

## Online Supplementary material

### **Supplementary methods:**

#### **Animals**

Male Sprague-Dawley rats (200-250 g) (Charles Rivers, St-Aubin-Lès-Elbeuf, France) were used. After arrival at the laboratory, the animals were allowed to acclimatize for one week in groups of 6 per cage with *ad libitum* access to food and water. The guidelines of the Committee for Research and Ethical Issue of the I.A.S.P. (Zimmermann, 1983) were followed. Great care was taken, particularly with regard to housing conditions, to avoid or minimise discomfort of the animals.

#### **Induction of mononeuropathy**

After determination of preliminary thresholds to paw pressure and delay of withdrawal to hindpaw immersion (the mean of 2 consecutive stable values which do not differ more than 10%) an unilateral peripheral mononeuropathy was induced, according to the method of Bennett and Xie (Bennett and Xie, 1988). Briefly, rats were anaesthetised with sodium pentobarbital (50mg/kg, i.p.) and four chromic gut (5-0) ligatures were tied loosely (with about 1 mm spacing) around the right common sciatic nerve. The nerve was constricted to a barely discerning degree, so that circulation through the epineural vasculature was not interrupted. The rats were used 7 days after ligation when mechanical and thermal hyperalgesia were established.

#### **Assessment of nociceptive reactions.**

**Paw pressure test.** Mechanical nociceptive thresholds, expressed in grams (g), were measured with an Ugo Basile analgesimeter (Ugo Basile analgesimeter, Bioseb, Paris, tip diameter of the probe: 1mm) by applying an increasing pressure (maximal pressure applied: 750g) to the right hind paw of unrestrained rats until a squeak (vocalization threshold) was obtained. Mean basal control pre-drug (healthy rats) or pre-injury (mononeuropathic animals) values were  $324\pm 21$  and  $336\pm 5$  g, respectively.

**Tail immersion test.** In healthy rats, the lower 5 cm portion of the tail was immersed into a 46°C hot-water bath (Ministat MHUB11, Bioblock Scientific, France) as described by Janssen et al (Janssen et al., 1963). The time in seconds for tail withdrawal from the water was taken as the latency time, with a cut-off set at 30 sec. The scores of two separate withdrawal latency determinations were averaged as basal control pre-drug values (mean value for the three treatment groups:  $12.6\pm 0.4$ sec). Careful attention was taken to ensure that the ambient temperature was maintained at 22-23°C.

**von Frey test.** To assess tactile allodynia, paw withdrawal threshold to a non-noxious tactile stimulus was determined using an automated electronic von Frey (Bioseb, France). The electronic von Frey employs a single nonflexible filament to which the experimenter applies an increasing force. The stimulus was applied on the plantar surface of the rat hind paw (Whiteside et al., 2004). The paw withdrawal threshold, determined twice and averaged, was taken as the score of allodynia. A cut-off force at 30 g was considered to be a noxious stimulus, as it elicited a response in naïve animals.

**Paw immersion test.** In mononeuropathic rats, the thermal nociception was tested by measuring the withdrawal latency elicited by immersion of the right hind paw into a 46°C hot-water bath as previously described (Attal et al., 1990). The time in seconds was taken as the latency time, with a cut-off set at 30 sec. The scores of two separate withdrawal latency determinations were averaged as basal control pre-injury values (mean value for the three treatment groups: 9.6±0.3 sec). Careful attention was taken to ensure that the ambient temperature was maintained at 22-23°C.

### **Expression of results and statistical analysis.**

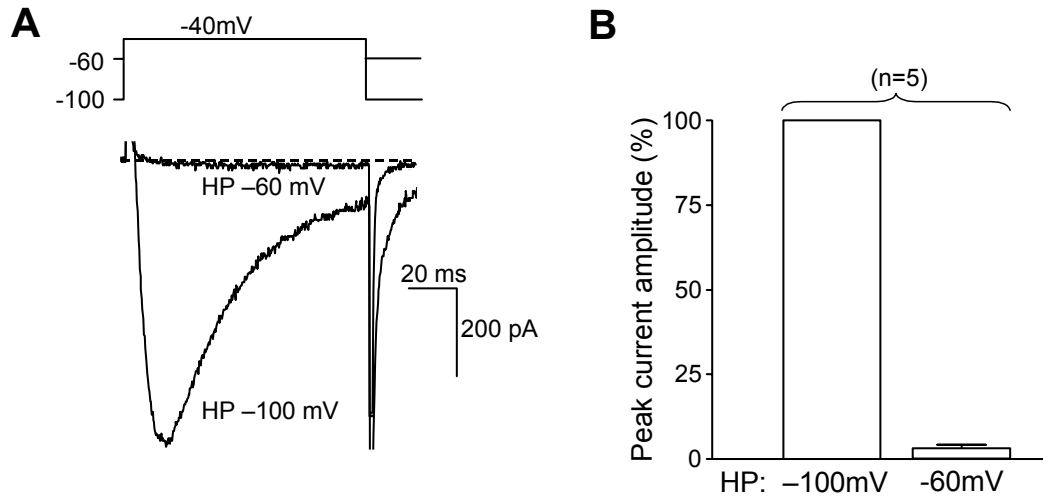
Results were expressed in grams (g) or in seconds (s) as mean ± S.E.M. for vocalization thresholds and latency times, respectively. Areas under the time course curves (AUC) of variations (post-drug - pre-drug values) of vocalization thresholds were calculated using the trapezoidal method. Data were analysed by a two-way analysis of variance (ANOVA) followed by the PLSD (predicted least statistical differences) Fischer t-test when the time course of the effect was compared. A one-way analysis of variance was used to compare the effect of different treatments as estimated by AUC. The significance level (\*) was  $P < 0.05$  for the two statistical analyses.

### **References:**

- Attal, N., Jazat, F., Kayser, V. and Guilbaud, G. (1990) Further evidence for 'pain-related' behaviours in a model of unilateral peripheral mononeuropathy. *Pain*, **41**, 235-251.
- Bennett, G.J. and Xie, Y.K. (1988) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain*, **33**, 87-107.
- Zimmermann, M. (1983) Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*, **16**, 109-110.

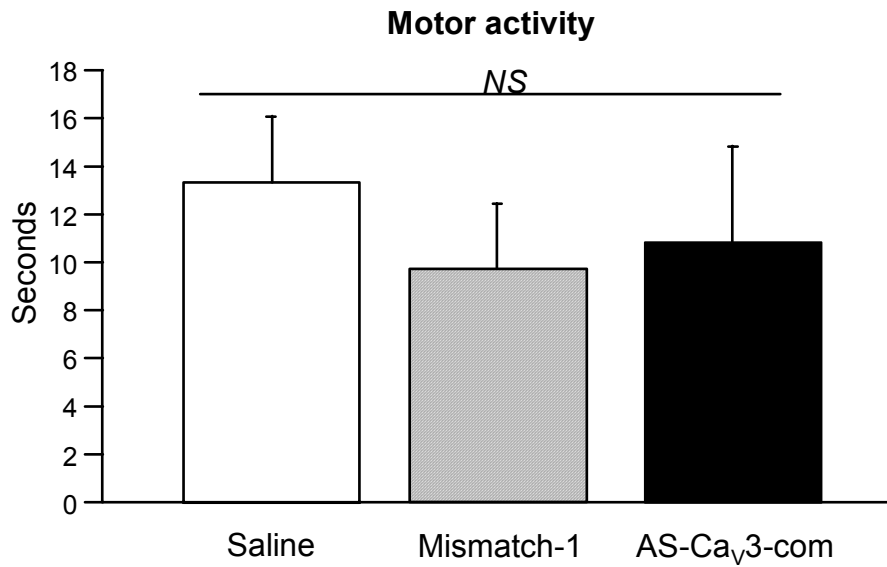
## Supplementary material :

### Supplement 1



**Figure S1 legend.** T-type calcium currents in small and medium diameter DRG cell bodies. A Example of currents evoked by depolarizing the DRG cell soma to  $-40\text{mV}$  for  $100\text{ms}$  from a holding potential of  $-100\text{mV}$  and  $-60\text{mV}$ . Note the nearly complete inactivation of the current when the holding potential is maintained at  $-60\text{mV}$ . B Normalized mean peak current amplitude at  $-40\text{mV}$  ( $\pm\text{SEM}$   $n=5$ ) for several cells expressing a low voltage activated T-type current. Note that inactivation of the T-type current does not reveal any other current component at this step depolarization to  $-40\text{mV}$ .

Supplement 2



**Figure S2 Legend.** Example of the lack of effect of the antisense ODN on spontaneous locomotor activity of freely moving animals taken from a three groups of neuropathic animals (CCI). The histogram shows that at the end of the 4days-ODN injection protocol, rats treated with the saline (open bar), the mismatch-1 (hatched bar), or AS- $Ca_v3$ -com (filled bar) had no significantly different locomotor activity (n= 5-7 animals per treatment group).

**Method: Assessment of motor activity.** Motor activity was monitored individually using an actimeter Actisystem (Apelex, Passy, France). The measurements were performed before and just after the last injection of ODNs or saline. Each rat was allowed to acclimate to the recording environment for 10 min and then the activity was continuously monitored for 10 min.

Supplement 3

**Table S1: Primers used for the Quantitative RT-PCR**

<b>Gene</b>	<b>Accession N° (Genbank)</b>	<b>Forward (F)/Reverse (R) primer</b>	<b>Amplicon size (in bp)</b>
Ca <sub>v</sub> 3.1	NM_031601	(F) 5' -CCAAACAGCGGGAGAGTCA-3' (R) 5' -AGAAGCTTGCCAGGGTGCTA-3'	78
Ca <sub>v</sub> 3.2	AF290213	(F) 5' -TCATCACTCACACGGCAGCTA-3' (R) 5' -TGGGCCACGTCCAGGTT-3'	67
Ca <sub>v</sub> 3.3	NM_020084	(F) 5' -CCAGGAAGGCTCAGGTTCTG-3' (R) 5' -TCCTCACTGCTCTGGAGTCCAT-3'	72
HPRT	NM_012583	(F) 5' -GCGAAAGTGAAAAAGCCAAGT-3' (R) 5' -GCCACATCAACAGGACTCTTG TAG-3'	76
Synaptophysin	NM_012664	(F) 5' -TCAGGACTCAACACCTCAGTGG-3' (R) 5' -AACACGAACCATAAGTTGCCAA-3'	74