# 各種神経障害性疼痛モデルの背景データ：機械的刺激による疼痛反応評価
# Background data on various neuropathic pain models: Evaluation of pain responses to mechanical stimulation

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## Introduction

In recent years, we have been receiving an increasing number of requests for pain evaluation tests, and we will introduce background data on various neuropathic pain models, including the streptozocin (STZ)-induced diabetic peripheral neuropathic pain (DPN) model, the anticancer drug-induced peripheral neuropathy (CIPN) model, and the spared nerve injury (SNI) model.

## Methods

This study was conducted as approved by the International Animal Care and Use Committee of NISSEI BILIS Co., Ltd., Shiga Laboratory.

### Materials and Methods

●Animals
✓*DPN model*; Male SD rats, 7 weeks old (The Jackson Laboratory Japan, Inc.)
✓*CIPN model*; Male BALB/c mice, 7 weeks old (Japan SLC, Inc.)
✓*SNI model*; Male SD rats, 9 weeks old (Japan SLC, Inc.)

●Model preparation
✓*DPN model*; STZ (Sigma) was dissolved in a citrate buffer solution at a concentration of 60 mg/mL and administered intravenously at dose of 60 mg/kg. Onset of diabetes was confirmed one week after STZ injection by measuring blood glucose levels in samples taken from the tail vein using a blood glucose meter (ARKRAY, Inc.). Only rats with a glucose level greater than 400 mg/dL were considered as diabetic.
✓*CIPN model*; Oxaliplatin (OHP, Nippon Kayaku Co., Ltd.) was dissolved in a 5% glucose solution at a concentration of 2 mg/mL and administered intraperitoneally at dose of 20 mg/kg.
✓*SNI model*; The common peroneal nerve and tibial nerve of rats were tightly ligated and the distal side was cut (see Figure 1). (Decosterd I, et al. 2000)

●Drug administration
✓*DPN model and CIPN model*; Duloxetine (Tokyo Chemical Industry Co., Ltd.) was dissolved in a water for injection at a concentration of 6 mg/mL and orally administered at dose of 30 mg/kg.
✓*SNI model*; Pregabalin (LYRICA OD Tablets, Viatris Inc.) was suspended in a water for injection at a concentration of 4 mg/mL and orally administered at dose of 20 mg/kg.

●Behavioural test
Pain response was assessed by using the "up and-down" method, as previously described by Dixon (1965) and Chaplan et al (1994). The animals were placed individually in a cage with a wire mesh bottom (see Figure 2). After acclimation period of at least 15 min (30 min for mice), calibrated von Frey filaments (North Coast Medical, USA; ranging from 0.4 to 15 grams for rats, from 0.04 to 2.0 grams for mice) were applied to the mid-plantar surface of the hindpaw for 3 to 4 seconds. A positive response was recorded if the animals showed withdrawal and guarding, "scratching" or "licking" of the stimulated paw. Data were calculated as the 50% withdrawal threshold in grams.

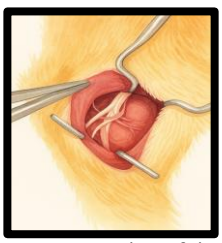
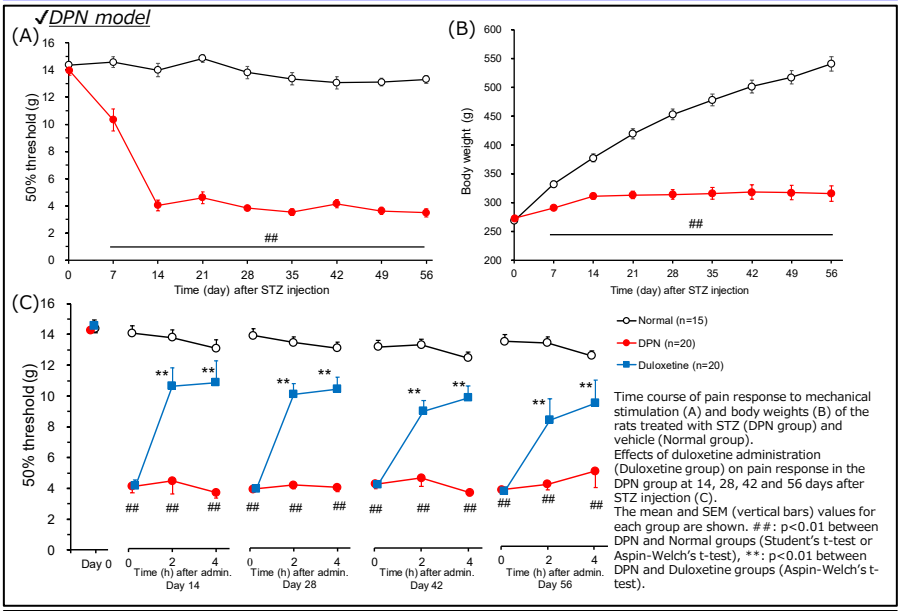


Figure 1. Branches of the sciatic nerve

Figure 2. von Frey test

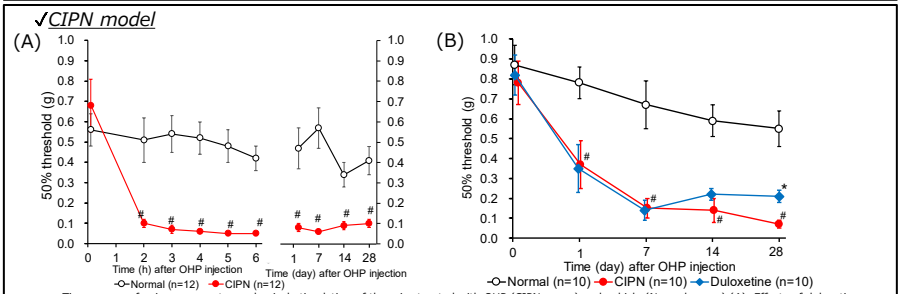
## Results

✓*DPN model*



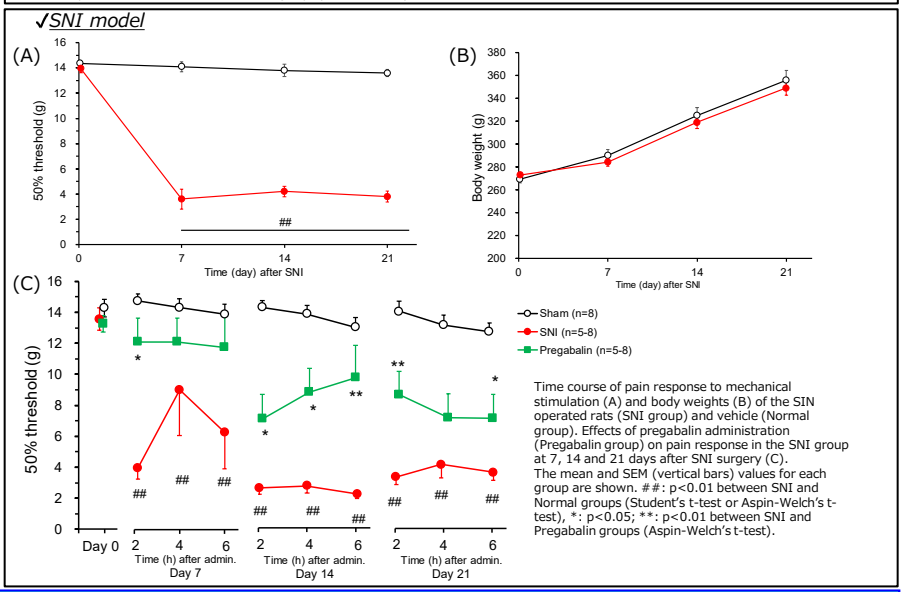

Time course of pain response to mechanical stimulation (A) and body weights (B) of the rats treated with STZ (DPN group) and vehicle (Normal group). Effects of duloxetine administration (Duloxetine group) on pain response in the DPN group at 14, 28, 42 and 56 days after STZ injection (C). The mean and SEM (vertical bars) values for each group are shown. ##: p<0.01 between DPN and Normal groups (Student's t-test or Aspin-Welch's t-test), **: p<0.01 between DPN and Duloxetine groups (Aspin-Welch's t-test).

✓*CIPN model*




Time course of pain response to mechanical stimulation of the mice treated with OHP (CIPN group) and vehicle (Normal group) (A). Effects of duloxetine administration (Duloxetine group) on pain response in the CIPN group at 1, 7, 14 and 28 days after OHP injection (B). The mean and SEM (vertical bars) values for each group are shown. #: p<0.01 between CIPN and Normal groups (Student's t-test or Aspin-Welch's t-test), *: p<0.05 between CIPN and Duloxetine groups (Student's t-test).

✓*SNI model*




Time course of pain response to mechanical stimulation (A) and body weights (B) of the SIN operated rats (SNI group) and vehicle (Normal group). Effects of pregabalin administration (Pregabalin group) on pain response in the SNI group at 7, 14 and 21 days after SNI surgery (C). The mean and SEM (vertical bars) values for each group are shown. ##: p<0.01 between SNI and Normal groups (Student's t-test or Aspin-Welch's t-test), *: p<0.05; **: p<0.01 between SNI and Pregabalin groups (Aspin-Welch's t-test).

## Conclusion

✓*DPN model*: The paw withdrawal threshold to mechanical stimulation was decreased after first week of STZ injection and lasted for 56 days after injection. Duloxetine 30 mg/kg increased this lowered threshold, suggesting its usefulness as a positive control drug.
✓*CIPN model*: The paw withdrawal threshold to mechanical stimulation was decreased after 2 hours of OHP injection and lasted for 28 days after injection. No clear antiallodynic effect was observed with duloxetine at 30 mg/kg.
✓*SNI model*: The paw withdrawal threshold to mechanical stimulation was decreased after 7 days of surgery and lasted for 21 days. Pregabalin 20 mg/kg increased this lowered threshold, suggesting its usefulness as a positive control drug.

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☑ The author has no conflict of interest to disclose with respect to this presentation.