EJN fens EUROPEAN JOURNAL OF NEUROSCIENCE

European Journal of Neuroscience, Vol. 33, pp. 2255–2264, 2011 Bial doi:10.1111/j.1460-9568.2011.07721.x

NEUROSYSTEMS

# Instability of spatial encoding by CA1 hippocampal place cells after peripheral nerve injury

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*Keywords*: multichannel recording, neuropathy, pain, rat

## Abstract

Several authors have shown that the hippocampus responds to painful stimulation and suggested that prolonged painful conditions could lead to abnormal hippocampal functioning. The aim of the present study was to evaluate whether the induction of persistent peripheral neuropathic pain would affect basic hippocampal processing such as the spatial encoding performed by CA1 place cells. These place cells fire preferentially in a certain spatial position in the environment, and this spatial mapping remains stable across multiple experimental sessions even when the animal is removed from the testing environment. To address the effect of prolonged pain on the stability of place cell encoding, we chronically implanted arrays of electrodes in the CA1 hippocampal region of adult rats and recorded the multichannel neuronal activity during a simple food-reinforced alternation task in a U-shaped runway. The activity of place cells was followed over a 3-week period before and after the establishment of an animal model of neuropathy, spared nerve injury. Our results show that the nerve injury increased the number of place fields encoded per cell and the mapping size of the place fields. In addition, there was an increase in in-field coherence while the amount of spatial information content that a single spike conveyed about the animal location decreased over time. Other measures of spatial tuning (in-field firing rate, firing peak and number of spikes) were unchanged between the experimental groups. These results demonstrate that the functioning of spatial place cells is altered during neuropathic pain conditions.

## Introduction

It has long been known that the hippocampus is crucial for learning and memory. One special feature of pyramidal complex-spike neurons found in CA1 and CA3 areas of the hippocampus is that they encode the animal location in the environment (Fox & Ranck, 1981). These place cells increase their firing rate when an animal is in a particular position within its environment (place field; O' Keefe & Dostrovsky, 1971; O' Keefe & Nadel, 1978), and this place field is usually stable across repeated visits to the same environment, even when the animal is removed from it for extended time periods (Muller *et al.*, 1987; Thompson & Best, 1990). Several studies have shown that changes in the spatial features or motivational content of the testing environment may disrupt the place field stability (Kobayashi *et al.*, 1997; Markus *et al.*, 1995; Moita *et al.*, 2004; Muller & Kubie, 1987; Wood *et al.*, 2000). On the other hand it has been shown that place field instability could also result from pharmacological manipulations of hippocampal functioning (Dragoi *et al.*, 2003; Kentros *et al.*, 1998; Rotenberg *et al.*, 2000) or from direct lesioning of the hippocampus or hippocampal-connected areas (Leutgeb & Mizumori, 1999; Liu *et al.*, 2003; McNaughton *et al.*, 1989; Mizumori *et al.*, 1994; Muir & Bilkey, 2001).

In addition to spatial information, the hippocampus has repeatedly been described as being involved in the regulation of several behavioural aspects of the adaptation to aversive situations, including pain (Khanna, 1997; Khanna & Sinclair, 1992; Soleimannejad *et al.*, 2006). Hippocampal neurons respond to noxious stimulation (Delgado, 1955; Halgren *et al.*, 1978; Khanna, 1997; Soleimannejad *et al.*, 2006; Tai *et al.*, 2006; Wei *et al.*, 2000; Zheng & Khanna, 2008), and human imaging studies have shown its activation by acute noxious stimulation (Bingel *et al.*, 2002; Ploghaus *et al.*, 2001, 2000). Moreover, partial hippocampectomy has been used (albeit with moderated success) as a treatment for human chronic pain syndromes (Gol & Faibish, 1967), while the inactivation of hippocampal synaptic transmission attenuated nociceptive behaviour in the rat formalin model (Khanna, 1997; McKenna & Melzack, 1992, 2001).

Despite all the knowledge gathered on how the hippocampus affects pain processing, little is known about how pain affects hippocampal functioning. It has been shown that chronic pain changes c-fos expression (Carter *et al.*, 2011; Ceccarelli *et al.*, 2003) and long-term potentiation (Kodama *et al.*, 2007; Ren *et al.*, 2011), and causes changes in hippocampal volume (Lutz *et al.*, 2008; Younger *et al.*, 2010), but it is still unknown whether pain affects crucial hippocampal functions such as the generation of spatial maps. Therefore, our goal

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*Received 2 February 2011, revised 13 March 2011, accepted 5 April 2011*

was to verify whether long-term neuropathic pain disrupts the stability of CA1 hippocampal place fields in the absence of spatial or motivational changes introduced in the familiar testing environment, or alterations in the performance capability of the animal. We recorded neuronal activity of freely moving rats in a simple spatial alternation task, performed before and after the induction of the spared nerve injury (SNI) model of neuropathic pain (Decosterd & Woolf, 2000).

## Materials and methods

### Subjects

Twelve adult male Sprague–Dawley rats weighing between 275 and 325 g were used in this study. The rats were maintained on a 12-h light–dark cycle, and both training and recording sessions ran at approximately the same time each day, during the light portion of the cycle. All procedures and experiments adhered to the guidelines of the Committee for Research and Ethical Issues of IASP (Zimmermann, 1983) and with the Ethical Guidelines for Animal Experimentation of the European Community Directive Number 86/609/ECC of 24 November of 1986, and were approved by national and local boards.

### Behavioral arena

The testing environment consisted of a U-shaped arena with three sections that were 72 cm long and 15 cm wide, and with opaque walls 30 cm high (Fig. 1); this arena was similar to an elevated runway previously used in a place cell stability study (Tropp *et al.*, 2005). Rats were trained to run an alternation schedule on the arena to receive food reinforcements at both ends of the runway; one chocolate-flavoured pellet was automatically delivered by a pellet dispenser (Coulbourn Instruments, Whitehall, PA, USA), depending on previous visit to the opposite end of the runway. Control of pellet dispensers was fully automated using the OpenControl software adapted to this particular task (Aguiar *et al.*, 2007). We preferred the use of this simple task in a linear runway instead of a more complex bifurcating arena that requires navigational decision-making because pain is known to affect both decision-making and spatial navigation in working memory-dependent tasks (Pais-Vieira *et al.*, 2009a; Hu *et al.*, 2010; our own unpublished results), and any perturbations of the navigational performance of the animals may lead to spontaneous place field remapping.

### Experimental schedule

During the training period, the rats were given two 15-min sessions per day to learn the alternation schedule until they performed 80 correct runway alternations within a period of 4 consecutive days (Tropp *et al.*, 2005). After reaching this performance criterion the animals were surgically implanted unilaterally with a multielectrode array for single-unit recording (see details below).

After 7 days of recovery from the implantation surgery, 12 animals ($n = 6$ in both sham and nerve-lesioned groups) were recorded over 4 consecutive days (hereafter named baseline or control period) while performing the runway alternation task in two daily sessions of 15 min. The detailed results from this control period sessions are presented in Table S1 as Supporting Information. On the following day the animals were briefly anesthetized and either surgically subjected to the SNI model of neuropathic pain (Decosterd & Woolf, 2000) or to a sham intervention with the same extent of skin incision and muscle dissection, performed in the left hindpaw contralateral to the location of the intracranial recording electrodes. Both groups of animals were then recorded for a period of 3 weeks (with recordings performed on days 1, 2, 3, 7, 10, 15 and 21 after SNI or sham intervention). The sensory threshold for noxious stimulation was measured using von Frey filaments (Somedic, Sweden) as previously described (Chaplan *et al.*, 1994). Von Frey testing was always performed after the second recording session of the day (with at least a minimum interval of 1 h after replacing the animals in their home cage). Testing was performed in an elevated chamber with a thin metallic mesh floor that allowed easy access to the plantar surface of the left hindpaw; filament series were run from the thinnest to the widest to detect the filament to which the animal withdrew the paw in at least six of 10 successive applications; we then performed another two series of 10 stimulations using the same filament (2-min intervals between sessions) and averaged the number of positive responses evoked by the three series.

### Multielectrode implantation

For the surgical implantation of the intracranial multielectrode array the animals were anesthetized with an intramuscular injection of a mixture of xylazine and ketamine (10 and 60 mg/kg, respectively). Anesthesia was maintained with small additional injections of ketamine (one-third of the initial dosage). Oxygen was supplied during the procedure via a face mask. The depth of anesthesia and paralysis of the musculature was assessed by regularly testing the corneal blink, hindpaw withdrawal and tail-pinch reflexes. After the anesthesia induction, each animal received a single dose of atropine sulphate (0.02 mg/kg, subcutaneous) and 1 mL of serum (sucrose 2% w/v in NaCl 0.9% w/v, subcutaneous) every hour throughout the surgery. Core body temperature was measured with a rectal thermometer and maintained at 37 °C by means of a homeothermic blanket system. The animal's head was shaved and cleaned using a triple application of alcohol (70%, v/v) and Betadine. A midline subcutaneous injection of 0.3 mL of 1% lignocaine (B Braun, Melsungen, Germany) was applied to the scalp for local analgesia. Anesthetized animals were secured in a stereotaxic frame using ear bars, and a midline incision was made caudally to the animal's eyes and ending between ears. The connective tissue was blunt-dissected, and the top of the skull was exposed and cleaned using hydrogen peroxide. After exposure of the scalp, holes were bored for fixation of four screws used for securing the array and for grounding. One craniotomy ($3 \times 3$ mm) was made for implantation of the multielectrode.

Each multielectrode array consisted of eight filaments of isonel-coated tungsten wire (35 $\mu$m diameter; California Fine Wire Company, Grover Beach, CA, USA) with impedances varying between 0.5 and 0.7 M$\Omega$ at 1 kHz. The multielectrode arrays were built in a $4 \times 2$ architecture, interspaced with 250 $\mu$m between rows and 400 $\mu$m between columns (Silva *et al.*, 2010). The arrays were rostrocaudally oriented and mounted in the holder of a hydraulic micropositioner (FHC Inc, Bowdoin, ME, USA) and slowly driven (50 $\mu$m/min) into the right hippocampal CA1 region after *dura mater* removal, while monitoring the neuronal activity. The following coordinates in mm relative to bregma (Paxinos & Watson, 1998) were used to center the arrays: −3.2 rostrocaudal, +2.2 mediolateral and −2.6 dorsoventral. After the electrodes were advanced to the correct position, the craniotomy was sealed with a layer of agar (4% in saline) and the array was cemented to the skull screws using dental acrylic. At the end of the implantation the animal was transferred to a recovery cage. The analgesic carprofen (7.5 mg/kg; Rimadyl, Pfizer Animal Health, Lisbon, Portugal) and the antibiotic amoxicillin (6 mg/kg; Clamoxyl, Pfizer Animal Health,

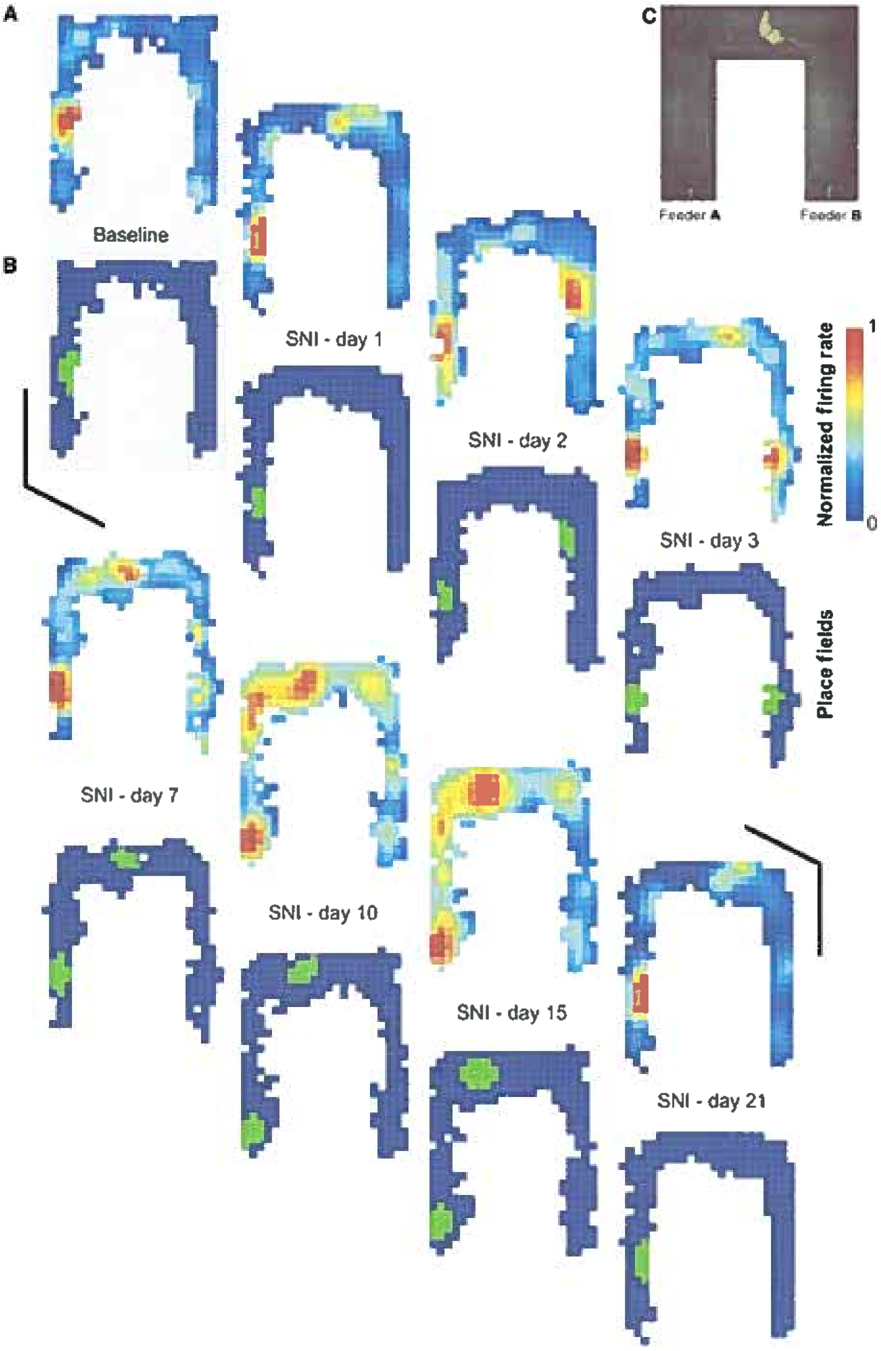


FIG. 1. Illustration of activity of a place cell during the testing environment navigation before (baseline) and after nerve injury (SNI). (A) Normalized firing rate maps. The bright red pixels correspond to regions where the cell fired at a higher rate, and the dark blue to a lower rate. (B) Correspondent encoded place fields (green pixels). (C) Photograph of the U-shaped testing environment. The rats were trained to alternate between feeders A and B in the maze for food reinforcement dispensed by pellet feeders located one at the end of each arm. The feeders dispensed a single food pellet when the animal performed a correct alternation.

Lisbon, Portugal) was administered subcutaneously every 24 h for 3 days while the animal recovered from surgery. Rats were allowed to recover for 1 week before the task re-training sessions began.

### *Neural recordings*

All neural activity signals from the implanted microelectrodes were recorded and processed using a Multineuron Acquisition Processor system (16-MAP; Plexon Inc., Dallas, TX, USA). Voltage–time threshold windows were used to identify single-unit waveforms. The differentiated neural signals were preamplified (10 000–25 000 ×), and digitized at 40 kHz. Up to two neuronal action potentials per recording channel were sorted online (SortClient 2008; Plexon Inc.) and validated by offline analysis (Offline Sorter 2.8.; Plexon Inc.) according to the following cumulative criteria: voltage thresholds > 2 SD of amplitude distributions; signal-to-noise ratio > 3; fewer than 1% of interspike intervals > 1.2 ms; and stability of the waveform shape as determined by a waveform matching template

algorithm and principal component analysis. In addition, the Waveform Tracker software (Plexon Inc.) was used to verify that the recorded units were stable across experimental sessions. This software ensures that the same neuron is recorded in consecutive sessions through the analysis of the projection of the waveform of each recorded unit onto its three-dimensional principal components analysis space. An overhead video tracking system (CinePlex; Plexon Inc.) was used to provide information about the animal location on the runway and synchronize the video recordings with the acquired neuronal data.

### *Data analysis*

Neural ensemble data were processed offline using NeuroExplorer 4 (NEX; Plexon Inc.) and exported to our own MatLab R14 routines for additional analysis (MathWorks, Natick, MA, USA). Statistical comparisons of the experimental groups were performed by repeated-measures two-way ANOVA and, when appropriate, a *post hoc* analysis was carried out using the Bonferroni test (Prism 5.0; GraphPad, San Diego, CA, USA). The level of significance was set as at 5%. Results are expressed as mean ± SEM.

### *Classification of cells*

Putative pyramidal complex-spike 'place cells' were classified as such if they had a spike width (peak-to-trough) > 450 $\mu$s and a signal-to-noise ratio > 3 : 1. These cells were characterized by complex bursts wherein they often fired two to five spikes with an interspike interval of approximately 5 ms. Complex bursts were identified with an autocorrelation function (Neuroexplorer; Plexon Inc.) that calculated the time between all spike pairs and distinguished between the firing patterns of place cells and those of theta cells (putative interneurons; Markus *et al.*, 1994). Data from recorded theta cells were not included in the present study. Place cells recorded in the testing environment typically presented a mean firing rate < 5 Hz and a maximal in-field firing rate < 30 Hz. Because most of the examined parameters are affected by low firing rates, cells that had firing rates < 0.1 Hz were not analyzed. Furthermore, because it was important to ensure that the same unit was being recorded in repeated sessions, only units that exhibited stable spike amplitudes and consistent waveforms within and between recording sessions were included in this study.

### *Firing properties*

Firing rate maps were prepared for each cell by dividing the recording environment into a 32 × 32-bin array, each bin corresponding to 20 × 20 pixels of video resolution. Average firing rate for each bin was calculated for each neuron by dividing the number of recorded spikes by the time spent in that bin. Place fields were designated as an area of 5–20 bins sharing adjacent edges, with a firing rate > 2 SD above of the average cell firing rate over the entire arena (Muller & Kubie, 1989). Place field size was defined as the number of bins sharing adjacent edges that comprised the place field.

Basic firing properties were calculated: (i) the average firing rate of the unit on testing environment; (ii) the average firing rate within the place field; (iii) the maximum peak of firing rate within the place field; (iv) spatial discrimination capability, the ratio between the mean firing rate inside the place field and the mean firing rate outside the place field; and (v) the coherence of the firing inside the place field, this coherence measure being a computed autocorrelation between the rate for each bin of the place field and the average rate of all in-field bins (Neuroexplorer; Plexon Inc.).

In order to provide a measure of spatial firing that does not require characterization of the place field, the data obtained from the recording sessions were analyzed using a measure of spatial information content measure (I, or specificity; Skaggs & McNaughton, 1996; Skaggs *et al.*, 1993). Specificity of the place field was calculated in terms of the amount of spatial information content (in bits) that a single spike conveyed about the animal's location and was calculated using the equation $I = \Sigma P_i (R_i/R).\log_2(R_i/R)$, where $i$ is the bin number, $P_i$ is the probability for occupancy of bin $i$, $R_i$ is the mean firing rate for bin $i$ and $R$ is the overall mean firing rate. A value of 0 indicates that no spatial information is conveyed, while a typical place cell generates a value close to 1 bit of information per spike.

The averaged position of a place field on the runway was defined by calculating the centre of mass (centroid) of the firing rate distribution within the place field boundaries (Mehta *et al.*, 1997). To assess place field expansion we calculated the size (in bins) of each field. In addition, to assess shifts in the place field position, we calculated the linear distance (in pixels) between field centroids of the current and previous recording sessions.

### *Histology*

At the end of all experiments, the rats were deeply anesthetized with a mixture of ketamine and xylazine, and the recording site was marked by injecting DC current (10–20 $\mu$A for 10–20 s) through one microelectrode, marking the area below the electrode tips. Afterwards animals were perfused through the heart with 0.01 M phosphate buffer (pH = 7.2) in 0.9% saline solution, followed by 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde for 4 h and stored in 30% sucrose before they were frozen and sectioned into 60-$\mu$m slices. Sections were stained with Cresyl violet for microscopic identification of the recording site (shown in Supporting Information Fig. S1).

## Results

### *Mechanical hypersensitivity after SNI*

All SNI animals developed mechanical allodynia as indicated by the significant decrease in the mechanical force needed to evoke paw withdrawal to Von Frey stimulation in the hindpaw ipsilateral to the lesion, but not in the contralateral hindpaw (Bonferroni, $P < 0.001$). In the SHAM group, no statistical differences were noted between before and after surgery sessions (Fig. 2A).

### *Behavioral task activity*

No evident variation was found in rat activity across training sessions, and no signs of satiation were observed in the 15-min duration of each session. ANOVA revealed no significant differences in the number of correct alternations (from feeder A to feeder B) between SNI and SHAM animals (ANOVA-RM, $F_{7,70} = 1.33$, $P = 0.2421$; Fig. 2B). However, there was a within-group time effect across pre- and post-surgery recording sessions (ANOVA-RM, $F_{7,70} = 8.33$, $P < 0.0001$), probably due to the decrease in correct alternations that occurred in both groups in the first 3 days after surgery.

These results show that the induction of chronic pain did not cause a significant change in task performance between control and pain groups; this is technically crucial for establishing that eventual alterations in hippocampal spatial encoding did not result from

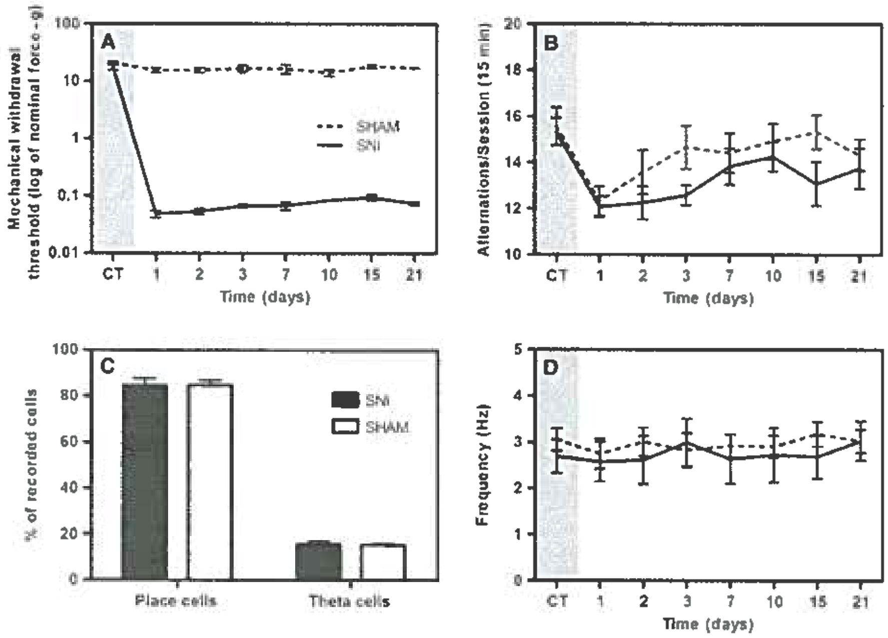


FIG. 2. Behavioural performance in the runway alternation task. (A) Level of sensitivity to mechanical stimulation evaluated using von Frey filaments. A large decrease was observed in the threshold required to induce a paw response in the SNI group. (B) Number of correct alternations on the U-shaped maze. Similar levels of behavioural task activity were observed for the two experimental groups. (C) The proportion of recorded neurons that were classified as place cells showed a similar distribution in the two experimental groups. Note that only neurons classified as place cells were considered in the analyses of the present study. (D) Average firing rate per place cell during the entire session remained unchanged after surgery. SNI, nerve lesion group; SHAM, control group. Values are mean ± SEM. Shaded area on the left of each graph represents the baseline control period (CT); the baseline plotted value is the collapsed average of the recording sessions in the 4 days before the nerve lesion, although it should be noted that in all statistical comparisons the values of the control days were used without any averaging. Comparisons between control and after surgery recording sessions based on two-way repeated-measures ANOVA, followed by Bonferroni's *post hoc* test.

changes in either locomotion or reward motivation, which are known to induce place cell instability by themselves.

### *Neuronal activity during task performance*

We used the Waveform Tracker software (Plexon Inc.) to ensure that individual neural recordings were correctly identified across experiments; only place cells that remained stable throughout the recording sessions in terms of waveform shape were considered in the present study (Supporting Information Fig. S1). Therefore, a total of 117 validated neurons were recorded from the CA1 region in six nerve-injured rats (SNI; $n = 58$ cells) and six control SHAM rats ($n = 59$ cells). Some cells were excluded from the final analysis because they did not exhibit the criterion properties of pyramidal complex cells described earlier (18 neurons, presumably theta cells). Consequently, 49/58 (84.48%) of cells from the SNI group and 50/59 (84.75%) cells from the SHAM group were determined to be place cells (Fig. 2C).

The mean firing rate was calculated for each place cell for the duration of the behavioural task. A two-factor repeated-measures ANOVA was used to compare the firing rates of cells from SNI and SHAM animals in pre- and post-surgery recording sessions (Fig. 2D), and revealed no significant differences in mean firing rate between the two experimental groups (ANOVA-RM, $F_{7,70} = 0.99$, $P = 0.4466$) and across the recording sessions (ANOVA-RM, $F_{7,70} = 1.03$, $P = 0.4225$).

### *Encoding of place fields*

CA1 pyramidal cells of the SNI group showed a significant increase in the number of encoded place fields. This increase was observed on a cell-by-cell basis and did not affect all cells simultaneously recorded in the same animal (Fig. 3). Repeated-measures ANOVA revealed that there were differences between the two groups ($F_{7,70} = 5.87$, $P < 0.0001$) and across time ($F_{7,70} = 3.27$, $P = 0.0460$). Moreover, *post hoc* analysis showed that the number of place fields in the SNI group was larger than in SHAM control animals (Bonferroni, $P < 0.01$ for day 7 after SNI and $P < 0.001$ for day 10 after SNI; Fig. 4A).

A two-factor repeated-measures ANOVA was used to compare the in-field firing activity of cells from SHAM and lesion animals in pre- and post-surgery recording sessions. There was no significant group effect for the place cells' in-field mean firing rate ($F_{7,70} = 1.03$, $P = 0.4201$) or in-field peak of firing ($F_{7,70} = 0.66$, $P = 0.7059$; Fig. 4B and C). However, over the recording sessions there was a significant effect of time in the in-field mean firing rate ($F_{7,70} = 6.99$, $P < 0.0001$) and in-field peak of firing ($F_{7,70} = 4.26$, $P = 0.0006$).

The recordings in the SNI and SHAM groups show that the internal place field coherence of place cells was affected after peripheral nerve injury with a significant increase after 7 days of lesion (Fig. 4D). Analysis revealed that there were differences between groups (ANOVA-RM, $F_{7,70} = 5.45$, $P < 0.0001$) and across the recording sessions (ANOVA-RM, $F_{7,70} = 4.74$, $P = 0.0002$). *Post hoc* analysis showed that values of field coherence after nerve injury were greater than those observed in the SHAM group (days 7 and 10; Bonferroni, $P < 0.05$).

There were also differences in the field size between the two experimental groups (ANOVA-RM, $F_{7,70} = 3.57$, $P = 0.0025$) and across the recording sessions (ANOVA-RM, $F_{7,70} = 2.63$, $P = 0.0179$; Fig. 4E). *Post hoc* analysis of field size revealed a significant increase for SNI group after nerve injury. However, no group interaction was found for field centroid movement (ANOVA-RM, $F_{7,70} = 1.38$,

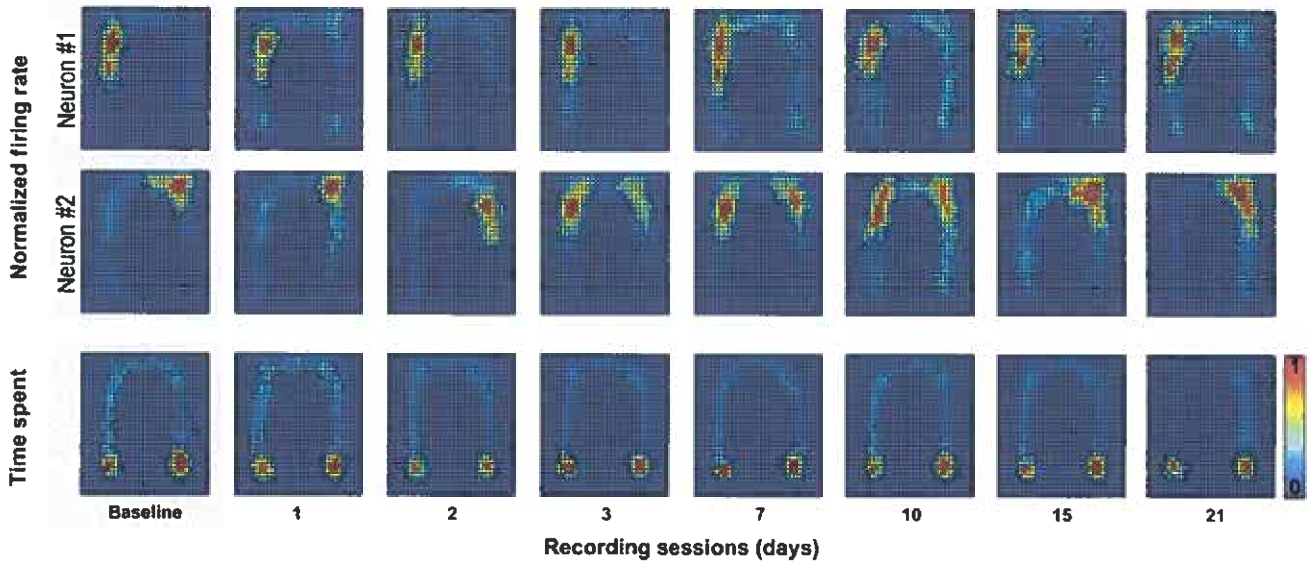


FIG. 3. Differential effect of pain over the remapping of place fields. The top two rows show the normalized firing rate per bin of two simultaneously recorded hippocampal place cells during the alternation task in the U-shaped runway. Note the similarity in the spatial firing activity of neuron 1 across recording sessions, and the encoding instability of neuron 2 in the days following the nerve lesion. The bottom row shows the time spent in each bin during the entire recording session, and demonstrates that the place field locations are commonly unrelated to the movement of the animal. Bright red represents bins with higher firing rate (top two rows) or where the animal spent more time (bottom row), and dark blue represent bins with lower firing rates (top two rows) or where the animal spent less time during the session (bottom row). The shaded plot on the left of each row represents the baseline control period; the baseline plotted values are the collapsed average of the activity in the 4 days before the nerve lesion.

$P = 0.2275$), although both groups presented changes over time (ANOVA-RM, $F_{7,70} = 13.10$, $P < 0.0001$; Fig. 4F).

### Spatial information content

The information content (bits/spike) of cells recorded from peripheral nerve injury animals (SNI group) was significantly lower than that of cells from SHAM control animals (Fig. 4G). ANOVA-RM analysis revealed there was a difference between groups ($F_{7,70} = 16.06$, $P < 0.0001$) and across the recording sessions ($F_{7,70} = 10.44$, $P < 0.0001$). *Post hoc* analysis revealed a significant decrease in information content encoded by the cells of the SNI group 10 days after nerve injury when compared with SHAM group (Bonferroni, $P < 0.05$).

### Spatial discrimination capability

Spatial discrimination capability, or the ratio between the mean firing rate inside the place field and the mean firing rate outside the place field, is shown in Fig. 4H. The results indicate that spatial discrimination did not differ between experimental groups (ANOVA-RM, $F_{7,70} = 1.13$, $P = 0.3565$) or over recording sessions (ANOVA-RM, $F_{7,70} = 1.79$, $P = 0.1037$).

## Discussion

The aim of the present study was to address for the first time whether the onset of an animal model of the chronic neuropathic pain condition affects the spatial encoding properties of hippocampal CA1 pyramidal cells. This type of cell is considered to be crucial for the continuously updated representation of space and individual position. However, this dynamic information is only one of several features stored in the hippocampal network (Eichenbaum *et al.*, 1999; Leutgeb *et al.*, 2005), and for this reason it is of primary interest to determine what factors, including pain, may contribute to disruption of the stability of place cells.

Our results show that pain causes instability in hippocampal place field encoding in the absence of changes in overall task performance. As intended, the onset of the pain model caused a transient reduction in task performance only in the days immediately following the surgery and this effect was equally observed in both control and pain animals. We specifically used this simple alternation task because it is not cognitively challenging and our results are in agreement with previous reports showing that pain has no impact on performance in simple spatial and nonspatial memory tasks (Apkarian *et al.*, 2004; LaBuda & Fuchs, 2000; Leite-Almeida *et al.*, 2009), although pain-related memory deficits may be observed in more complex memory tasks (Dick & Rashiq, 2007; Millecamps *et al.*, 2004; Leite-Almeida *et al.*, 2009). Moreover, the transient effect in performance (Fig. 2B) is not temporally correlated with the peak of place field instability (Fig. 4A), suggesting that the late onset instability of CA1 place fields is not caused by motor impairment or reduced motivation for task completion.

It is important to note that, although the task used in this study is not strictly hippocampus-dependent, several single-unit recording studies have also used tasks which are not hippocampus-dependent, such as forced-choice tasks, to examine place field characteristics, showing that place cells present environment re-mapping even on nonhippocampal tasks (Markus *et al.*, 1995; Muller & Kubie, 1987; Ranck, 1973).

### Chronic pain changed CA1 place cell activity

Basic firing properties of CA1 place cells remained stable after peripheral nerve lesion. These properties included mean firing rate

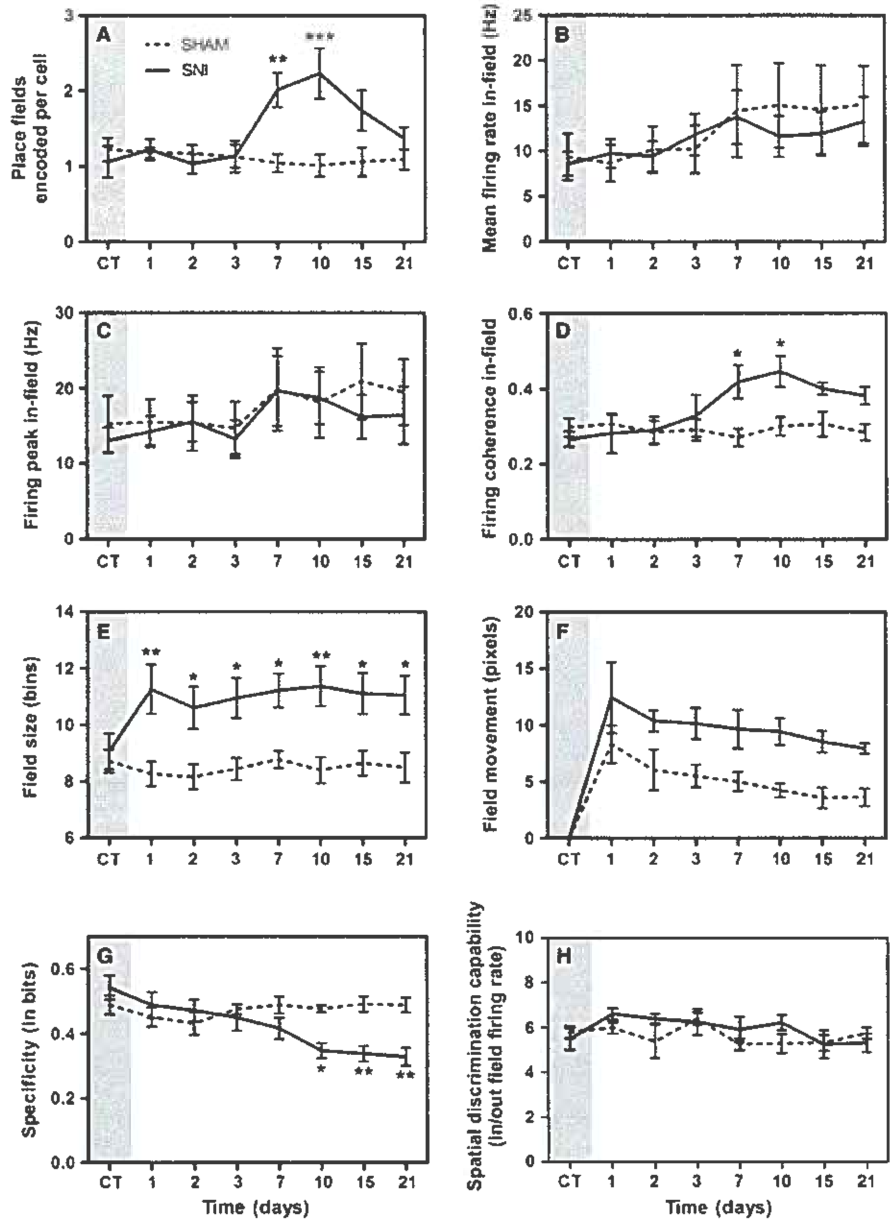


FIG. 4. Spatial place field properties across recording sessions. (A) Number of place fields encoded per recorded place cell. (B) Place field firing coherence. (C) Average in-field firing rate. (D) maximum firing rate for in-field peak. (E) Average size, in bins, of the place fields. (F) Place field shifts of centroid location, in pixels. (G) Specificity, or spatial content information (in bits), conveyed per spike of place cells. (H) Spatial discrimination capability (in-field/out-field firing ratio) between groups. SNI, nerve lesion group; SHAM, control group. Values are mean ± SEM. Shaded area on the left of each graph represents the baseline control period (CT); the baseline plotted value is the collapsed average of the recording sessions in the 4 days before the nerve lesion, although it should be noted that in all statistical comparisons the values of the control days were used without any averaging. Comparisons between control and after surgery recording sessions based on two-way repeated-measures ANOVA, followed by Bonferroni's *post hoc* test. $*P < 0.05$; $**P < 0.01$, and $***P < 0.001$.

activity and spatial discrimination capability. Indeed, the fact that the spatial discrimination was similar across the two groups implies a proportional firing activity in both experimental conditions. However, the analysis of spatial information content, or specificity (Skaggs *et al.*, 1993), a measure of spatial firing that does not require that a particular region be defined as a place field, revealed significantly lower values in the neurons recorded from nerve-lesioned rats, indicating that the activity of these neurons provided less information about spatial location. It should also be noted that the spatial information content is only minimally affected by proportional changes in the firing rate.

### *Chronic pain disrupted place field stability*

Spatial tuning characteristics such as number of place fields encoded per cell and in-field firing coherence changed after nerve lesion, with a peak effect observed 7 days after the lesion, of two place fields encoded per cell. Interestingly, 21 days after nerve lesion the majority of the cells returned to the baseline encoding of only one place field in average. A possible hypothesis to explain this transient effect is a reduction in pain threshold associated with nerve-injured animals; however, no statistically significant differences in data were encountered between days 15 and 21 in the von Frey test. In addition, it is

important to note that the second place field occupied a location which was not adjacent to the first one; this suggests that pain-induced field remapping and field expansion are different phenomena. It has been reported that place fields may be modified during a single recording session, and this may represent the within-session continuous acquisition of novel spatial information (Mehta *et al.*, 1997, 2000). This hypothesis does not apply to our results because we only started the control recording sessions after several training sessions, and during all control recording sessions the properties of place field remained stable (see Supporting Information Table S1).

### *Mechanisms of place field instability*

Apart from studies involving lesioning of the hippocampus or hippocampus-connected areas (McNaughton *et al.*, 1989; Muir & Bilkey, 2001), to our best knowledge no previous studies have shown disruption of place fields in the absence of changes to the testing environment. Stressful stimuli were shown to alter the in-field firing rate stability of place cells but not the stability of the field's location within a familiar environment (Kim *et al.*, 2007), although the data presented by the authors suggest a shift in pre- vs. post-stress location. A similar study has shown that the stability of place fields in a familiar environment does not change across the estrous cycle (Tropp *et al.*, 2005).

Several studies have shown that place field instability is accompanied by an overall increase in the size of preexisting place fields, and it has been reported that this size expansion is diminished in aged rats (Barnes *et al.*, 1997; Shen *et al.*, 1997) and abolished by selective blockade of NMDA receptors (Ekstrom *et al.*, 2001). Moreover, NMDA-dependent long-term potentiation processes are important for maintaining the stability of place fields (Kentros *et al.*, 1998; Shapiro & Eichenbaum, 1999).

Only incomplete and sometimes conflicting data exist on the molecular mechanisms of interplay between pain and hippocampal plasticity; it has been shown that chronic neuropathy reduces CA1 long-term potentiation (Kodama *et al.*, 2007; Ren *et al.*, 2011) while the opposite effect has been described after acute peripheral injection of formalin (Zhao *et al.*, 2009). In addition, recent studies have shown that chronic pain reduces the hippocampal levels of BDNF (Duric & McCarson, 2005; Hu *et al.*, 2010; Al-Amin *et al.*, 2011), which is known to be a key regulator of hippocampal synaptic plasticity (Minichiello, 2009). It must be noted that to our best knowledge no direct connection has been demonstrated between neurotrophin levels in the hippocampus and the stability of place cells, but it is expected that the modulation of molecules important for synaptic plasticity also leads to changes in the circuitry of hippocampal spatial encoding.

Finally, it has been proposed that hippocampal remapping may result from memory interference between concurrent sets of experiences (Colgin *et al.*, 2008). This is in agreement with the idea that evoked or spontaneous pain perception causes an interference with ongoing cognitive functions (Seminowicz & Davis, 2007; Moriarty *et al.*, 2011), disrupting the attentional processes that are crucial for learning and memory (Boyette-Davis *et al.*, 2008; Pais-Vieira *et al.*, 2009b).

## Conclusion

In summary, our data suggest that peripheral nerve injury (SNI) induces a relative instability of hippocampal CA1 place cells' spatial features. The present data indicate that nerve lesion induces a clear reduction in the speciality measure, indicating that place cells provided less information about spatial location after lesion. Our findings also demonstrate place field disturbances, namely in the number, size and in-field firing coherence. These changes are probably caused by hippocampal structural adaptive mechanisms that occur during the onset of the painful condition, which may disturb the mnemonic processes that rely on the integration and consolidation of spatial reference memory.

## Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Stability of waveform shapes of two hippocampal place cells simultaneously recorded from the same channel (yellow and green) across experimental sessions (A). Note that the waveform shape of each place cell remained stable throughout the recording sessions. Only units with a $> 3:1$ signal to noise rate were considered. (B) Illustration of the Unit A firing activity recorded from a rat running on the U-shaped task encoding a spatial place field (area with peak of firing). Maximum firing rate is indicated by red and occupancy with no firing by blue. (C) Offline analysis of 3-D PC cluster stability from the channel shown across the whole recording sessions using the WaveTracker software (Plexon Inc., Dallas, TX, USA). In this view (D), 2-D PC clusters are projected as function of time (Z-axis). Stability across time and absence of overlap between units isolated from the same channel were used as extra-selection criteria. (E) Location of implanted multielectrode arrays for nine rats used in this study. The black dots indicate the location of the centre of the array in CA1 region.

Table S1. Statistical summary for SHAM and SNI-group comparison across all measurements during the eight recording sessions of the control period.

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## Acknowledgements

This work was supported by grants from the Portuguese Foundation for Science and Technology – FCT: FCT SFRH/42500/2007, FCT PTDC/SAU-NEU/100733/2008; and BIAL Foundation: BIAL Project 126/08.

## Abbreviations

SNI, spared nerve injury.

## Conflict of interest

The authors do not have any conflicts of interest.

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