

Increased responses in the somatosensory thalamus immediately after spinal cord injury



E. Alonso-Calviño^a, I. Martínez-Camero^{a,1}, E. Fernández-López^a, D. Humanes-Valera^{a,2}, G. Foffani^{a,b,c}, J. Aguilar^{a,*}

^a Hospital Nacional de Paraplégicos, Servicio de Salud de Castilla-La Mancha, 45071 Toledo, Spain

^b CINAC, HM Puerta del Sur, Hospitales de Madrid, Madrid, Spain

^c CEU-San Pablo University, Madrid, Spain

ARTICLE INFO

Article history:

Received 27 August 2015

Revised 26 November 2015

Accepted 14 December 2015

Available online 17 December 2015

Keywords:

Thalamocortical plasticity

Spinal cord injury

Somatosensory system

Nucleus ventralis posterior lateralis of the thalamus

Electrophysiology

Evoked responses

Spontaneous activity

ABSTRACT

Spinal cord injury (SCI) involves large-scale deafferentation of supraspinal structures in the somatosensory system, producing well-known long-term effects at the thalamo-cortical level. We recently showed that SCI provokes immediate changes in cortical spontaneous and evoked responses and here, we have performed a similar study to define the immediate changes produced in the thalamic ventro-postero-lateral nucleus (VPL) that are associated with the forepaw and hindpaw circuits. Extracellular electrophysiological recordings from the VPL reflected the spontaneous activity and the responses to peripheral electrical stimulation applied to the paws. Accordingly, the activity of the neuronal populations recorded at specific thalamic locations that correspond to the forepaw and hindpaw circuits was recorded under control conditions and immediately after thoracic SCI. The results demonstrate that peripheral inputs from both extremities overlap on neuronal populations in the somatosensory thalamus. In addition, they show that the responses of thalamic neurons to forepaw and hindpaw stimuli are increased immediately after SCI, in association with a specific decrease in spontaneous activity in the hindpaw locations. Finally, the increased thalamic responses after SCI have a state-dependent component in relation with cortical activity. Together, our results indicate that the thalamic changes occurring immediately after SCI could contribute to the cortical changes also detected immediately after such spinal lesions.

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Spinal cord injury (SCI) produces large-scale deafferentation at the supraspinal level that induces physiological changes in different brain structures. The main changes after SCI have been described at the cortical and thalamic levels (Endo et al., 2007; Gustin et al., 2009; Wrigley et al., 2009; Aguilar et al., 2010; Qi et al., 2011; Ghosh et al., 2010, 2012; Liang and Mendell, 2013) although other studies have shown changes at the brainstem level (Jain et al., 2000; Onifer et al., 2007; Liao et al., 2015). All these changes are related to the anatomical and physiological reorganization that occurs, through which the deafferented region enhances its responses to stimulation of body regions above the level of the lesion, referred to as the intact area.

Changes at the cortical level in response to SCI have been studied in detail using different experimental approaches and over a wide range of time windows, from immediately after the lesion (hours and days) to weeks and months (Endo et al., 2007; Wrigley et al., 2009; Aguilar et al., 2010; Ghosh et al., 2010, 2012; Moxon et al., 2014). We recently described the cortical changes that occur immediately after SCI using simultaneous electrophysiological recordings from cortical forepaw and hindpaw locations (Aguilar et al., 2010; Humanes-Valera et al., 2013; Yague et al., 2014). In these studies we showed that two main physiological phenomena occur in the somatosensory cortex: first, an immediate change occurs in the brain that involves a switch from spontaneous delta activity (1–4 Hz) to slow-wave activity (<1 Hz; Aguilar et al., 2010); second, an increased evoked response appears in the intact cortical FP representation in response to peripheral FP stimulation that has two different components: a state-dependent component (related to the change in spontaneous activity) and a state-independent one (Humanes-Valera et al., 2013; Yague et al., 2014).

At the thalamic level, the long-term changes in neuronal activity after SCI have been described in terms of channelopathies that mainly affect the spontaneous activity, related with pain or phantom limb sensations (Hains et al., 2005; Waxman and Hains, 2006; Hains and

* Corresponding author.

E-mail address: jdaguilar@sescam.jccm.es (J. Aguilar).

¹ Present address: Instituto de Biomedicina de Sevilla, Avda. Manuel Siurot, s/n 41013 Sevilla, Spain.

² Present address: Department of Systems Neuroscience, Institute of Physiology, Faculty of Medicine, Ruhr-University Bochum, D-44801 Bochum, Germany.

Available online on ScienceDirect (www.sciencedirect.com).

Waxman, 2007). But only few studies focused on thalamic spontaneous activity as well as the evoked responses that are expanded into the thalamic deafferented region (Gerke et al., 2003; Jain et al., 2008; Liang and Mendell, 2013). Moreover in these studies there is no clear relationship between the spontaneous activity and the evoked responses after SCI, and there is little information about the immediate effects of SCI at the thalamic level (Liang and Mendell, 2013).

The increased evoked responses recorded immediately after SCI in the somatosensory cortex could, at least in part, have a subcortical origin, for example at the thalamic level (Humanes-Valera et al., 2013). Moreover, the strong thalamo-cortico-thalamic loop could be involved in the change of spontaneous activity described at the cortical level after SCI, which could influence thalamic spontaneous activity after a lesion (Crunelli and Hughes, 2010).

In order to establish the thalamic participation in the immediate changes that SCI produces at the supraspinal level, we studied the responses recorded in the ventro-postero-lateral (VPL) nucleus of the thalamus to peripheral forepaw and hindpaw stimulation in under control conditions and immediately after thoracic SCI.

2. Materials and methods

Experiments were performed in accordance with the European Union guidelines (Directive 2010/63/EU) and they were approved by the Ethical Committee for Animal Research at the “Hospital Nacional de Paraplégicos” (Toledo, Spain). A total of 50 male Wistar rats (300–400 g) were used in the experiments, which basically consisted in recording the extracellular activity in the thalamic VPL. The main experimental approach was similar to that used in our previous studies, in which electrophysiological recordings from the somatosensory cortex were collected in anesthetized rats during control conditions and immediately after SCI (Aguilar et al., 2010; Yague et al., 2011, 2014; Humanes-Valera et al., 2013).

2.1. Experimental protocol

Animals were anesthetized with urethane (1.5 g/kg i.p.) and they were placed in a stereotaxic frame (SR-6 Narishige Scientific Instruments, Japan), checking their body temperature with a rectal probe and controlling it automatically with a heating pad (Cibertec SL, Madrid, Spain). The surgical intervention involved a thoracic laminectomy (at T9–T10), keeping the dura mater intact until the moment of performing a complete transection of the spinal cord. Lidocaine 2% was applied over the body surface in contact with the stereotaxic frame and over the areas for incisions. The skin of the head was softly removed and the skull was exposed. A craniotomy was performed on the right side using the coordinates of the somatosensory cortex and somatosensory thalamus (AP, 1 to –4; ML, 1 to 4) and

the cisterna magna was opened with a small incision on the dura mater in order to decrease intracranial pressure and guarantee the stability of recordings. The dura mater of the brain was removed after craniotomy, and tungsten electrodes were lowered into the somatosensory cortex and thalamus following the coordinates of Chapin and Lin (1984) and the Paxinos and Watson (2007) atlas of the rat brain. The correct location of the electrodes in the cortex was determined by assessing the responses to tactile stimulation with a cotton swab stick in the rat's forepaw or hindpaw, and by ensuring a good quality of averaged neural responses to electrical stimulation in the forepaw or hindpaw. Cortical recordings of the paw's representation were used to check the spontaneous activity relative to the degree of anesthesia under control conditions and monitoring the immediate changes previously described by our lab (Aguilar et al., 2010; Humanes-Valera et al., 2013). Moreover, cortical recordings from the hindpaw representation were used to ensure that the spinal cord had been completely sectioned as a lesion at the thoracic level impedes any evoked responses (Fig. 1). Thalamic locations were defined in a similar manner: once the electrode had been lowered into the somatosensory thalamus, peripheral stimulation (tactile and electrical) was applied in order to check the response to the given extremity (forelimb or hindlimb) in terms of magnitude and latency.

A total of 55 electrophysiological recordings in the thalamic VPL forepaw location (FP-location; $n = 35$) or thalamic VPL hindpaw location (HP-location; $n = 20$) were obtained from 50 experiments. It should be noted that two electrodes for thalamic recordings were located in the same experiments on VPL in 5 animals (FP and HP locations) in order to record the activity in both VPL locations simultaneously. Once the final locations were defined, the electrodes were fixed in place and were not moved throughout the entire experiment.

Once the cortical and thalamic recordings had been located, data were collected from each thalamic location (FP-location and HP-location) by applying the stimulation protocol under pre-lesion conditions (control protocol; Fig. 1). Using an experimental protocol (Fig. 1) similar to what we described previously, bipolar needle electrodes were located subcutaneously in the wrist of each paw, one pole in each side of the paw allowing applying peripheral stimulation to both extremities (forepaw and hindpaw) contralateral to the hemisphere recorded. The electrical stimulation parameters were: 1 ms pulse duration at 0.5 Hz frequency delivered to each extremity. Two different intensities were applied: low intensity (0.5 mA) and high intensity (5 mA). Low-intensity stimuli were intended to activate only a fraction of the available fibers, mainly low-threshold primary fibers running through the lemniscal pathway, from the dorsal column to the brainstem (Lilja et al., 2006). High-intensity stimuli were intended to activate the maximum number of fibers, including high-threshold primary fibers that make a synapse in the dorsal horns of the spinal cord, in turn activating the spinothalamic tract (Lilja et al., 2006; Yague et al., 2011).

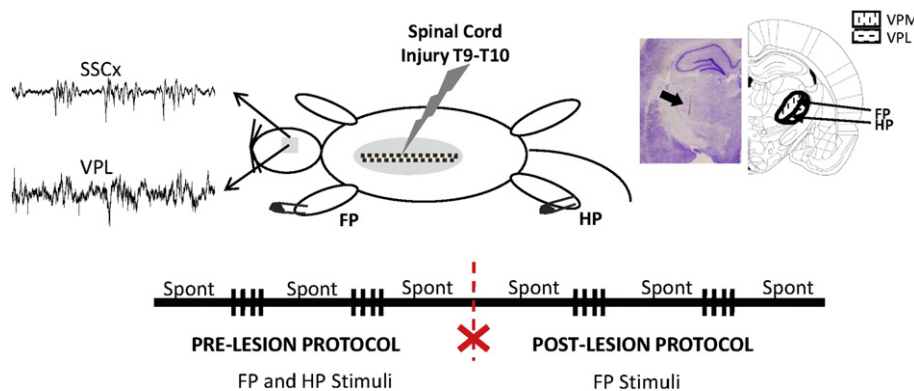


Fig. 1. Schematic representation of experimental design. Cortical and thalamic extracellular recordings were performed simultaneously. Thalamic recordings were made in the ventral-posterior-lateral nucleus (VPL), where electrodes were placed on the forepaw and/or hindpaw location (FP-location and HP-location). A protocol of peripheral stimulation in the forepaw and hindpaw was applied under control conditions and after spinal cord injury. Schematic representation of the thalamic location of electrodes in the VPL nucleus and the histological confirmation by using Nissl staining is shown.

After a protocol was obtained under control conditions from each animal, a complete transection of the spinal cord at the T9–T10 thoracic level was performed with a spring scissors. Immediately after a lesion we confirmed the complete transection of the spinal cord by the lack of responses evoked by maximal intensity (10 mA) stimulation of the hindpaw or alterations to spontaneous activity. Electrophysiological recordings were continuously acquired during the transection to confirm the stability of the recordings from control conditions to post-lesion conditions. Between 10 and 30 min after the transection, we started the post-lesion protocol, recording spontaneous activity and evoked responses by peripheral forepaw stimulation.

Electrophysiological recordings were obtained using tungsten electrodes with a 4–5 M Ω impedance at 1 kHz (TM31C40KT and TM31A50KT, World Precision Instruments Inc., Sarasota, FL, USA) that were connected to a modular system of a preamplifier, filter and amplifier (Neurolog System, Digitimer Ltd., UK). Raw signals were amplified ($\times 500$) and filtered (0.5 Hz–3 kHz) online, and the signals were converted into digital signals with an A/D converter (1401 CED Cambridge Electronic Designs, UK). Spike2 software was used for acquisition and posterior analysis, and all the data were stored on a computer for posterior off-line analysis. The raw signals were treated off-line in order to quantify the responses and the spontaneous activity.

2.2. Histology

At the end of the experiments, animals were transcardially perfused with heparinized saline followed by 4% paraformaldehyde. Then the brain was removed and post-fixed in the same fixative solution for 24 h at 4 °C. The next day the brain was washed 3 times with PBS and sections were obtained with a vibrating blade microtome (Microm HM 650 V (Microm International GmbH, Walldorf, Germany) at 50 μ m and Nissl stained to obtain histological confirmation of the correct electrode location (Fig. 1).

2.3. Data analysis

2.3.1. Thalamic responses

Somatosensory thalamic responses to peripheral stimulation were quantified using three different methods: 1) averaged local field potentials (LFPs); 2) averaged rectified multiunit activity (rMUA); and 3) peri-stimulus-time histograms (PSTH) of the action potentials from neuronal population activity. Time-stamps of stimuli were used as the reference to average the raw recordings of field potentials in order to obtain the averaged LFPs. The amplitude of LFP responses was quantified as peak-to-peak amplitude. Raw signals were filtered at 300–3 kHz to isolate the spike activity from the neuronal populations (MUA) closest to the recording electrode. Once the MUA was obtained, it was rectified and smoothed (by a median filter $t = 0.5$ ms) by applying a median filter (0.5 ms) in order to obtain a rectified MUA (rMUA). The rMUA signal was then averaged across stimuli to obtain the local thalamic responses of the neuronal population recorded at each location. The spikes were extracted by thresholding the MUA signal at 5 standard deviations above the noise in order to select the few neurons recorded closest to the electrode. Every selected spike was considered an event in a new channel that was used to construct the PSTH by using the time-stamps of stimuli as reference and the time-stamps of spikes as measurement to create the histogram with a bin value of 1 ms. The magnitudes of rMUA and PSTH responses were quantified as background-subtracted area within 30 ms post-stimulus, flooring negative values to zero.

2.3.2. Thalamic spontaneous activity

The spike events to quantify the firing rate were obtained during spontaneous activity, using the representative times of 100 s from each recording in control conditions and after SCI in each animal. Spikes were obtained by thresholding the MUA signal at 5 standard deviations above the noise.

2.3.3. Spontaneous activity in the somatosensory cortex

Cortical recordings were filtered at 300 Hz–3 kHz in order to isolate the spike activity from the neuronal population closest to the recording electrode. The filtered signal was rectified to obtain the rMUA and we used the mean voltage value extracted from the cortical rMUA over 300 s of spontaneous activity. Slow-wave activity had a mean rMUA lower than that under delta activity (Aguilar et al., 2010). In addition, changes between delta activity that are predominant during normal doses of anesthesia and slow-wave activity were controlled visually and labeled off-line.

2.4. Statistical analysis

The data were analyzed using Statistica.Ink software (Statsoft Ibérica, Lisboa, Portugal), which was used to perform *t*-tests (when there was one factor with two levels) and two-way analyses of variance (ANOVA). Tukey Honest Significant Test was used for post-hoc comparisons. Outliers were excluded when values exceeded 3 standard deviations from the mean. All results were considered significant at $p < 0.05$.

3. Results

We first analyzed the neuronal responses to electrical stimulation for each electrode location in control conditions (before performing the SCI). We stimulated the forepaw and hindpaw at two different electrical intensities (see [Materials and methods](#) and [Fig. 2](#)) in order to determine by the neuronal responses the electrode location inside the somatotopic arrangement of the thalamus. This analysis allowed us to determine the overlap of peripheral inputs over the neuronal population recorded at each electrode location.

After the control condition in intact animals, we analyzed the neuronal responses for each electrode location immediately after performing the SCI. The comparisons of neuronal responses obtained under control conditions and after SCI in each experiment were intended to study the immediate changes that occur at the thalamic level.

3.1. Spatial tuning of the extremities in the somatosensory thalamus

In order to first determine the thalamic location for each electrode, we created an index of spatial tuning (IST). We defined the IST as the ratio between the averaged response magnitude to forepaw stimulation divided by the sum of the averaged response magnitudes to forepaw and hindpaw stimulation. For this purpose we used two different signals, rMUA and PSTH, in order to determine which of these two signals were most informative about spatial thalamic responses to both contralateral extremities [Fig. 2A](#).

Then the IST has a range between 0 and 1, where the values between 0.5 and 1 indicate an electrode location in the forepaw thalamic region (FP-location) and conversely, IST values between 0 and 0.5 indicate an electrode location in the hindpaw thalamic region (HP-location) ([Fig. 2A](#)).

The electrical stimulation protocol was applied in both extremities (forepaw and hindpaw) at two different intensities (low intensity and high intensity). Low intensity stimulation grouped the majority of IST for rMUA and PSTH values in two ranges located at the extremes of the distribution, which is between 0 and 0.1 and 0.9–1 ([Figs. 3A and B](#)). This distribution of IST values resembles an all-or-nothing response to only one extremity (forepaw or hindpaw), which can be explained by the classic receptive field definition obtained using tactile stimulation. However, when high intensity stimulation was applied the IST distribution showed an increased representation of values between 0.1 and 0.9 in both cases of rMUA and PSTH magnitudes ([Figs. 3A and B](#)). These results demonstrated that there is a thalamic neuronal population in the ventral–posterior–lateral nucleus (VPL) where peripheral inputs from both extremities (forepaw and hindpaw) overlap ([Fig. 3C](#)).

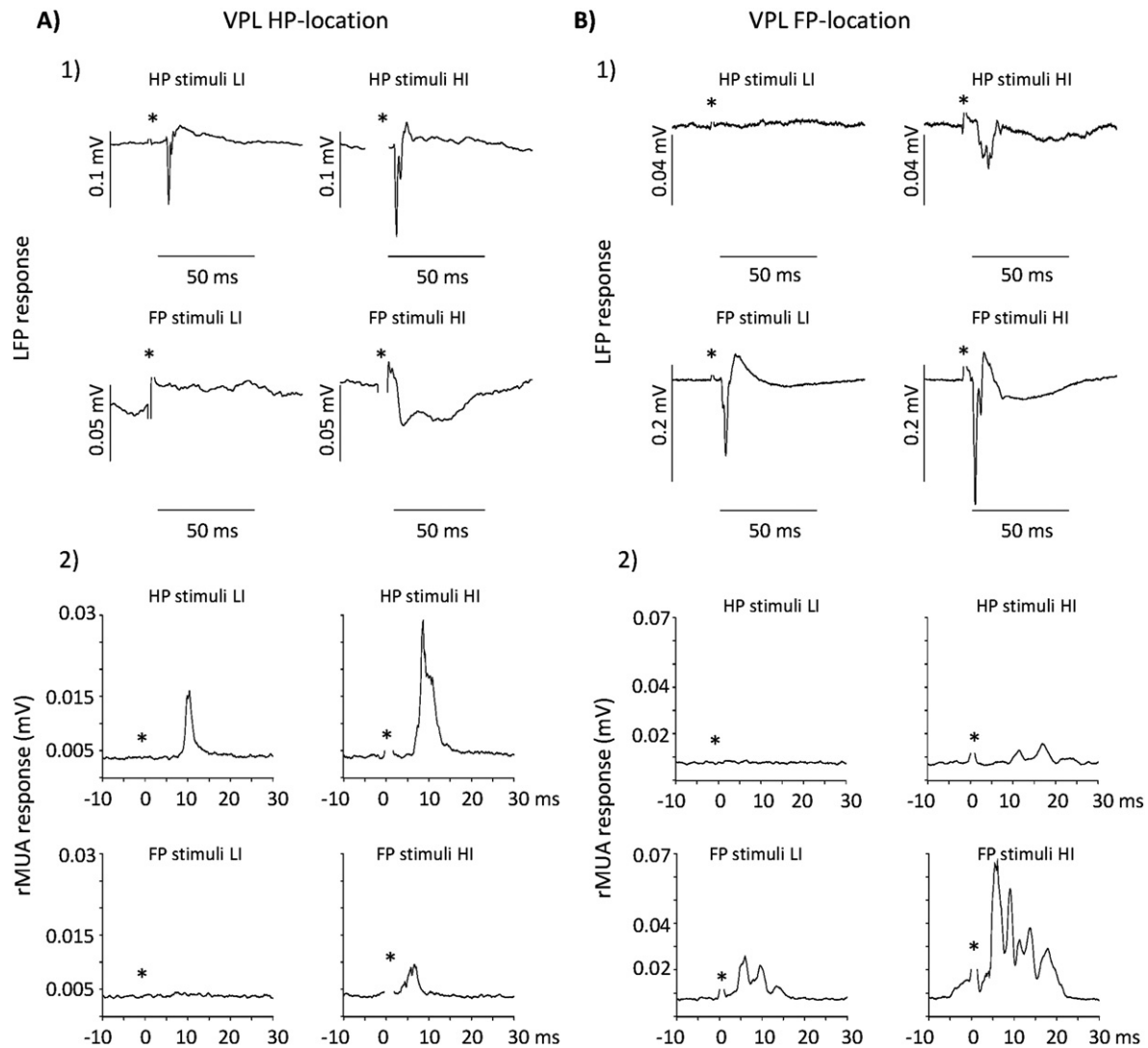


Fig. 2. Examples of averaged local field potentials (LFP) and rectified multiunit activity (rMUA) of thalamic responses recorded on VPL HP-location (A) and VPL FP-location (B) to peripheral electrical stimulation at two different intensities (LI is low intensity and HI is high intensity) in both contralateral extremities (HP stimulation and FP stimulation). A1) LFP responses on VPL HP-location to stimulation on hindpaw at low and high intensity stimulation (upper panels), and to forepaw at low and high intensity stimulation (lower panels). A2) rMUA responses on VPL HP-location to stimulation on hindpaw at low and high intensity stimulation (upper panels), and to forepaw at low and high intensity stimulation (lower panels). B1) LFP responses on VPL FP-location to stimulation on hindpaw at low and high intensity stimulation (upper panels), and to forepaw at low and high intensity stimulation (lower panels). B2) rMUA responses on VPL FP-location to stimulation on hindpaw at low and high intensity stimulation (upper panels), and to forepaw at low and high intensity stimulation (lower panels). Note the interesting neuronal responses in HP-location by forepaw high intensity stimulation, and in the same way the neuronal responses in FP-location to hindpaw high intensity stimulation. (*) Indicates the artifact of electrical stimulation.

We analyzed the percentage of recordings, using the rMUA signal, in which the electrodes with IST corresponding to FP-location or HP-location in the VPL receive inputs from the other paw (which is defined as “overlap”). It was observed that 61.8% of multiunit recordings showed overlap of inputs from both extremities (30.9% corresponding to FP-location rMUA recordings and 30.9% of recordings corresponding to HP-location rMUA recordings) (Fig. 3D).

To quantify more precisely the physiological overlap between the representations of the extremities in the somatosensory thalamus, in each recording we defined the value 0 (i.e.: no overlap, $IST < 0.1$ or > 0.9) when we observed a response to only one extremity and that of 1 (i.e.: overlap, $0.1 < IST < 0.9$) when a response to both extremities could be observed. Subsequently, these data were analyzed using two-way independent measures ANOVA: first factor rMUA/PSTH; second factor stimulation intensity low/high. The physiological overlap between the FP-locations and HP-location in the VPL was greater for the rMUA than for the PSTH (factor rMUA/PSTH, $F(1, 216) = 5.5$, $p = 0.0198$), and for the high intensity stimuli as opposed to the low-intensity stimuli (factor stimulation intensity, $F(1, 216) = 22.0$, $p < 0.0001$).

To verify that the results obtained with the ANOVA did not depend on any possible violation of the statistical assumptions, we repeated the same analysis with a generalized linear model, using a binomial distribution and the logit link function. The results produced were essentially the same (factor rMUA/PSTH – Wald = 5.3, $p = 0.0213$; factor stimulation intensity – Wald = 19.3, $p < 0.0001$).

Thus, our data demonstrate that the massive recruitment of peripheral fibers by high intensity stimulation (5 mA) unmasks the overlap of peripheral inputs from both contralateral extremities (forepaw and hindpaw) onto a neuronal population in the somatosensory thalamus (as measured by the rMUA). Due to the clear results obtained with the rMUA compared to the PSTH to study the evoked responses in the ventral posterior nucleus of the thalamus (VPL) to different peripheral inputs, we decided to use the rMUA signal from a local neuronal population for the following sections in relation to the effects of a SCI in the somatosensory thalamus.

Interestingly, this arrangement could be the basis of the physiological reorganization after complete deafferentation of a body region due to SCI.

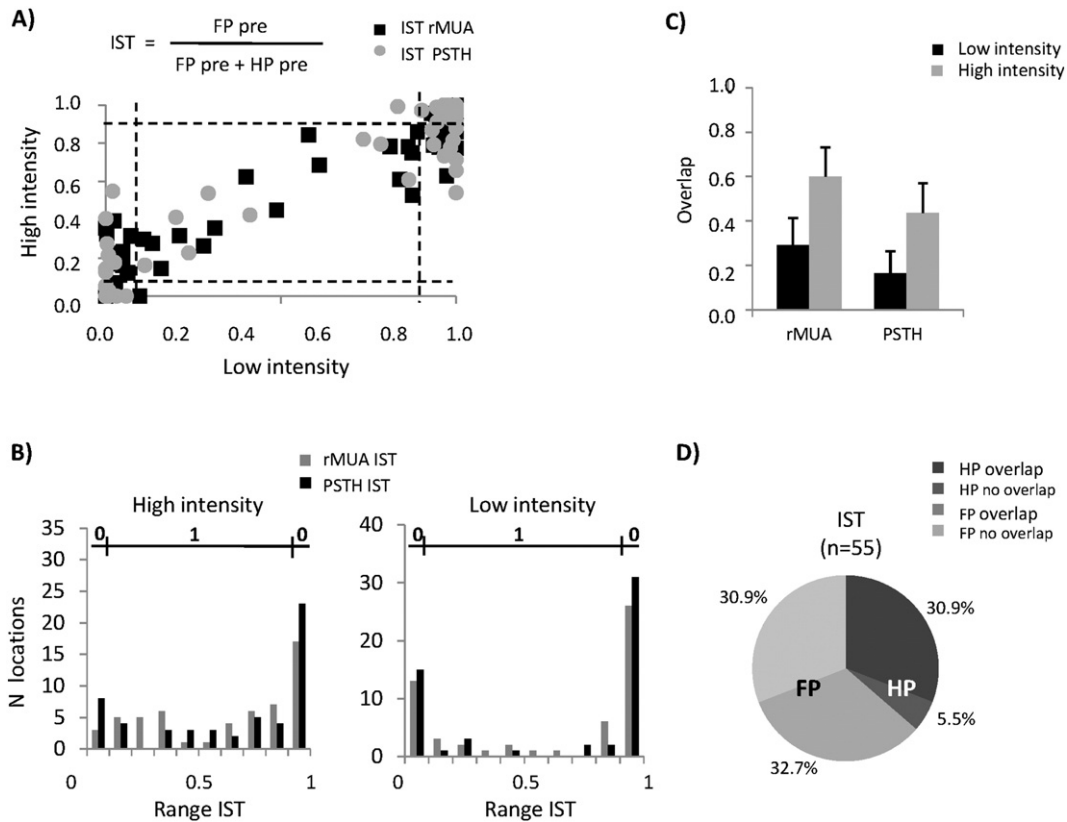


Fig. 3. Analysis of thalamic neuronal population of VPL in response to stimulation in different extremities (forepaw and hindpaw) in order to quantify the overlapping of peripheral inputs onto the same neuronal population. A) Definition of Index of Spatial Tuning (IST) for the VPL: ratio between the response magnitude to forepaw stimulation (FP pre) divided by the sum of the same value and the response magnitude to hindpaw stimulation (HP pre). Scatter plot shows the distribution of all IST obtained at low and high intensity stimulation using values of PSTH and rMUA. B) Distribution of IST values for high (left histogram) and low intensities (right histogram) of all neuronal population recorded from VPL thalamic locations. It can be observed that high intensity stimulation produces higher representation in middle values indicating “overlapping” of peripheral input onto the neuronal population. C) Normalized histogram of all neuronal populations recorded in thalamic VPL with IST values related to peripheral overlapping ($0.1 < \text{IST} < 0.9$). D) Schematic representation of the percentage of recordings made in HP-location and FP-location showing values of IST related to overlap and non-overlap peripheral inputs.

3.2. Changes in somatosensory thalamic responses after SCI

We previously demonstrated that SCI provokes immediate changes in the somatosensory cortex (Aguilar et al., 2010; Humanes-Valera

et al., 2013). Thus, we also studied the thalamic electrophysiological responses to peripheral forepaw and hindpaw stimulation under control conditions, and after SCI. Electrophysiological recordings were obtained from the FP-locations ($n = 35$) and HP-locations ($n = 20$). In light of the

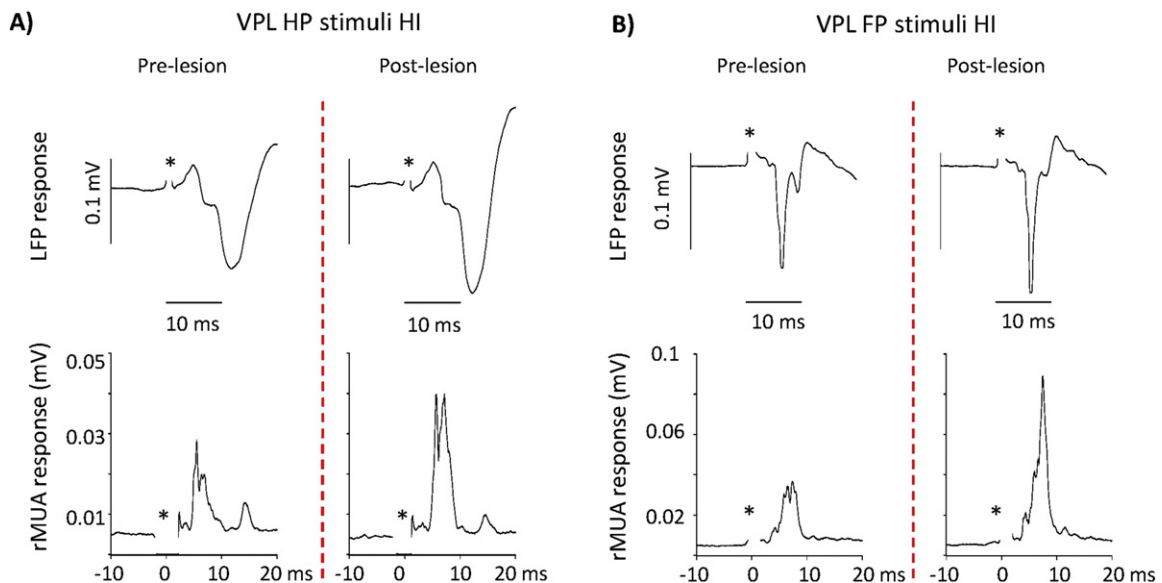


Fig. 4. Examples of averaged LFP (upper panels) and rMUA (lower panels) in response to forepaw stimulation at high intensity in two conditions (pre-lesion and post-lesion). A) Example of recording in VPL HP-location. B) Example of recording in VPL FP-location. Note the increased responses in both locations after SCI (post-lesion). (*) indicates the electrical stimulation artifact.

initial responses under control conditions (see above), in these studies we only used the responses obtained following high intensity stimulation in the forepaw and hindpaw in order to optimize the activation of the neuronal populations in the VPL.

Thalamic responses were recorded from a fixed electrode location under control conditions (i.e. before of SCI) by averaging the LFP and rMUA signals to the electrical stimulation of the forepaw. Then, a SCI was performed at thoracic level (T9–T10) and immediately after the SCI the same experimental protocol of stimulation was applied in order to record the neuronal activity in the same thalamic location (i.e. the same neuronal population) than in control conditions (Figs. 4 and 5A). This arrangement allowed us to do the comparisons between the responses recorded at each thalamic location under control conditions and after SCI (Fig. 5). All statistical comparisons were made by two-way ANOVA, constructed with time pre-lesion/post-lesion repeated measures as the first factor and the thalamic electrode location in the FP-location or HP-location as the second factor (determined by IST). The comparison of the LFP data (Fig. 5B1) showed that the magnitude of the responses increased after SCI (time $F(1, 53) = 13.7$, $p = 0.0005$) irrespective of the FP-location or HP-location (time \times location $F(1, 53) = 1.2$, $p = 0.28$). However, and as expected, we observed that when the stimulation was applied in the forepaw the response magnitude recorded in the FP-location was higher than the response magnitudes recorded in the HP-location (location $F(1, 53) = 4.8$, $p = 0.0321$). Analyzing the rMUA gave similar results as the LFP comparisons (Fig. 5B2), an increased magnitude of the responses after SCI at both the FP-location and HP-location (time $F(1, 49) = 7.6$, $p = 0.0083$) (time \times location $F(1, 49) = 0.0$, $p = 0.99$). Likewise, the response at the FP-location was

higher in magnitude than at the HP-lactation (location $F(1, 49) = 37.6$, $p = 0.0001$). Nevertheless, these results together point to a clear effect of SCI on the neural population responses in VPL to peripheral stimulation above the lesion level.

The result that the enhanced responses after SCI did not depend on the thalamic location could be confounded by the higher responses to forepaw stimuli recorded at FP-locations compared to HP-locations. To exclude this possibility, we created a ratio of the pre- to post-lesion responses for each VPL recording location by dividing the magnitude of the responses obtained after SCI by that of the control responses at each VPL location (Fig. 6). A ratio of 1 means that no change was found between the pre- and post-lesion responses (see the black line in Figs. 6A, B), whereas a ratio > 1 reflects a higher amplitude/magnitude of thalamic VPL response after SCI and a value < 1 reflects a smaller amplitude/magnitude of thalamic VPL response after SCI (Fig. 6). As the absence of a change in the responses was marked by the value 1, we applied a One Sample Test in order to determine if SCI produced changes in the magnitude of thalamic responses (i.e.: a value other than 1). The comparisons obtained with LFPs and rMUAs confirmed that the amplitude/magnitude of thalamic responses were higher in the VPL immediately after SCI (LFP One Sample Test $p = 0.001$ in Fig. 6A; rMUA One Sample Test $p = 0.019$ in Fig. 4B). Note that in Figs. 6A and B, the average of population ratios obtained from the response magnitudes using the two methods are marked with blue lines, which in all three plots appears to be > 1 .

In a posterior refinement of the comparisons we wanted to address whether the increase in the magnitude of the responses was distinct at the two VPL locations (FP-location and HP-location). Both measures

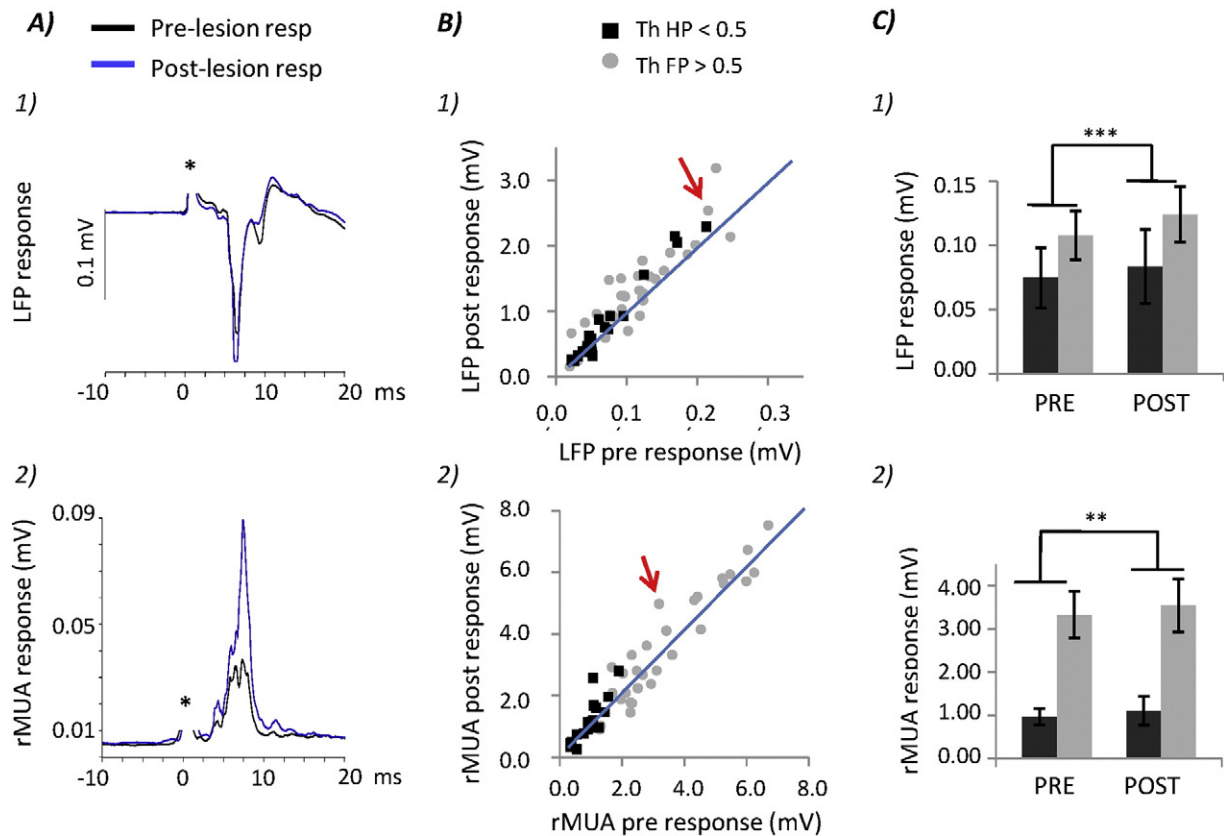


Fig. 5. Thalamic VPL responses under control conditions (black traces) and immediately after SCI (blue traces). A) Examples of representative thalamic responses in the same VPL-location under control conditions and after SCI. A1) Averaged LFP responses under both conditions. A2) Averaged rMUA responses under both conditions. B) Scatter plot of responses obtained from thalamic locations under control conditions and after SCI. Diagonal blue line indicates no change, while above the blue line are the VPL locations that showed increased responses after SCI and below the blue line are the VPL locations that showed decreased responses after SCI. B1) Scatter plot of the neuronal populations recorded using LFP responses in pre-lesion and post-lesion conditions. B2) Scatter plot of the neuronal populations recorded using rMUA responses in pre-lesion and post-lesion conditions. C) Population data of responses of VPL thalamic locations under control conditions and after SCI. C1) Data are related to LFP responses. C2) Data are related to rMUA responses. Error bars determine the CI of 95%.

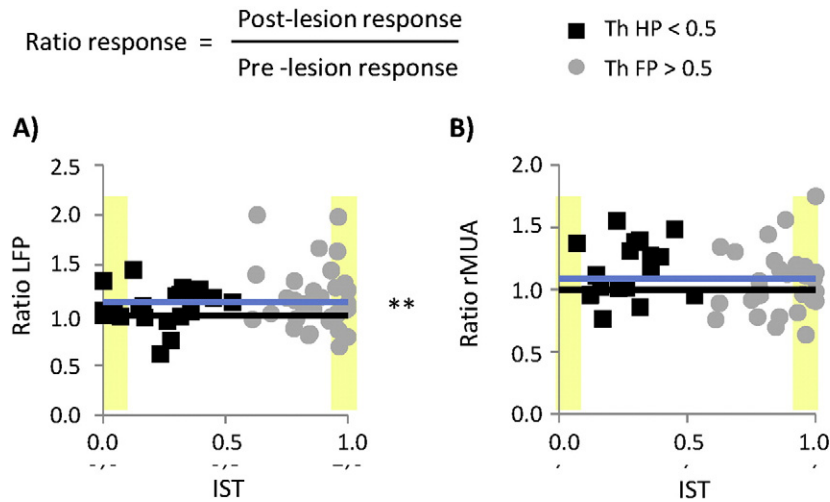


Fig. 6. Ratio of the thalamic responses depending on the IST: A) data of the magnitudes of the LFP response; B) data of the magnitude of the rMUA response. The black lines indicate a ratio of 1, which means no change between the pre- and post-lesion responses. The blue lines are the average ratios of the entire population that indicate an increased population response. Yellow areas indicate the IST values related to the neuronal population on VPL locations with non-overlapping of peripheral inputs. The asterisks indicate significant differences between the average ratios and the value reflecting no changes (one sample *t*-tests **p* < 0.05, ***p* < 0.01).

(LFP, rMUA) indicated that the greater magnitude of thalamic responses did not differ in function of the location (LFP ratio, *p* = 0.35; rMUA ratio, *p* = 0.31).

Response latencies were studied in order to determine possible changes between control conditions and after SCI. We used the LFP signal to measure the latencies, and we observed that on average there is an increased latency of the responses recorded in HP-locations and FP-locations after SCI (paired *t*-test for HP-location *p* = 0.0227; paired *t*-test for FP-location *p* = 0.0482).

An increased physiological response (measured by LFP and rMUA) to peripheral stimulation after a complete thoracic SCI can be detected in the thalamic VPL nucleus. These functional changes affect the intact VPL area (FP-location in our model) and the deafferented VPL area (HP-location in our model).

3.3. Thalamic spontaneous activity after complete spinal cord injury

The results we obtained previously at the cortical level demonstrated a change in the state of spontaneous activity in the somatosensory cortex after SCI (Aguilar et al., 2010). Here we studied the thalamic activity in the VPL nucleus from the same perspective of massive deafferentation by a SCI. We have assessed the immediate effect of SCI on

spontaneous neuronal activity of VPL locations that keep intact its peripheral inputs (FP-locations) and the spontaneous neuronal activity in the VPL locations that were affected by massive deafferentation after SCI (HP-location).

We used the firing rate of the neuronal population recorded at each VPL (FP location or HP location) under control conditions and after SCI as a measure of spontaneous activity (Fig. 7A). Taking into account the possible differential activity between the FP-location and HP-location in the VPL due to the SCI, we studied these effects with a two-way ANOVA in which the first factor was the activity under control conditions (pre-lesion) and after SCI (post-injury), and the VPL location was included as the second factor (FP-location vs HP-location). The spontaneous activity in the thalamic VPL nucleus decreased immediately after SCI (thalamic firing rate pre/post lesion; *F*(1, 51) = 26.9; *p* < 0.0001; Fig. 7B) and more specifically, the VPL spontaneous activity decreased depending on the location (FP-location vs HP-location) (pre/post injury × location (*F*(1, 51) = 8.1; *p* = 0.0063). We applied a Tukey post-hoc test to compare the effects of SCI on the VPL FP-location and HP-location, showing that SCI provoked a decrease in spontaneous activity in the VPL HP-location (*p* = 0.0002). By contrast, no changes in spontaneous activity were evident after SCI at the VPL FP-location (*p* = 0.199; Fig. 7B).

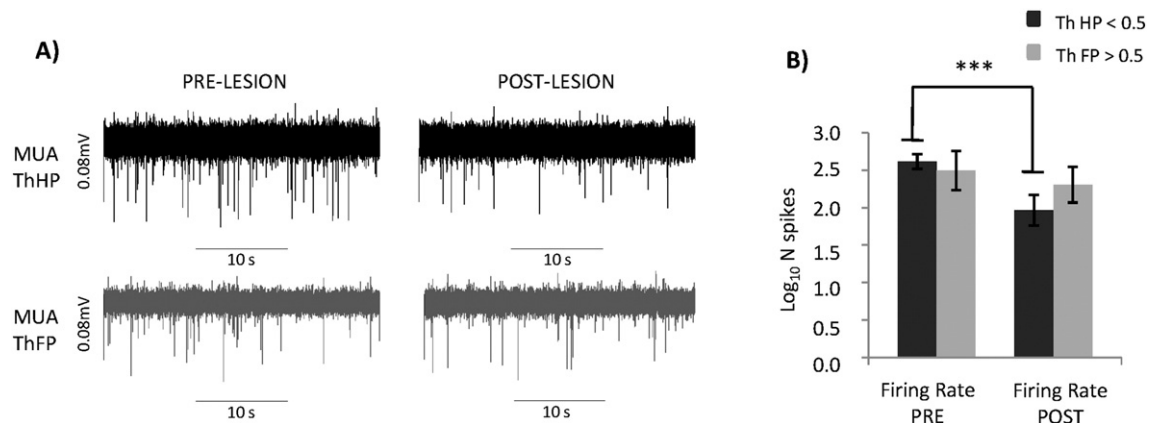


Fig. 7. A) Examples of the filtered thalamic recordings showing multi-unit activity for the VPL HP-locations and VPL FP-locations under control conditions (A1) and after SCI (A2). B) Population data comparing pre- and post-lesion firing rates at the VPL HP-locations and VPL FP-locations. The firing rate was lower after SCI at both thalamic locations (FP-location and HP-location), although this difference was only statistically significant at the HP sites: ****p* < 0.001.

3.4. The relationship between cortical states and increased thalamic evoked responses after spinal cord injury

To study the relationship between the cortical activity in the somatosensory cortex and the VPL thalamic activity, we first simultaneously recorded spontaneous activity in the somatosensory cortex and in the VPL in a set of animals (at only one VPL site, the FP-location or HP-location; $n = 36$). This approach allowed us to determine the state of the somatosensory cortex under control conditions and after SCI as well as its relation with thalamic responses under both conditions. We wanted to determine if the increasing of evoked responses at thalamic level were state-dependent or state-independent in relation to the change of cortical states that occurs immediately after SCI (Aguilar et al., 2010). In this context, we considered the cortical activity under control conditions as the reference to determine the state of the somatosensory system prior to SCI and the cortical activity immediately after SCI.

We measured the magnitude of the thalamic responses (LFP and rMUA) evoked by peripheral stimulation in two different conditions: 1) when the somatosensory cortex displayed delta activity (indicating high spontaneous activity) in control conditions, which develops into slow-wave activity immediately after SCI (Figs. 8A1 and A2) when the somatosensory cortex displayed slow-wave activity under control conditions and immediately after SCI, which did not affect the spontaneous activity (Figs. 8A2).

We used two-way ANOVA to analyze the data on the evoked responses (LFP and rMUA) in 16 animals recorded in the first state (delta activity prior to SCI and slow-wave activity post-SCI) and on the evoked responses in 20 animals that were recorded in the second state (slow-wave activity pre- and post-SCI). We took as the first repeated measures factor the time relative to the lesion (pre/post lesion) and the cortical state as the second factor (rapid pre-lesion activity to slow-wave activity post-lesion/slow-wave activity pre- and post-lesion). Our results confirmed that the LFP responses in the VPL increased after a lesion (time, $F(1, 34) = 23.25$, $p < 0.0001$; Figs. 8B, C) in a state-dependent manner (time \times state, $F(1, 34) = 4.87$, $p = 0.0341$). The post-hoc Tukey test showed that the LFP responses increased after SCI in animals that displayed delta activity before the lesion ($p = 0.0004$) but not in animals with slow-wave activity before the lesion ($p = 0.22$; Fig. 8C).

4. Discussion

In the present work we studied the immediate effects that a complete SCI at a thalamic level produces on the neuronal population of the thalamic VPL nucleus. The main results can be summarized as follows: 1) a massive recruitment of peripheral fibers by high intensity stimulation (5 mA) unmasks the overlap of peripheral inputs from both contralateral extremities (forepaw and hindpaw) onto a neuronal population in the VPL nucleus of the thalamus; 2) the thalamic VPL nucleus shows increased physiological responses to peripheral stimulation immediately after a complete thoracic SCI; these rapid functional changes have been recorded in the VPL area FP-location that is intact in our model and to the deafferented VPL area HP-location that is deafferented in our model; 3) the spontaneous neuronal activity is reduced only in the deafferented HP-location of the thalamic VPL nucleus after a thoracic SCI; and 4) the increased responses of thalamic VPL neurons is partially dependent on the changes of cortical states after SCI, moreover there is a part of the increased thalamic responses after SCI that is independent of cortical states.

Together, our results indicate that functional neuronal changes in the thalamic VPL nucleus immediately after SCI could be associated with the immediate cortical changes also provoked by such lesions (Aguilar et al., 2010).

4.1. Thalamic responses to peripheral inputs: specific location vs overlapping receptive fields

It is well known that in the somatosensory thalamus, the ventrobasal complex has a somatotopic arrangement (Emmers, 1965; Francis et al., 2008). The size of the receptive fields changes in function of the body surface and in different locations of the VPL, showing some degree of overlap from different digits of the paw and for the limb of the same extremity (Emmers, 1965; Rasmussen, 1996; Francis et al., 2008). However, there is only some evidence of overlapping receptive fields in VPL related to FP-location and HP-location (Francis et al., 2008). Thus, the first aim of our study was to quantify the overlap of peripheral inputs from contralateral extremities in the neural thalamic population of VPL in order to obtain a clearer idea of the contribution of peripheral inputs to the physiological responses in the VPL nucleus. This interesting overlap of peripheral inputs from the forelimb and hindlimb onto

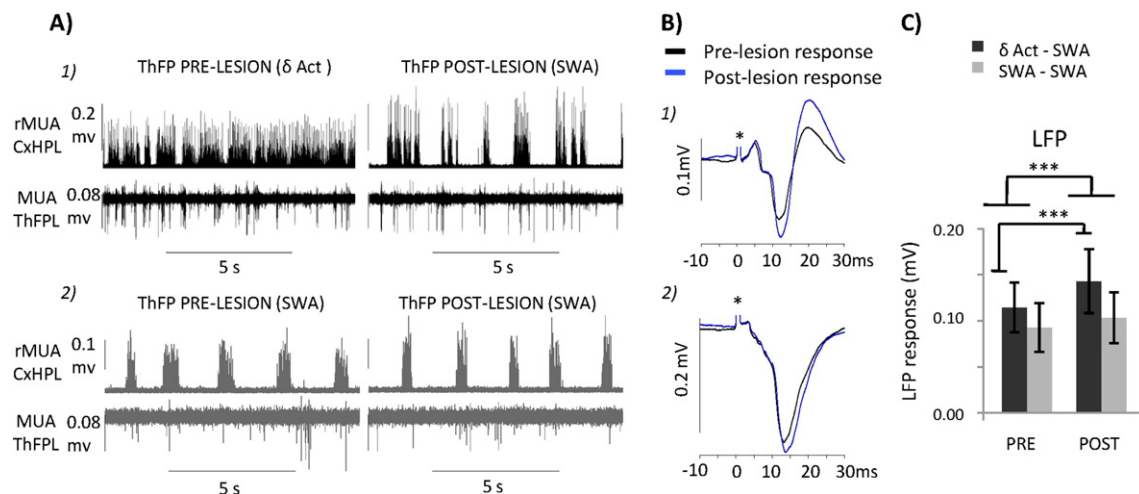


Fig. 8. A) Individual examples of simultaneous recordings in the somatosensory cortex (cortical HP-location) and thalamic VPL FP-location. A1) Example of a change in the cortical activity after SCI. A2) Example of a cortical status that does not change after SCI as it already displays slow-wave activity. B) Individual comparisons of the averaged LFP in thalamic VPL-location in response to peripheral stimulation under control conditions (black traces) and after SCI (blue lines). The upper panel in B shows an example of the recordings obtained in an experiment where a change in cortical activity occurred after SCI. The lower panel in B shows an example of the recordings obtained in an experiment where no change in cortical activity occurred after SCI. The population data of the magnitude of the response under control conditions and after SCI based on LFP. Black bars indicate the experiments where a change in cortical status occurred after SCI, and the gray bars indicate experiments where no change in cortical activity occurred after SCI. Asterisks indicate statistical differences: ** $p < 0.01$; *** $p < 0.001$.

specific thalamic populations of neurons could be important in the rapid functional reorganization after SCI as in other massive deafferentations (Rasmusson, 1996).

We created an index of spatial tuning (IST) in order to characterize the relationship between responses recorded from a given VPL neuronal population to forepaw and hindpaw stimulation. We used the IST as an index of location, to demonstrate that there is an important overlap of forepaw and hindpaw peripheral inputs over an important thalamic neuronal population.

It is remarkable that the thalamic overlapping of peripheral inputs can only be detected by maximizing the recruitment of peripheral fibers, as achieved by applying high intensity stimulation to the paws. Similar results were described for the activation of adjacent digits in a raccoon (Rasmusson, 1996). By contrast, low intensity stimulation provokes physiological responses that are mainly constrained to just one paw.

It is noteworthy in our results that we used two different analyses in order to select the neuronal activity from a reduced population (PSTH analysis) or from a wider neuronal population (rMUA analysis). We observed that the distribution of IST values for PSTH (even at high intensity stimulation) showed most of the values in the extremes of the IST values ($IST < 0.1$ and $IST > 0.9$) indicating a low probability of physiological overlapping. However, the distribution of IST values for rMUA data showed more values in the middle of the distribution ($0.1 < IST < 0.9$). It was confirmed by the percentage of multiunit recordings from FP-locations and HP-locations that showed overlap (30.9% each of them). Similarly a work in the auditory system reveals the importance of experimental methods (type of neurophysiological signals as single unit vs MUA or LFP; stimulation at threshold intensity vs suprathreshold intensity) to fully understand the classical physiological responses as well as the less classical physiological responses that physiologically characterize the system in different conditions (Guo et al., 2012). We can conclude from this comparison that the phenomenon of physiological overlapping in the thalamic VPL nucleus depends on two main factors: the amount of peripheral inputs recruited or activated and the population size that we are selecting from recordings. Our results indicate that the physiological overlapping observed in the VPL is a phenomenon that has to be studied at the level of the neuronal population and not at the level of single units or a small selected group of neurons.

Moreover, it should be noted that our IST was obtained under anesthesia, which on the one hand makes the physiological data relevant by revealing a thalamic interaction between distant peripheral inputs but on the other hand, it doesn't allow us to assess any functional role during different behavioral states. However, it has been demonstrated that cholinergic and noradrenergic activities, which arise in different behavioral states, modulate the receptive fields of thalamic neurons (Aguilar and Castro-Alamancos, 2005). Thus, it is possible that under natural behavioral states, as in the vigilant state, thalamic interaction of natural somatosensory inputs from the forepaw and hindpaw could affect a widespread population of neurons.

Based on this thalamic overlapping of peripheral inputs, we propose this to be a possible physiological mechanism for the intra-thalamic reorganization after SCI. Indeed, this mechanism of thalamic sensory overlapping could be part of the natural sensorimotor integration that occurs during walking in quadruped mammals.

4.2. Increased thalamic responses after SCI

Our experimental model involves complete transection of the spinal cord at the thoracic level, which produces a massive deafferentation of the supraspinal somatosensory structures while preserving the peripheral inputs from body regions above the lesion. For that reason, it creates an immediate imbalance in the thalamic VPL nucleus, where the region corresponding to the forelimbs (FP-location) and upper trunk maintains its principal peripheral inputs, while the region corresponding to the

hindlimbs (HP-location) and lower trunk is prevented from transmitting its principal peripheral inputs.

Our results support the idea that in the rat thalamus, the response of the neuronal population in different VPL locations is the result of a balance of contralateral peripheral inputs from body regions that mainly correspond to the forelimb and hindlimb. Somatosensory thalamocortical neurons receive principal inputs at the somatic and proximal dendrites, and secondary inputs through the distal dendrites (Ma et al., 1987). In this scenario, the neuronal population assigned to the thalamic HP-locations will experience increased responses to forepaw stimulation due to their lack of principal inputs from the hindpaw. Therefore, inputs in thalamic HP-location after thoracic SCI will be received from the forepaw peripheral inputs through distal dendrite contacts. This increased neuronal response of HP-location may be mostly related to the neuronal populations that display more peripheral overlapping, as described above.

Because the somatosensory thalamus does not have inhibitory interneurons (Barbaresi et al., 1986; Harris and Hendrickson, 1987), direct local lateral inhibition is not possible when sensory inputs from the periphery reach the thalamus. The phenomenon of lateral inhibition has been described in the somatosensory thalamus, and in the dorsal column nuclei of cats (Canedo and Aguilar, 2000; Aguilar et al., 2003; Soto et al., 2004) and rats (Nuñez and Buño, 1999), but exclusively in reference to receptive fields in the same extremity (i.e.: different digits in the same paw). However, peripheral stimulation of the forepaw in rats that produces direct evoked responses in the cuneate nucleus has not been shown to evoke direct or indirect responses in the gracilis nucleus, which only receives peripheral inputs from the lower trunk and hindlimbs. For that reason, our demonstration of thalamic overlapping of peripheral inputs from distant body regions, i.e.: overlapping in the thalamus of inputs from the contralateral dorsal column nuclei, could represent the basis for thalamic and cortical reorganization in the early moments and in the long-term after SCI.

It might be expected that after complete thoracic SCI, the neuronal population with principal inputs from the forepaw will be not affected. However, we previously described an increased cortical response in the FP-location after the same type of SCI (Aguilar et al., 2010; Humanes-Valera et al., 2013). Thus, we show here that in a similar way to the cortical effects, the responses of the thalamic neuronal population FP-locations are increased immediately after SCI. These increased responses in the neuronal population of FP-location could be due to unmasking of latent synapses, as we proposed for the cortical changes (Humanes-Valera et al., 2013). The reduction in the peripheral inputs affecting the thalamic neuronal population of HP-location could dampen the tonic inhibition through the reticular thalamic nucleus and/or through a reduction in extra-synaptic GABA. As such, the somatosensory thalamocortical neurons could increase their input resistance, making these neurons more responsive to peripheral inputs in the FP-location (Cope et al., 2005).

Finally, the increased latencies of responses after SCI indicate that the local mechanism at the thalamic level related to neuronal excitability, as neuromodulatory tone, extra-synaptic GABA tone etc., could be involved in the physiological changes that take place immediately after SCI. More precise data are needed to know the underlying cellular mechanisms of these rapid functional changes.

In general, the higher amplitude/magnitude of responses in thalamic recordings from the FP-location and HP-location of the VPL immediately after thoracic SCI could partially explain the cortical changes in the evoked responses observed under the same experimental conditions (Aguilar et al., 2010; Humanes-Valera et al., 2013).

4.3. Thalamic change of spontaneous activity immediately after SCI

Given the immediate changes in spontaneous activity we described previously in the somatosensory cortex using the same experimental approach as that employed here (Aguilar et al., 2010), we set out to study the spontaneous activity of neuronal populations in the VPL

immediately after SCI. We found that the spontaneous activity of thalamic neuronal populations was affected in a different manner by the SCI. More in detail, our results demonstrated that the neuronal population recorded at HP-locations decreased its firing rate immediately after thoracic transection of the spinal cord. However the spontaneous activity of the neuronal population at FP-locations was not affected by the SCI.

The reduction in firing rate related to the spontaneous activity of the thalamic neuronal population at the HP-location could be explained by the loss of tonic synaptic inputs of direct peripheral afferents from body areas located caudal to the spinal cord transection, as it has been demonstrated for neurons in dorsal column nuclei immediately after peripheral deafferentation (Northgrave and Rasmusson, 1996). In addition, a possible reduction in local tonic GABA inhibition could explain the reduced firing rate by setting neurons in a quiescent region of resting membrane potential (Cope et al., 2005).

In the case of the neuronal population at the FP-location, we detected a reduction in their firing rate that did not reach statistical significance. Differences in the firing rates between neuronal populations of FP-location and HP-location might reflect the different mechanism underlying a physiological imbalance that takes place between VPL locations. In this sense, a recent work demonstrated that a partial deafferentation of the VPL by thoracic spinal hemisection induces changes in the spontaneous activity of an important neuronal population that was deafferented by a lesion (Liang and Mendell, 2013). Moreover, the same work shows that an acute hemisection leaves a population of non-deafferented neurons with an unaltered spontaneous activity. In our case, this non-deafferented population could be related to the neuronal populations at VPL FP-location. Nevertheless, we use a different lesion model that could logically produce different results and effects on supraspinal structures. Thus we have demonstrated that it suffers an immediate decrease in spontaneous activity after complete SCI (Aguilar et al., 2010). Then, in our experimental model of thoracic transection of SCI the main mechanism involved in the reduction of the thalamic firing rate is the loss of principal peripheral inputs as it occurs in the HP-location. Meanwhile, the reduction in cortical activity induced by the same model of SCI does not affect the firing rate of the thalamic FP-location.

4.4. Thalamic responses depend on the cortical status after SCI

We previously assessed whether the increased responses following SCI were dependent on the cortical state of activity (Aguilar et al., 2010; Humanes-Valera et al., 2013), and for that reason in these experiments we took the cortical state as a reference of the general brain state in control conditions (before SCI) and immediately after SCI. In addition, the somatosensory cortex exerts a powerful influence on the somatosensory thalamus (Sherman and Guillery, 2006). For that reason, we assessed whether the thalamic responses were affected by the overall cortical status before and after SCI, in order to clarify potential cortical state-dependent influences. Accordingly, experiments in which a change in cortical states was detected were analyzed separately from experiments in which no such changes were observed.

Our results demonstrate that the VPL thalamic responses increase after SCI in both locations (FP-location and HP-location) by peripheral stimulation above the lesion level (in the forepaw). When the averaged LFP was used, the increased responses were detected in the experimental group that showed a change in the cortical activity. A physiological explanation is that cortical delta activity is related to a more active and depolarized thalamic neuronal population, and cortical slow-wave activity is related to a slightly-hyperpolarized state of the thalamic neuronal population. Therefore, the responses of a thalamic neuronal population to peripheral stimulation from a more hyperpolarized state, should produce an increased population depolarization that finally is recorded as an increased magnitude of LFP thalamic response.

On the other hand, our results demonstrated that there are increased thalamic responses when rMUA was used and the cortical state of slow-wave activity was not affected between pre and post SCI. In that case the result is similar to the cortical state-independent changes that we have described previously (Humanes-Valera et al., 2013). This effect can be due to a reduction of local inhibition (as it has been explained in Discussion 4.3) or a reduction on neuromodulatory tone of acetylcholine. Both possible mechanisms lead to an increased input resistance that could induce increased responses to the peripheral inputs.

Then, the responses of neuronal populations in the thalamus behave similarly to a cortical neuronal population after SCI, with a state-dependent mechanism and a state-independent mechanism. In this context, the present results could at least partially underlie our previous results regarding enhanced cortical responses after SCI (Aguilar et al., 2010; Humanes-Valera et al., 2013). Thus, we propose that early changes in the thalamic neuronal VPL populations after SCI contribute to the cortical changes observed immediately after SCI.

5. Conclusions

The problem of thalamic changes after SCI has been studied using different models of spinal lesion, different time scales, different stimulation protocols, etc. Moreover previous works have been focused on different anatomical and physiological targets, as spontaneous activity, the connections between neuronal population inside the thalamus, relation with different brain structures and the behavioral data in reference to pain (Gerke et al., 2003; Gustin et al., 2009; Wrigley et al., 2009; Seminowicz et al., 2012; Liang and Mendell, 2013). In this context our results point to a joint role of the thalamic and cortical activity that develops immediately after SCI, and that could be in the origin of physiological alterations of the somatosensory system after SCI. The thalamic and cortical changes due to a SCI must therefore be studied together in order to understand the complexity of brain reorganization provoked by such lesions in the somatosensory system.

In summary, our results demonstrate that there is an important overlapping of peripheral inputs in the thalamic VPL nucleus that could represent the basis for the functional changes observed immediately after complete transection of the spinal cord at the thoracic level. In addition, SCI triggers immediate functional changes in the somatosensory thalamus (VPL), which could underlie the previously described cortical changes after the same type of SCI. Together, the functional thalamic and cortical changes described here and in our previous study could be related to the origin of long-term pathological problems after SCI.

Acknowledgements

This work was supported by Fondo de Investigación Sanitaria del Instituto de Salud Carlos III, co-funded by FEDER (PI08/1810 and PI11/02451) and by Ministerio de Economía y Competitividad of Government of Spain, co-funded by FEDER (SAF2012/40109).

References

- Aguilar, J.R., Castro-Alamancos, 2005. Spatiotemporal gating of sensory inputs in thalamus during quiescent and activates states. *J. Neurosci.* 25 (47), 10990–11002. <http://dx.doi.org/10.1523/JNEUROSCI.3229-05.2005>.
- Aguilar, J., Humanes-Valera, D., Alonso-Calviño, E., Yague, J.G., Moxon, K.A., Oliviero, A., Foffani, G., 2010. Spinal cord injury immediately changes the state of the brain. *J. Neurosci.* 30 (22), 7528–7537. <http://dx.doi.org/10.1523/JNEUROSCI.0379-10.2010>.
- Aguilar, J., Rivadulla, C., Soto, C., Canedo, A., 2003. New corticocuneate cellular mechanism underlying the modulation of cutaneous ascending transmission in anesthetized cats. *J. Neurophysiol.* 89 (6), 3328–3339. <http://dx.doi.org/10.1152/jn.01085.2002>.
- Barbaresti, P., Spreafico, R., Frasconi, C., Rustioni, A., 1986. GABAergic neurons are present in the dorsal column nuclei but not in the ventroposterior complex of rats. *Brain Res.* 382, 305–326. [http://dx.doi.org/10.1016/0006-8993\(86\)91340-5](http://dx.doi.org/10.1016/0006-8993(86)91340-5).
- Canedo, Aguilar, J., 2000. Spatial and cortical influences exerted on cuneothalamic and thalamocortical neurons of the cat. *Eur. J. Neurosci.* 12, 2515–2533. <http://dx.doi.org/10.1046/j.1460-9568.2000.00107.x>.

- Chapin, J.K., Lin, C.S., 1984. Mapping the body representation in the SI cortex of anesthetized and awake rats. *J. Comp. Neurol.* 229 (2), 199–213.
- Cope, D.W., Hughes, S.W., Crunelli, V., 2005. GABA_A receptor-mediated tonic inhibition in thalamic neurons. *J. Neurosci.* 25 (50), 11553–11563. <http://dx.doi.org/10.1523/JNEUROSCI.3362-05.2005>.
- Crunelli, V., Hughes, S.W., 2010. The slow (<1 Hz) rhythm of non-REM sleep: a dialogue between three cardinal oscillators. *Nat. Neurosci.* 13 (1), 9–17.
- Emmers, R., 1965. Organization of the first and the second somesthetic regions (SI and SII) in the rat thalamus. *J. Comp. Neurol.* 124, 215–227.
- Endo, T., Spenger, C., Tominaga, T., Brené, S., Olson, L., 2007. Cortical sensory map rearrangement after spinal cord injury: fMRI responses linked to Nogo signalling. *Brain* 130 (11), 2951–2961. <http://dx.doi.org/10.1093/brain/awm237>.
- Francis, J.T., Xu, S., Chapin, J.K., 2008. Proprioceptive and cutaneous representation in the rat ventral posterolateral thalamus. *J. Neurophysiol.* 99, 2291–2304. <http://dx.doi.org/10.1152/jn.01206.2007>.
- Gerke, M.B., Duggan, A.W., Xu, L., Siddall, P.J., 2003. Thalamic neuronal activity in rats with mechanical allodynia following contusive spinal cord injury. *Neuroscience* 117 (3), 715–722. [http://dx.doi.org/10.1016/S0306-4522\(02\)00961-2](http://dx.doi.org/10.1016/S0306-4522(02)00961-2).
- Ghosh, A., Haiss, F., Sydekum, E., Schneider, R., Gullo, M., Wyss, M.T., Mueggler, T., Baltes, C., Rudin, M., Weber, B., Schwab, M.E., 2010. Rewiring of hindlimb corticospinal neurons after spinal cord injury. *Nat. Neurosci.* 13 (1), 97–104. <http://dx.doi.org/10.1038/nn.2448>.
- Ghosh, A., Peduzzi, S., Snyder, M., Schneider, R., Starkey, M., Schwab, M.E., 2012. Heterogeneous spine loss in layer 5 cortical neurons after spinal cord injury. *Cereb. Cortex* 22 (6), 1309–1317. <http://dx.doi.org/10.1093/cercor/bhr191>.
- Guo, W., Chambers, A.R., Darrow, K.N., 2012. Robustness of cortical topography across fields, laminae, anesthetic states, and neurophysiological signal types. *J. Neurosci.* 32 (27), 9159–9172. <http://dx.doi.org/10.1523/JNEUROSCI.0065-12.2012>.
- Gustin, S.M., Wrigley, P.J., Siddall, P.J., Henderson, L.A., 2009. Brain anatomy changes associated with persistent neuropathic pain following spinal cord injury. *Cereb. Cortex* 20 (6), 1409–1419. <http://dx.doi.org/10.1093/cercor/bhp205>.
- Hains, B.C., Waxman, S.G., 2007. Sodium channel expression and the molecular pathophysiology of pain after SCI. *Prog. Brain Res.* 161, 195–203.
- Hains, B.C., Saab, C.Y., Waxman, S.G., 2005. Changes in electrophysiological properties and sodium channel Nav 1.3 expression in thalamic neurons after spinal cord injury. *Brain* 128 (Pt10), 2359–2371.
- Harris, R.M., Hendrickson, A.E., 1987. Local circuit neurons in the rat ventrobasal thalamus — a GABA immunocytochemical study. *Neuroscience* 21 (1), 229–236.
- Humanes-Valera, D., Aguilar, J., Foffani, G., 2013. Reorganization of the intact somatosensory cortex immediately after spinal cord injury. *PLoS ONE* 8 (7), e69655. <http://dx.doi.org/10.1371/journal.pone.0069655>.
- Jain, N., Florence, L.S., Qi, H.X., Kaas, J.H., 2000. Growth of new brainstem connections in adult monkeys with massive sensory loss. *Proc. Acad. Nat. Sci.* 97 (10), 5546–5550. <http://dx.doi.org/10.1073/pnas.090572597>.
- Jain, N., Qi, H.X., Collins, C.E., Kaas, J.H., 2008. Large scale of reorganization in the somatosensory cortex and thalamus after sensory loss in macaque monkeys. *J. Neurosci.* 28, 11042–11060. <http://dx.doi.org/10.1523/JNEUROSCI.2334-08.2008>.
- Liang, L., Mendell, L.M., 2013. Bilateral transient changes in thalamic nucleus ventroposterior lateralis after thoracic hemisection in the rat. *J. Neurophysiol.* 110 (4), 942–951. <http://dx.doi.org/10.1152/jn.00998.2012>.
- Liao, C.C., DiCarlo, G.E., Gharbawie, O.A., Qi, H.X., Kaas, J.H., 2015. Spinal cord neurons inputs to the cuneate nucleus that partially survive dorsal column lesion: a pathway that could contribute to recover after SCI. *J. Comp. Neurol.* <http://dx.doi.org/10.1002/cne.23783>. Epub ahead of print.
- Lilja, J., Endo, T., Hofstetter, C., Westman, E., Young, J., Olson, L., Spenger, C., 2006. Blood oxygenation level-dependent visualization of synaptic relay station of sensory pathways along the neuraxis in response to graded sensory stimulation of a limb. *J. Neurosci.* 26 (23), 6330–6336. <http://dx.doi.org/10.1523/JNEUROSCI.0626-06.2006>.
- Ma, W., Peschanski, M., Ralston 3rd, H.J., 1987. The differential synaptic organization of the spinal and lemniscal projections to the ventrobasal complex of the rat thalamus. Evidence for convergence of the two systems upon single thalamic neurons. *Neuroscience* 22 (3), 925–934.
- Moxon, K.A., Oliviero, A., Aguilar, A., Foffani, G., 2014. Cortical reorganization after spinal cord injury: always for good? *Neuroscience* 283, 78–94. <http://dx.doi.org/10.1016/j.neuroscience.2014.06.056>.
- Northgrave, S.A., Rasmusson, D.D., 1996. The immediate effects of peripheral deafferentation on neurons of the cuneate nucleus in raccoons. *Somatosens. Mot. Res.* 13 (2), 103–113.
- Núñez, A., Buño, W., 1999. In vitro electrophysiological properties of rat dorsal column nuclei neurons. *Eur. J. Neurosci.* 11 (6), 1865–1876. <http://dx.doi.org/10.1046/j.1460-9568.1999.00605.x>.
- Onifer, S.M., Nunn, C.D., Decker, J.A., Payne, B.N., Wagoner, M.R., Puckett, A.H., Massey, J.M., Armstrong, J., Kaddumi, E.G., Fentress, K.G., Wells, M.J., West, R.M., Calloway, C.C., Schnell, J.T., Whitaker, C.M., Burke, D.A., Hubscher, C.H., 2007. Loss and spontaneous recovery of forelimb evoked potentials in both the adult rat cuneate nucleus and somatosensory cortex following contusive cervical spinal cord injury. *Exp. Neurol.* 207 (2), 238–247.
- Paxinos, G., Watson, C., 2007. *The rat brain in stereotaxic coordinates*. Academic Press, Amsterdam.
- Qi, H.X., Chen, L.M., Kaas, J.H., 2011. Reorganization of somatosensory cortical areas 3b and 1 after unilateral section of dorsal columns of the spinal cord in squirrel monkeys. *J. Neurosci.* 31 (38), 13662–13675. <http://dx.doi.org/10.1523/JNEUROSCI.2366-11.2011>.
- Rasmusson, D.D., 1996. Changes in the response properties of neurons in the ventroposterior lateral thalamic nucleus of the raccoon after peripheral deafferentation. *J. Neurophysiol.* 75 (6), 2441–2450.
- Seminowicz, D.A., Jiang, L., Ji, Y., Xu, S., Gullapalli, R.P., Masri, R., 2012. Thalamocortical asynchrony in conditions of spinal cord injury pain in rats. *J. Neurosci.* 32 (45), 15843–15848. <http://dx.doi.org/10.1523/JNEUROSCI.2927-12.2012>.
- Sherman, S.M., Guillery, R.W., 2006. *Exploring the Thalamus and its Role in Cortical Function*. The MIT Press, Cambridge, Massachusetts, USA.
- Soto, C., Aguilar, J., Martín-Cora, F., Rivadulla, C., Canedo, A., 2004. Intracuneate mechanisms underlying primary afferent cutaneous processing in anesthetized cats. *Eur. J. Neurosci.* 19 (11), 3006–3016. <http://dx.doi.org/10.1111/j.0953-816X.2004.03432.x>.
- Waxman, S.G., Hains, B.C., 2006. Fire and phantoms after spinal cord injury: Na⁺ channels and central pain. *Trends Neurosci.* 29 (4), 207–215.
- Wrigley, P.J., Press, S.R., Gustin, S.M., Macefield, V.G., Gandevia, S.C., Cousins, M.J., Middleton, J.W., Henderson, L.A., Siddall, P.J., 2009. Neuropathic pain and primary somatosensory cortex reorganization following spinal cord injury. *Pain* 141 (1–2), 52–59. <http://dx.doi.org/10.1016/j.pain.2008.10.007>.
- Yague, J.G., Foffani, G., Aguilar, J., 2011. Cortical hyperexcitability in response to preserved spinothalamic inputs immediately after spinal cord hemisection. *Exp. Neurol.* 227, 252–263. <http://dx.doi.org/10.1016/j.expneurol.2010.11.011>.
- Yague, J.G., Humanes-Valera, D., Foffani, G., Aguilar, J., 2014. Functional reorganization of the forepaw cortical representation immediately after thoracic spinal cord hemisection in rats. *Exp. Neurol.* 257, 19–24. <http://dx.doi.org/10.1016/j.expneurol.2014.03.015>.