paw readings were taken at 4h post treatment which corresponded to peak effect of PGB against tactile (Von Frey) allodynia. PGB failed to influence any of the paw measures.

## Results

### C. Characterisation of SNI surgery on burrowing – effect of pregabalin and carbamazepine.

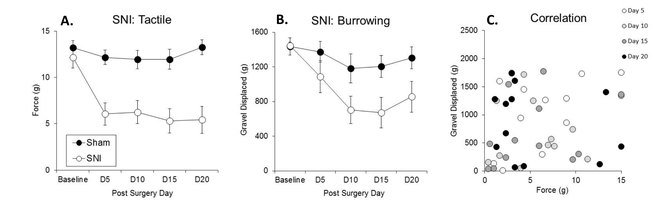



***Figure 3.*** Effect of SNI surgery on **(A)** the threshold withdrawal response to a tactile (Von Frey) stimulus, **(B)** the amount of gravel displaced in a 1h burrow test. Rats were surgically prepared on day 1, and at day 5, 10, 15 and 20 the rats were assessed. A presurgical baseline measure was also taken 2 days prior to surgery. **(C)** Lack of correlation between the threshold withdrawal response and burrowing score for individual rats tested over D5 to D20.

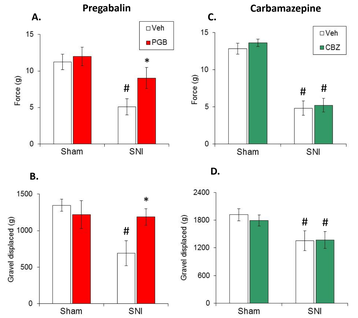



***Figure 4.*** Effect of **(A,B)** PGB (10mg/kg IP; 4h ptt.), **(C,D)** CBZ (30mg/kg IP; 30min ptt.) against the reduced threshold to a tactile (Von Frey) stimulus and the burrowing deficit in SNI prepared rats. PGB treatment attenuated the tactile allodynia and burrowing deficit induced by SNI surgery. In contrast CBZ treatment failed to affect either measure. * P<0.05 vs. respective SNI/vehicle treated group. # P<0.05 vs. sham/vehicle treatment group.

### D. Characterisation of chronic pregabalin treatment on evoked responses in SNI rats

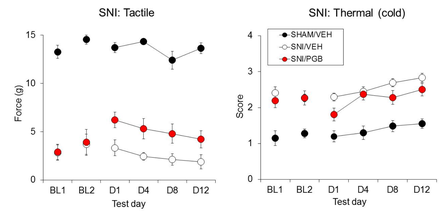
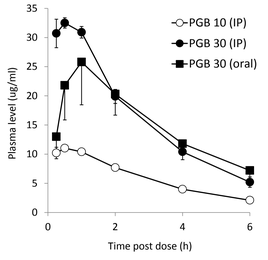



***Figure 5.*** Effect of chronic (2 x 10 mg/kg IP/day x 12 days) PGB treatment on tactile and cold allodynia in SNI prepared rats. At pre-drug baseline (BL1 and BL2) there was no difference between SNI groups. On day 1 of dosing PGB produced a significant attenuation of the withdrawal sensitivity to a tactile and cold stimulus. The magnitude of this effect was not affected by repeated dosing as revealed by retesting on treatment days 4, 8 and 12. Measurement of PGB plasma levels taken on day 13, 4h post dosing showed no significant difference between chronic and acute treatment (chronic: 3.7±0.2 µg/ml, acute: 3.1±0.4 µg/ml; NS).

### E. Characterisation of the pharmacokinetic property of pregabalin




| Parameter | Units | Dose (mg/kg) & Route 10 IP | 30 IP | 30 oral |
|---|---|---|---|---|
| n | | 3 | 6 | 4 |
| $t_{max}$ | h | 0.5 ± 0.0 | 0.54 ± 0.25 | 1.0 ± 0.0 |
| $C_{max}$/Dose | kg*ng/mL/mg | 1130 ± 32 | 1100 ± 24 | 860 ± 245 |
| Apparent half-life | h | 2.3 ± 0.03 | 2.3 ± 0.2 | 4.2 ± 2.2 |
| $AUC_{0-inf}$/Dose | h*kg*ng/mL/mg | 4260 ± 246 | 3800 ± 296 | 4480 ± 1320 |
| $MRT_{0-inf}$ | h | 3.43 ± 0.05 | 3.22 ± 0.25 | 5.71 ± 2.04 |

***Figure 6.*** **(A)** Time course of pregabalin plasma concentration following either oral (30 mg/kg) or intraperitoneal (10 and 30 mg/kg) doses to male, Sprague-Dawley (SD) rats. Plasma samples were collected at 0.25, 0.5, 1, 2, 4, 6, 24 h and pregabalin was quantified by LC-MS/MS analysis. N=3-6 rats per treatment. **(B)** Table for pharmacokinetics parameters calculated from the concentration vs. time curve.

## Summary and conclusions

1. **Consistent with an anticonvulsant profile, both PGB and CBZ prevented MES-induced seizures, CBZ being the more potent. Testing both drugs in the SNI model under an equivalent dosing regimen identified PGB to be the more effective, based on efficacy (tactile and cold allodynia) and tolerability.**
2. **SNI surgery produced enduring changes to paw position and gait. PGB failed to affect any of these changes which may suggest they are unrelated to pain per se.**
3. **SNI surgery produced a deficit in burrowing behaviour. Consistent with effect against evoked measures, PGB but not CBZ reversed the burrowing deficit. These findings support burrowing as a potentially useful means to study the effect of drugs against chronic aspects to theSNI model.**
4. **Chronic PGB treatment did not result in greater efficacy against the evoked hypersensitivity responses. No tolerance developed to PGB.**
5. **PK studies revealed PGB (10 mg/kg IP) to be effective at plasma levels corresponding to therapeutically relevant concentrations (4-8 µg/ml).**
6. **Overall these data support improved efficacy and tolerability for PGB compared to CBZ in the SNI model, which is consistent with findings for both drugs reported in other nerve injury models and also clinical experience (Dworkin et al, 2009). There is also good correspondance between plasma [PGB] across species.**

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