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Research Paper

Slow-wave activity homeostasis in the somatosensory cortex after spinal cord injury

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ABSTRACT

The cortical reorganization after spinal cord injury (SCI) involves a series of physiological changes that drive the expansion of the intact cortical area to the deafferented cortical area. These changes have always been studied under a stimulus-response paradigm, which demonstrates that the deafferented cortex becomes more responsive to stimulation of body regions above the level of the lesion. However, less is known about how permanent large-scale deafferentation affects spontaneous activity in the somatosensory cortex, an important physiological feature related to the processing of peripheral inputs and perception. Here we studied the spontaneous activity at two sites of the somatosensory cortex, corresponding to forepaw and hindpaw, and at three different time points after SCI: acute SCI, one week post-SCI and chronic SCI (1–3 months after injury). Electrophysiological recordings from anesthetized rats were obtained in conditions of slow-wave activity in order to compare features of the neural populations in periods of cortical up-states. Our data demonstrate that acute SCI reduces the excitability of cortical neurons during up-states in both the forepaw and the hindpaw cortex. One week after SCI, the properties of cortical neurons were similar to those under control conditions, indicating a homeostatic plasticity. Finally, chronic SCI increased neural activity during up-states, while reduced up-state frequency in the cortex. We conclude that SCI induces different homeostatic changes in cortical slow-wave depending on the time after lesion. This temporal evolution of spontaneous activity could help better understand the cortical plasticity associated with acute or chronic SCI.

1. Introduction

Spinal cord injury (SCI) is a central nervous system (CNS) lesion that produces massive deafferentation of brain structures like the motor cortex (MCx) and primary somatosensory cortex (SSCx). Under natural conditions, the SSCx receives information from the skin, muscles and joints, and all this information travels through ascending somatosensory pathways in the spinal cord (dorsal columns and spinothalamic tract) to reach the SSCx. This constant source of peripheral input helps maintain and modulate the spontaneous activity of cortical populations of neurons (Steriade, 2000; Lemieux et al., 2014). In this context, deprivation of peripheral sensory inputs provokes an immediate reduction in the spontaneous activity in the primary sensory cortices, such as the visual (Hengen et al., 2013; Keck et al., 2013) and the auditory system (Teichert et al., 2017). Importantly, both these sensory systems recover their spontaneous cortical activity within a short period of 2–3 days, a phenomenon identified as homeostatic plasticity (Hengen et al., 2013; Keck et al., 2013; Teichert et al., 2017). Following a similar rationale, recent data from our laboratory demonstrate that SCI decreases the spontaneous activity in both thalamus and SSCx (Alonso-Calviño et al., 2016; Humanes-Valera et al., 2017), changing the brain state to a slow-wave activity (Aguilar et al., 2010). Slow-wave activity has been related to neuronal plasticity in the cerebral cortex (Wilhelm et al., 2014; Timofeev and Chauvette, 2017). However, it remains unclear whether slow-wave activity contributes to the long-term homeostatic changes that take place in the SSCx after SCI (days-weeks-months).

Accordingly, in this study we assessed how the slow-wave activity of neuronal populations in the SSCx evolves from acute to chronic stages after SCI. As such, we obtained electrophysiological recordings from two different sites in the rat SSCx: one corresponding to the forepaw somatosensory cortex (CxFP) that will preserve its peripheral inputs after SCI; and a second location corresponding to the hindpaw

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somatosensory cortex (CxHP) that is affected by complete deafferentation of its peripheral inputs in both the acute and chronic states of SCI. All the animals were submitted to a state of cortical slow-wave activity by controlling the level of anesthesia (Humanes-Valera et al., 2013). In this state of slow-wave activity, neurons are exclusively active during the up-state and therefore, data were obtained by quantifying the physiological features of the up-state. As a result, the spontaneous activity of neuronal populations in the SSCx during slow-wave activity was reduced in the up-states in the acute period after SCI, becoming balanced and stronger in more chronic stages of SCI. Altogether, our data suggest that neuronal populations in the SSCx undergo homeostatic changes related to the transition from acute to chronic SCI.

2. Materials and methods

Experiments were carried out in accordance with the International Council for Laboratory Animal Science and the European Union 2010/63/EU guidelines. The experimental protocol was approved by Ethical Committee for Animal Research at the Hospital Nacional de Parapléjicos (Toledo, Spain). A total of 19 male Wistar rats (300-450 g) were used for the experiments on acute SCI to compare the pre-lesion state with that immediately after the lesion (Fig. 1A and C). Animals submitted to acute experiments were divided in two subsets as follow: first subset of 9 animals was submitted to a single pre-lesion period followed by a spinal cord lesion (Fig. 1C upper trace); in second subset of 10 animals the recording period prior lesion was two times the one performed in first subset, for this reason were named pre-lesion-1 and pre-lesion-2, finally those animals were submitted to spinal cord injury (Fig. 1C lower trace). Note that the two consecutive pre-lesion recording periods in the second subset were intended to discard possible effects of the protocol time duration over the cortical activity. Data from the second subset was also considered as sham group and only pre-lesion-1 and pre-lesion-2 periods were compared in order to confirm that experiment duration wasn't responsible for changes in spontaneous activity. The data from this experimental protocol were included along with that from the group of injured animals, reaching a total of 19 rats studied.

A second set of experiments was carried out on 40 male Wistar rats (300-450 g) to study the effects of chronic SCI (Fig. 1D). Animals with chronic SCI were divided into 3 groups: 1) 11 animals were used as controls (control); 2) 10 animals were studied one week after SCI; and 3) 19 animals were studied as chronic SCI (1 to 3 months after injury: Fig. 1D). Prior to SCI, two animals were housed in standard cages, with ad libitum access to food and water, and maintained at 23 °C on a 12 h light/dark cycle. After the SCI lesion, animals were housed individually in a non-enhanced environment and handled for manual voiding of the bladder. This experimental group was used previously in a different chronic study to examine cortical evoked responses to peripheral stimulation (Humanes-Valera et al., 2017).

The general experimental approach in acute experiments (anesthesia, surgery and electrophysiological recordings) was similar to that employed in our previous studies (Humanes-Valera et al., 2013, 2017; Alonso-Calviño et al., 2016), and any additional details will be indicated below. All the surgical interventions for chronic SCI and the protocols for the animal's posterior care were carried out at the animal facility of the Hospital Nacional de Parapléjicos (SESCAM, Toledo. Spain). In addition, the veterinary services at our institution helped in the post-surgical care of the animals.

2.1. Electrophysiological recordings

Animals were anesthetized with urethane (1.5 g/kg i.p.) and their body temperature was kept constant at 36.5 °C using a heated pad (Cibertec SL, Madrid, Spain). The animals were placed in a stereotaxic frame (SR-6 Narishige Scientific Instruments, Tokyo, Japan) and lidocaine 2% was applied over the area of the incision. The skull was exposed and a craniotomy was performed on the right side of the cranium over the SSCx at coordinates: AP 2 to -3; ML 1 to 5 (Paxinos and Watson, 2007). The cisterna magna was opened to decrease intracranial pressure and improve the stability of the recordings. Small incisions in

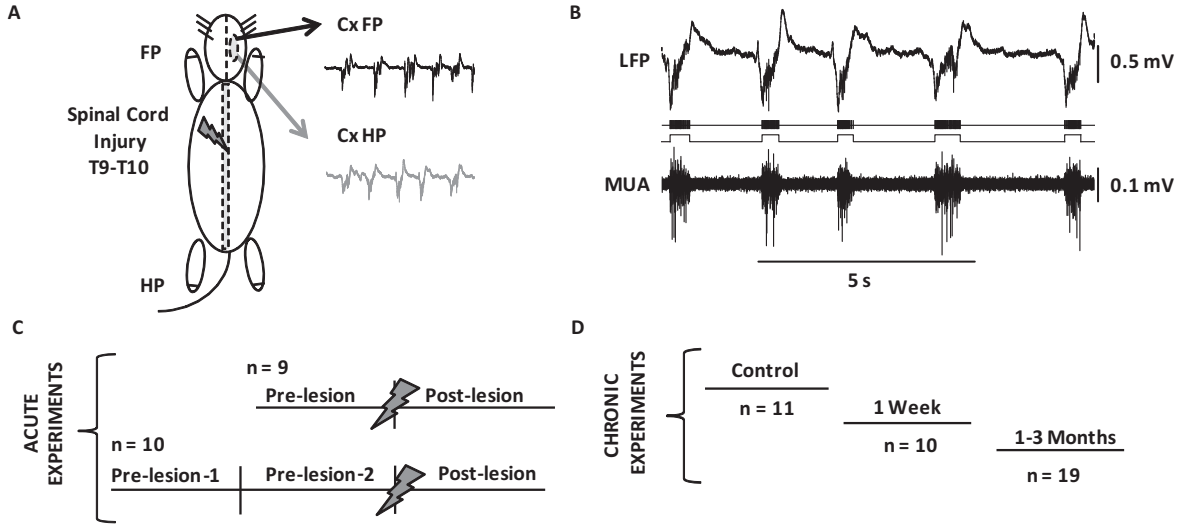

Fig. 1. Experimental design. A) Schematic representation of the experimental approach, indicating the simultaneous electrophysiological recordings from the somatosensory cortex corresponding to the forepaw (CxFP) and hindpaw (CxHP) representations, the spinal cord lesion was performed at thoracic level (T9-T10) as indicated (grey arrow). B) Example of a raw electrophysiological recording from the SSCx showing the slow-wave activity (LFP, upper trace), and the same signal filtered to reflect the activity of neuronal population in spikes (MUA, lower trace). Quantification of the spikes passing the voltage threshold (events channel in the middle) and the detection of the duration of the up-states (two levels channel between recordings). Schematic of the animal groups used in this study for (C) acute and chronic (D) experiments (see Materials and Methods). Chronic experiments were performed on different groups of animals: intact animals, 1 week, 1–3 months post SCI. Acute experiments were performed on two different groups. First group, 9 animals to study the pre-lesion vs post-lesion condition; Second group, 10 animals were recorded twice over the pre-lesion time, the first of these were considered pre-lesion-1 and the second, pre-lesion-2. This comparison was made to avoid any effects of time in the recordings. Finally, the same 10 animals were submitted to SCI and this group was assessed along with the previous 9 animals. D) Chronic experiments were performed on different groups of animals: intact animals, 1 week, 1 month, and 3 months after SCI.

the dura mater were made to allow the recording electrodes to be lowered into the cerebral cortex. Two tungsten electrodes were lowered into layer V of the SSCx at the coordinates described in Chapin and Lin, 1984, one in the forepaw area (AP 0.5 mm; ML 4 mm; D 1.1–1.6 mm) and the other in the hindpaw area (AP -1 mm; ML 2.5 mm; D 1.1–1.6 mm). The ground and reference electrodes were placed in the parietal muscular tissue, on opposite sides. In the acute experimental SCI group, the electrode locations in the cortex were optimized by assessing the responses to tactile stimulation of the rat's forepaw and hindpaw with a cotton swab.

Extracellular recordings were obtained using tungsten electrodes (TM31C40KT, 4-MΩ impedance at 1 kHz or TM31A50KT, 5-MΩ impedance at 1 kHz: World Precision Instruments Inc., Sarasota FL, USA). All recordings were pre-amplified in the DC mode, low pass filtered (< 3 kHz) and amplified using a modular system (Neurolog, Digitimer Ltd). Analogue signals were converted into digital data at a 20 kHz sampling rate and with 16–bit quantization via a CED power 1401 apparatus controlled by Spike2 software (v6, Cambridge Electronics Design, Cambridge, UK). The data was analyzed using Spike2 software.

Once the recording electrodes were situated at the correct location, spontaneous cortical activity was settled to slow-wave activity (< 1 Hz) in all animals, establishing a baseline to make consistent comparisons between pre- and post-lesion in the different experimental groups (Fig. 1A-B; see Humanes-Valera et al., 2013, 2017).

2.2. Data analysis

We analyzed similar periods of spontaneous activity in each group to compare acute (900 s pre-lesion and post-lesion) and chronic SCI (300 s for the control state; 1 week after SCI and 1–3 months after SCI). The raw signal was used to evaluate the slow-wave activity and to perform a Fast Fourier Transform analysis (FFT) that confirmed that the main frequency of the recordings produced peak values below 1 Hz. In addition, we extracted the multiunit activity (MUA) by band-pass filtering the raw signals (300–3000 Hz), the filtered signal showing a typical alternation of up- and down-states with a frequency < 1 Hz, a feature of the neural population that determines slow-wave activity (Fig. 1B).

The filtered signal was used to extract action potentials corresponding to the cortical neural population close to the electrode. Action potentials were selected using a voltage threshold placed at 5 times the standard deviation of the background noise for each recording (Rey et al., 2015). Action potentials crossing the voltage threshold were considered as events, and were located in a new data channel that was used to quantify spontaneous activity of neuronal population in different experimental conditions. Cortical neurons exhibit two differentiated states during slow-wave activity: up-states, periods corresponding to synchronized depolarization of the majority of the neural population; and down-states, periods where the large majority of cortical neurons are hyperpolarized and silent (Fig. 2A). Each up-state was identified as a burst of neural activity using the semi-automatic built-in script Bursts.s2s running on Spike2 v7.12 that allows the characterization of different parameters such as: 1) the frequency of up-states during slow-wave activity; 2) the total number of spikes per up-state; 3) the frequency of spikes during up-states (firing rate/Up); 4) the duration of the up-states (Up-duration). In addition the mean firing rate of neurons was initially calculated for the total time of recordings. All these parameters will be analyzed in order to compare control conditions with acute and chronic SCI.

2.3. Statistical analysis

Statistical analyses were performed using Statistica.Ink software (Statsoft Ibérica, Lisboa, Portugal). Acute experiments were evaluated using a Two-Way Analysis of Variance (ANOVA), with TIME as a repeated-measures factor (two levels: PRE- and POST-lesion) and CORTEX as an independent-measures factor (two levels: FP and HP). Sham experiments were evaluated through a Three-Way Analysis of Variance (ANOVA) with TIME as a repeated-measures factor (two levels: PRE- and POST-lesion) and CORTEX (two levels: FP and HP) and LESION (two levels: Real and Sham) as two independent-measures factors. Chronic Experiments were evaluated with a Two-Way Analysis of Variance (ANOVA), with CORTEX (two levels: FP and HP) and TIME (three levels: pre-lesion, 1 week post-lesion and 1–3 months post-lesion) as two independent-measures factors.

A Tukey Honest Significant Difference Test was used for all post-hoc comparisons and additional comparisons between the means were performed with a *t*-test. All results were considered significant at $p < 0.05$.

Confidence intervals in the figures were computed based on the current error term from the ANOVA table. Marginal means and standard deviation of all results are provided in Tables 1 and 2.

3. Results

3.1. Immediate effects of SCI on neuronal dynamics during up-states

We initially quantified the activity of the cortical neuronal populations through the mean firing rate, i.e.: the spikes per second (spikes/s) obtained from a fixed recording time pre- and post-lesion (900 s). A decrease in the mean firing rate was evident immediately post-lesion when compared to the pre-lesion group (2-way repeated measures ANOVA, TIME $F(1,36) = 23.7$; $p = 0.00002$: Fig. 2A, B). This effect was not specific to either the intact CxFP or the deafferented CxHP (TIME X CORTEX $F(1,36) = 0.07$; $p = 0.79$: Fig. 2B).

Slow-wave activity characterizes a cortical state in which neurons show oscillatory activity (< 1 Hz) between two differentiated states, up-states (or active periods) and down-states (or silent periods). During slow-wave activity cortical neurons only show discharges during up-states, while neuronal activity is absent during down-states. Thus, there are two possible explanations for the reduction in the mean firing rate: a reduction in the frequency of the up-states and/or a reduction in the number of spikes per up-state. When the frequency of up-states was quantified, no differences were found neither between the pre-lesion and the immediate post-lesion state (TIME $F(1,36) = 1.14$; $p = 0.29$; TIME X CORTEX $F(1,36) = 2.6$; $p = 0.12$; Fig. 2A, C) nor between cortices (CORTEX $F(1,36) = 2.2$; $p = 0.14$). However, a decrease was evident in the number of spikes per up-state at both cortical locations, the intact CxFP and the deafferented CxHP (TIME $F(1,36) = 29.3$; $p = 0.000004$; TIME X CORTEX $F(1,36) = 0.3$; $p = 0.56$; Fig. 2D).

The reduction in the number of spikes in the up-states could be a consequence of a reduced up firing rate (spikes/s), which in association with slow-wave activity represents the firing rate/up-state or a decrease in the duration of the up-state. By quantifying these features, we found a reduced up firing rate immediately post-lesion in both CxHP and CxFP (TIME $F(1,36) = 9.7$; $p = 0.003$; TIME X CORTEX $F(1,36) = 1.6$; $p = 0.22$) without differences between cortical locations (CORTEX $F(1,36) = 2.8$; $p = 0.1$). Similarly, a shorter duration of the up-state immediately post-lesion was observed (TIME $F(1,36) = 21,4$; $p = 0.00005$; TIME X CORTEX $F(1,36) = 1.4$; $p = 0.25$; Fig. 2E and F) confirming that SCI immediately reduces the excitability of neuron populations in the SSCx. It is worth noting that CxFP before SCI presented higher activity than CxHP in terms of mean firing rate ($F(1,36) = 6.6$; $p = 0.015$), spikes per up ($F(1,36) = 6.1$; $p = 0.02$) and up duration ($F(1,36) = 16.5$; $p = 0.0002$), which correlates with anatomical and physiological findings showing that CxFP is larger than CxHP. (Goloshevsky et al., 2011; Morales-Botello et al., 2012).

No statistical differences were found between both conditions of pre-lesion (group $n = 9$) and pre-lesion-2 (group $n = 10$) (unpaired *t*-test: mean firing rate $p > 0.56$; up-frequency $p > 0.08$; spikes per up > 0.70; up firing rate $p > 0.77$; up duration $p > 0.48$), which allowed us to use animals from pre-lesion (n = 9) and pre-lesion-2

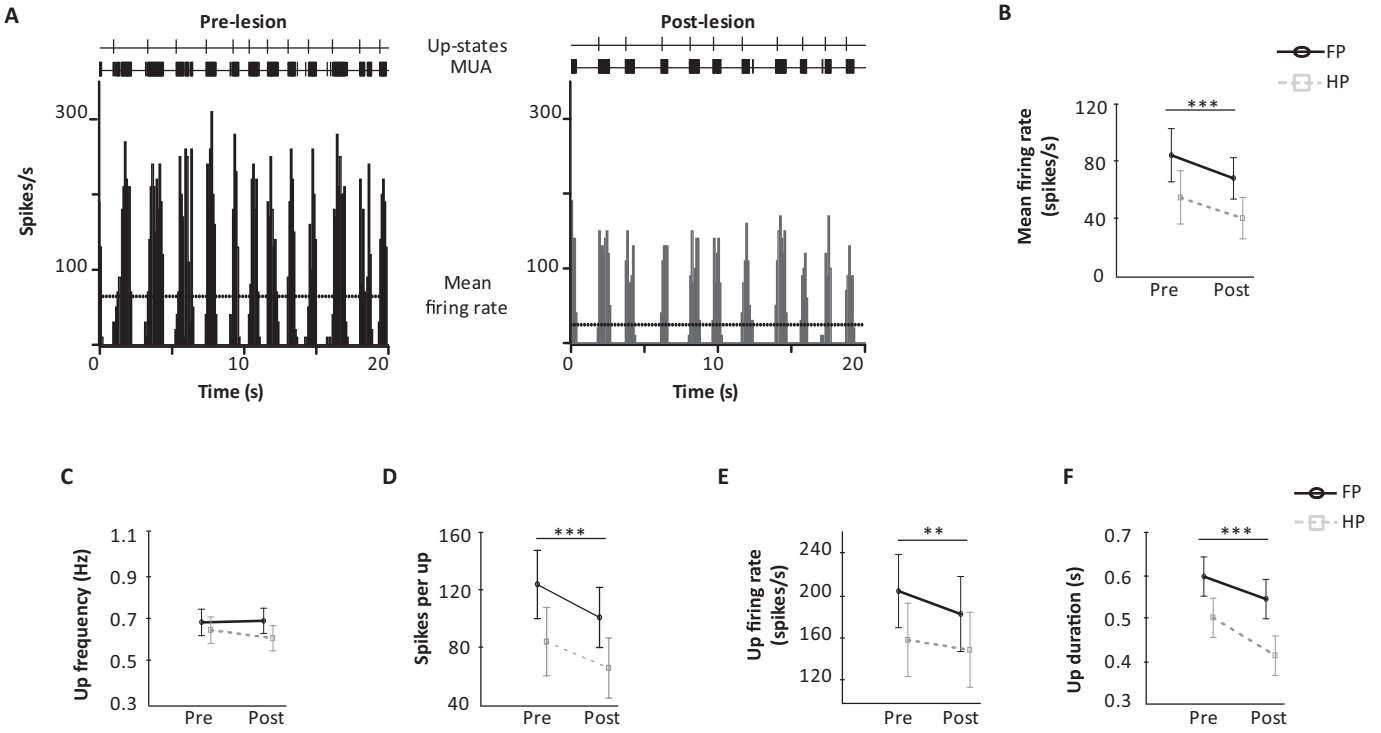

Fig. 2. Immediate effects of SCI on cortical neurons. A) Representative example of the MUA analysis for the control (pre-lesion) state and acute (post-lesion) SCI. *Upper trace* (Up-states), point marks indicating the onset of each up-state. *Middle trace* (MUA), marks corresponding to every action potential extracted from filtered recordings (note how action potentials are grouped inside up-states). *Bottom graph*, example histograms showing the mean frequency from 20 s MUA recording). The mean firing rate was calculated from the entire time of recordings, as shown by the dotted lines on the histograms. B) Analysis of the population data regarding the mean firing rate showing a general decrease in cortical activity: CxFP (dark line); CxHP (grey-dotted line). C) Population data of up-states frequency recorded in the CxFP (dark line) and CxHP (grey-dotted line). No changes were observed between pre- and post-lesion. D) Population data of the averaged spikes per up-state, the data confirm a general decrease in MUA in both CxFP and CxHP. E) Population data of the firing rate per up-state showed a decrease at both the cortical locations F) Quantification of the duration of the up-states shows a decrease after acute SCI. Analysis of the acute experiments representing the means, and the error bars are the mean ± 95% Conf. Interval: $^{**}p < 0.01$; $^{***}p < 0.001$.

(n = 10) as pre-lesion for acute comparisons ($n = 19$) (see Fig. 1C).

So far, our data shows that SCI immediately decreases spontaneous activity in SSCx. To verify if these results were not induced by the long time period of recording activity, we performed the same experimental protocol in the pre-lesion-1 and pre-lesion-2 groups as described in Materials and Methods. Comparisons showed that the mean firing rate did not change between the pre-lesion-1 and pre-lesion-2 periods, yet it did decrease post-SCI (3-way repeated measures ANOVA, Interaction TIME X LESION $F(1,34) = 9.1$; $p = 0.0047$; Tukey for pre-lesion-1/pre-lesion-2, $p = 0.99$; Tukey for pre-lesion-2/post-lesion, $p = 0.002$: Fig. 3A). This effect was not specific to either cortex locations (CxFP and CxHP) (TIME X CORTEX X LESION $F(1,34) = 1.3$; $p = 0.25$). Importantly, no differences in the frequency of up-states were observed (interaction TIME X LESION $F(1,34) = 0.03$; $p = 0.85$; TIME X CORTEX X LESION $F(1,34) = 2.3$; $p = 0.14$: Fig. 3B). Likewise, no differences were evident in the other features of the up-states between the 10 pre-lesion-1 and pre-lesion-2 animals, whereas the differences found previously between the pre- and post-lesion states were confirmed in these animals: 1) spikes per up-state (TIME X LESION $F(1,34) = 14.2$; $p = 0.0006$; Tukey pre-lesion-1/pre-lesion-2, $p = 0.92$; Tukey pre-lesion-2/post-lesion, $p = 0.0004$: Fig. 3C); This effect was not specific to either cortex (TIME X CORTEX X LESION $F(1,34) = 0.3$; $p = 0.57$). 2) Up-state firing rate (TIME X LESION $F(1,34) = 6.6$; $p = 0.014$; Tukey pre-lesion-1/pre-lesion-2: $p = 0.91$; Tukey pre-lesion-2/post-lesion: $p = 0.029$: Fig. 3D); This effect was not specific to either cortex (TIME X CORTEX X LESION $F(1,34) = 0.02$; $p = 0.89$) 3) Up-state duration (TIME X LESION $F(1,34) = 10.3$; $p = 0.03$; Tukey pre-lesion-1/pre-lesion-2, p = 0.99; Tukey pre-lesion-2/post-lesion, $p = 0.008$: Fig. 3E). This effect was not specific to either cortex (TIME X CORTEX X LESION $F(1,34) = 1.8$; $p = 0.18$).

These results indicate that under our experimental conditions of slow-wave activity, acute SCI dampens the spontaneous activity of neuronal populations in the SSCx. Specifically, acute SCI reduces facets of the up-state like the firing rate per up-state and the duration of the up-state. Overall, it appears that SCI provokes an immediate reduction in neuronal excitability in the SSCx.

3.2. The effects of chronic SCI on neuronal dynamics during cortical up-states

To study the effects of a chronic SCI on the spontaneous activity of neuronal populations in the SSCx, we used a set of 40 animals that were divided into three experimental groups: 11 animals were used a control group; 2) 10 animals were studied one week after SCI; and 3) 19 animals were studied in the chronic state, 1–3 months after SCI (Fig. 1D).

Spontaneous activity was recorded during 300 s in all three animal groups, in which neuronal dynamics of slow-wave activity was assessed following the same parameters than described for acute SCI (Fig. 4A). The statistical comparison of regarding mean firing rate showed no differences between groups (2-way independent measures ANOVA, TIME $F(2,74) = 0.3$; $p = 0.69$; TIME X CORTEX $F(2,74) = 0.2$; $p = 0.83$; Fig. 4B), consistent with previous reports (see Humanes-Valera et al., 2017). No differences were found between both cortical locations (CORTEX $F(1,74) = 0.01$; $p = 0.91$). Although, the frequency of up-states was not different between the control animals (named as "pre" in Fig. 4B-F) and one week after SCI, it was significantly lower 1–3 months after SCI (TIME $F(2,74) = 15.7$; $p = 0.000002$; Tukey pre-SCI/1w-SCI: $p = 0.99$; Tukey pre-SCI/1-3 m-SCI: $p = 0.0001$; Tukey

Table 1
Averages and SD from all parameters extracted from animals submitted to acute SCI.

	Acute experiments (n = 19)				Real (n = 9)				Sham (n = 10)			
Mean firing rate (spikes/s)	Before		After		Before		After		Before		After	
	FP	HP	FP	HP	FP	HP	FP	HP	FP	HP	FP	HP
Mean	84.33	54.90	68.19	40.40	78.05	53.02	64.86	34.27	82.77	58.73	79.86	63.16
± SD	43.64	35.80	35.20	26.20	29.79	26.93	27.72	23.92	46.01	25.88	44.24	31.43
Up frequency (Hz)	Before		After		Before		After		Before		After	
	FP	HP	FP	HP	FP	HP	FP	HP	FP	HP	FP	HP
Mean	0.68	0.64	0.69	0.61	0.62	0.60	0.65	0.55	0.64	0.64	0.63	0.63
± SD	0.15	0.11	0.14	0.11	0.11	0.11	0.12	0.11	0.06	0.04	0.07	0.08
Spikes per Up	Before		After		Before		After		Before		After	
	FP	HP	FP	HP	FP	HP	FP	HP	FP	HP	FP	HP
Mean	123.53	83.68	100.53	65.21	127.22	88.22	102.22	62.00	132.90	93.60	132.30	100.60
± SD	55.06	47.02	50.46	38.92	44.22	48.61	40.43	44.18	77.45	45.20	79.35	53.42
Up firing rate (spikes/s)	Before		After		Before		After		Before		After	
	FP	HP	FP	HP	FP	HP	FP	HP	FP	HP	FP	HP
Mean	205.29	159.84	183.91	150.69	205.51	164.34	181.19	145.97	193.81	168.84	196.67	174.84
± SD	82.50	62.73	79.78	70.06	56.15	63.06	54.31	68.39	93.67	57.90	94.46	57.75
Up duration (s)	Before		After		Before		After		Before		After	
	FP	HP	FP	HP	FP	HP	FP	HP	FP	HP	FP	HP
Mean	0.60	0.50	0.54	0.41	0.61	0.51	0.56	0.39	0.65	0.54	0.64	0.55
± SD	0.09	0.11	0.12	0.08	0.11	0.11	0.11	0.10	0.11	0.11	0.15	0.11

Table 2
Averages and SD from all parameters extracted from animals submitted to chronic SCI.

	Control (n = 11)		1 Week (n = 10)		1–3 Months (n = 19)	
Mean firing rate (spikes/s)	FP	HP	FP	HP	FP	HP
Mean	36.97	32.01	38.57	38.27	38.61	41.97
± SD	20.34	21.72	25.48	19.93	26.11	30.52
Up frequency (Hz)	FP	HP	FP	HP	FP	HP
Mean	0.69	0.60	0.65	0.65	0.49	0.52
± SD	0.17	0.08	0.09	0.12	0.11	0.11
Spikes per Up	FP	HP	FP	HP	FP	HP
Mean	52.82	51.64	60.90	62.70	82.84	83.95
± SD	27.56	29.38	41.50	36.46	60.36	61.60
Up firing rate (spikes/s)	FP	HP	FP	HP	FP	HP
Mean	89.65	93.47	89.43	97.09	94.09	109.84
± SD	41.56	50.79	45.56	42.58	50.89	67.34
Up duration (s)	FP	HP	FP	HP	FP	HP
Mean	0.58	0.57	0.63	0.64	0.85	0.75
± SD	0.09	0.18	0.21	0.38	0.43	0.34

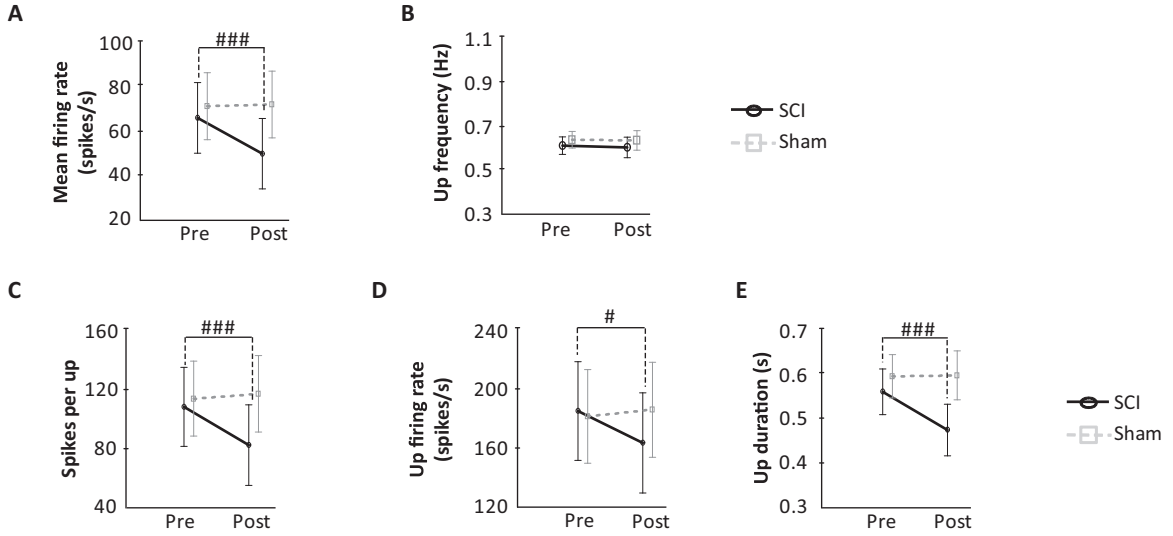

Fig. 3. Sham experimental protocols do not modify up-states features. Populational data of animals from the first group (n = 9, black line, SCI) and the second group of acute SCI (see explanation about groups on Materials and Methods) when submitted only to sham protocol (pre-lesion-1 vs pre-lesion-2; dotted grey line). A) Mean firing rate showing decrease in activity in SCI group (black line), but not in sham group (dotted grey line). B) Averaged up frequency plot showing no changes. C) Spikes per up-state were only decreased in the first group of acute SCI (black line), and no differences in the second group submitted to sham protocol (grey dotted line). In the same way, when parameters studied were up-state firing rate (D) and up-state duration (E), a decrease in both parameters was only observed in the group of acute SCI (black lines) while no changes were observed in the group submitted to sham protocol (dotted grey line). The data represents the mean and the error bars (mean ± 95% Conf. Interval): $\#p < 0.05$; $\#\#\#p < 0.001$, Tukey Honest Significant Test was used for post-hoc comparisons.

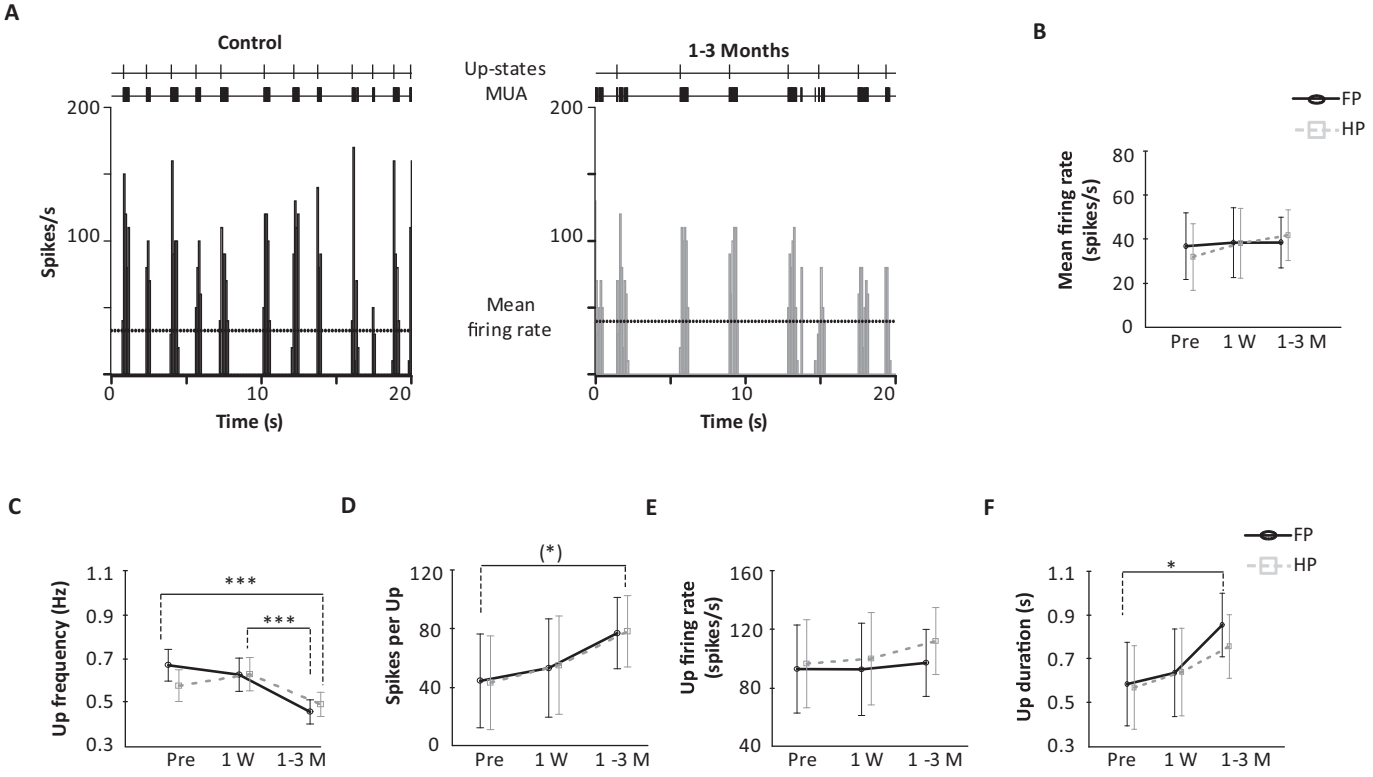

Fig. 4. Chronic effects of SCI on neuronal activity in the somatosensory cortex. A) Representative example of the MUA analysis in control condition and chronic SCI 1–3 months post-lesion. Upper traces (up-states) show the identification of every up-state. Middle traces (MUA), events corresponding to the MUA extracted from the filtered recordings. Botton graphs. Representative histograms showing an example of mean frequency of MUA (20 s examples). The mean firing rate was calculated from the entire time of the recordings, as shown by the dotted lines in the histograms. B) Analysis of the population data for the mean firing rate showed no changes in the CxFP (dark line) and CxHP (grey-dotted line). C) Population data of the up-state frequency recorded in the CxFP (dark line) and CxHP (grey-dotted line). There was a decrease in the up-state frequency at both cortical locations 1–3 months after SCI. D) Population data of the spikes per up-state confirming a general increase in the MUA at both cortical locations. E) Population data of the firing rate per up-state demonstrating no change at the two cortical locations. F) The duration of the up-state increases in chronic animals 1–3 months post-SCI.The x-axis indicates the Pre- (control group), 1w (one week after SCI) and 1-3 m (1–3 months after SCI). The chronic experiments are represented as the means and the error bars represent the mean ± 95% Conf. Interval: $(*)p < 0.1$; $*p < 0.05$; $***p < 0.001$.

1w-SCI/1-3 m-SCI: p = 0.0001: Fig. 4C). This effect was not specific to either cortex (TIME X CORTEX F(2,74) = 2; $p = 0.14$) and no differences were found between cortical locations of CxFP and CxHP (CORTEX F(1,74) = 0.4; $p = 0.5$).

As mentioned above, the majority of cortical neurons only produce discharges during the up-states under slow-wave activity. Thus, the most feasible hypothesis to maintain the same general firing rate when there are fewer up-states is for the neuronal population discharge to increase during up-states. As such, the number of spikes per up-state was quantified in each experimental group and the comparisons showed that no differences were found between groups; however, the number of spikes per up-state showed a tendency to increase (see Fig. 4D indicate by (* $p < 0.1$)) in group of 1–3 months after SCI (2-way independent measures ANOVA, TIME F(2,74) = 3,1; $p = 0.048$; Tukey pre-SCI/1w-SCI, $p = 0.80$; Tukey pre-SCI/1-3 m-SCI, $p = 0.052$; Tukey 1w-SCI/1-3 m-SCI, $p = 0.25$: Fig. 4D). This effect was not specific to either cortex (TIME X CORTEX F(2,74) = 0.005; p = 0.99) and no differences were found between cortices (CORTEX F(1,74) = 0.002; $p = 0.96$). There are two different possibilities that might explain the increase in the number of spikes per up-state: a higher firing rate per up-state and/or a longer duration of the up-state. We found that the firing rate per up-state was not statistically different between these three groups (TIME F(2,74) = 0.3;$p = 0.71$; TIME X CORTEX F (2,74) = 0.1; p = 0.9), although there was a mild tendency towards a higher firing rate in rats 1–3 months after SCI. As in previous comparisons, no differences were found between different cortical locations. (CORTEX F(1,74) = 0.5; $p = 0.46$). Importantly, significant differences were detected in the duration of the up-states, whereby these up-states were longer in the chronic SCI rats 1–3 months post-lesion (2-way independent measures ANOVA, TIME F(2,74) = 4.1; $p = 0.019$; Tukey pre-SCI/1w-SCI, p = 0.8; Tukey pre-SCI/1-3 m-SCI, $p = 0.024$; Tukey 1w-SCI/1-3 m-SCI, $p = 0.14$: Fig. 4F). In the same way than previous results, the effect was not specific to cortical locations, (TIME X CORTEX F(2,74) = 0.2; p = 0.8) and no differences were found between cortical locations CxFP and CxHP (CORTEX F(1,74) = 0.2; $p = 0.62$).

Together, these results show that during slow-wave activity, in the SSCx there is an initial depression of neural activity within the up-states in the acute period post SCI, which is followed by homeostatic recovery of normal slow-wave activity after one week. The neuronal hyperexcitability within the up-states was balanced by a reduction of up-state frequency 1–3 months after lesion.

4. Discussion

The data presented here demonstrate that after the large-scale deafferentation produced by SCI in the somatosensory system, slow-wave activity in the cerebral cortex temporally evolves from an immediate reduction in neural excitability under acute conditions to neural hyperexcitability in the up-states, as is evident 1–3 months after spinal lesion. However, the frequency of the up-states decreased 1–3 month after SCI.

We consider that these changes in cortical neuronal dynamics after acute and chronic SCI might reflect homeostatic changes to cortical circuits that initially serve to re-establish control conditions of neuronal excitability. However, these changes ultimately provoke cortical neuronal hyperexcitability during up-states.

4.1. Neuronal Dynamics changes during up-states in the somatosensory cortex in acute condition of SCI

Classical studies of the effects of SCI on the SSCx have focused on the stimulus-response paradigm, revealing how cortical excitability evolves from days to weeks after spinal lesion (Endo et al., 2007; Kaas et al., 2007; Kao et al., 2009; Ghosh et al., 2009; Humanes-Valera et al., 2017). However, neurophysiological effects are initiated at the cortical level immediately after SCI, due to peripheral deafferentation triggering changes in cortical states from delta to slow-wave activity, as demonstrated in an experimental model of acute SCI in anesthetized rats (Aguilar et al., 2010; Yagüe et al., 2011, 2014; Humanes-Valera et al., 2013). In this regard, we previously showed a relationship between slower cortical activity after acute SCI and an enhanced cortical response to peripheral stimulation (Aguilar et al., 2010; Humanes-Valera et al., 2013; Yagüe et al., 2014). Here we wanted to go a step further and thus, we took advantage of the cortical slow-wave activity that can be preserved pre- and post-SCI as a model to study the effects of SCI on the dynamics of cortical neuronal populations. In this sense, we characterized neuronal activity that takes place in the up-states (the mean firing rate and the firing rate per up-state), and the properties of the up-states at the cortical level in terms of duration (length) and frequency, both under control conditions and after SCI.

The initial reduction in spontaneous activity of cortical neuronal populations during up-states could be directly related to the reduction in spontaneous activity in the somatosensory thalamus after SCI (Alonso-Calviño et al., 2016). This idea is only partly supported by results from in vitro and in vivo experiments, in which disrupting thalamocortical connections between the somatosensory thalamus and SSCx provokes a reduction in the up-states in the cortex (Rigas and Castro-Alamancos, 2007; David et al., 2013). Nevertheless, we assume that if the somatosensory thalamus decays its intrinsic activity due to peripheral damage, this should be reflected in the SSCx (David et al., 2013).

The changes in neuronal dynamics that characterize up-states during slow-wave activity after SCI could be considered to be independent of the general state of the cortex because the main frequency was below 1 Hz in all the experiments (Steriade et al., 1993). Therefore, these neuronal changes will not affect the general cortical state, which is consistent with the state-independent physiological changes described previously in the study of cortical evoked responses (Humanes-Valera et al., 2013).

The dampened neuronal dynamics in the cortex after acute SCI could have pathophysiological implications, reflecting part of a complex neuronal response at the cortical level. Indeed, they are likely to be related to the reduced cortical activity described in humans using non-invasive techniques < 30 days after SCI (early stages: Hou et al., 2014; Zhu et al., 2015). Our data show that spontaneous neuronal activity at the cortical level is affected immediately after SCI, provoking less excitability in the neuronal population. We consider that these results from acute effect of SCI on spontaneous activity of somatosensory cortex could be the beginning of physiological modifications that later evolves into an adaptive process of neuronal plasticity.

4.2. Evolution of cortical neuronal dynamics under slow-wave activity from acute to chronic SCI stages

Slow-wave activity has been related to neuronal plasticity in the cerebral cortex (Wilhelm et al., 2014; Timofeev and Chauvette, 2017) and therefore, changes in the features of up-states could reflect different stages of cortical plasticity due to SCI. The results presented here describe an evolution in neuronal dynamics during cortical up-states from acute to chronic phases of SCI. Remarkably, after the reduction in neuronal activity associated with acute SCI, such changes were no longer evident one week after SCI, and there appears to have been a complete reversion to control conditions. Similar results over comparable periods (from acute to chronic phases after injury) have been described in different animal models using experimental models of sensory deprivation in vivo affecting the same or different sensory systems (Hengen et al., 2013; Keck et al., 2013; Teichert et al., 2017; Endo et al., 2007). Following a similar rationale, our results suggest that neuronal populations in the SSCx engage in homeostatic plasticity within a time window of one week (Bishop and Zito, 2013).

In addition, we studied longer periods to assess chronic responses to

SCI (1–3 months), in line with other attempts to study the cortical effects of SCI in rodents (Endo et al., 2007; Quiton et al., 2010). Interestingly, we found that the changes in the activity of cortical neurons did not cease one week after SCI, once homeostatic plasticity was established. Therefore, it appears that severe and permanent sensory deprivation may open a second phase of plasticity that overrides the natural (control) state.

This plasticity may be relevant to the management of neuropathic pain in animal models of SCI, which has been associated with neural hyperexcitability both at the cortical (Quiton et al., 2010) and thalamic level (Hains et al., 2006; Masri et al., 2009; Whitt et al., 2013).

Using a stimulus-response paradigm, we previously described dual plasticity in the cortex in chronic phases after SCI, with dampened and enhanced responses evoked on a short and long post-stimulus time scale respectively (Humanes-Valera et al., 2017). Here, by focusing on spontaneous slow-wave activity we found two main effects associated with chronic SCI: an increase in neuronal activity during up-states, and a decrease in the frequency of these up-states. These opposing effects suggest that additional homeostatic mechanisms may exist to control the overall cortical activity after chronic SCI. We propose that the effects we observed in spontaneous neuronal activity parallel the dual cortical plasticity we described previously (Humanes-Valera et al., 2017).

Finally, from a translational perspective, our results may be related to data obtained from patients with chronic SCI. In this regard, different authors have described that alterations in brain activity after SCI in patients show slower EEG frequencies (Tran et al., 2004; Herbert et al., 2007; Endo et al., 2007; Wydenkeller et al., 2009). Changes in spontaneous activity and evoked responses in patients with chronic SCI have been related to pathologies like neuropathic pain and structural changes in the brain after SCI (Wydenkeller et al., 2009; Jutzeler et al., 2015). However, the data gathered from animal models and studies in humans do not clarify if the cortical plasticity after SCI helps improving functional recovery or if it triggers other pathological states, such as chronic pain (for a review see Moxon et al., 2014).

4.3. Mechanistic considerations

Interestingly, the effects of acute and chronic SCI do not only affect the deafferented cortex (CxHP), but also the intact somatosensory cortex (CxFP) appears to be affected in a similar manner (see Figs. 2 and 4). In the somatosensory cortex, as in other sensory cortices, the spontaneous activity emerges from contributions of specific peripheral inputs, cortico-cortical networks and the action of neuromodulatory systems with origin in other non-cortical brain structures that regulate the overall cortical activity and neuronal excitability (Lee and Dan, 2012; Favero et al., 2012; Castro-Alamancos and Gulati, 2014). All these factors are affected by a SCI, but may be that each of them are involved in the control or in the modulation of different aspects of the cortical excitability at distinct time points after a SCI, leading to the observed differences between acute and chronic SCI data. Moreover, some of these factors might be responsible for the similar changes in spontaneous activity observed in CxFP and CxHP after SCI. We consider that the main difference created by SCI is the lack of peripheral inputs to CxHP while CxFP maintain its specific inputs intact. This differential effect over spontaneous activity has been reported at thalamic level (Alonso-Calviño et al., 2016), however we can not find differences in spontaneous activity at cortical level between CxFP and CxHP, which indicates that cortex have a more complex regulation of spontaneous activity. In this way, the other two factors that contribute to cortical activity, such as cortico-cortical networks and neuromodulatory systems that affect in the same way both cortical locations CxHP and CxFP, could be responsible for similar physiological changes observed in both cortical locations under acute and chronic stages of SCI. Regarding cortico-cortical connections, it has been demonstrated that cortical up-states propagate from the point of origin to other cortical locations (Volgushev et al., 2006; Reyes-Puerta et al., 2016). In this scenario the cortico-cortical connections between CxHP and CxFP make possible that alterations in up-states properties with origin in CxHP can be transmitted to local up-states produced in CxFP, and reciprocally, the activity of up-states originated in CxFP finally affects the up-states locally evoked in CxHP. This possible mechanism could be reinforced from acute to chronic stages of SCI. Moreover, the similar effects of SCI over spontaneous activity of CxFP and CxHP could also be assigned to the neuromodulatory systems, which directly modulate the general cortical activity and in particular the up-states generation and some of its properties (Mena-Segovia et al., 2008; Castro-Alamancos and Gulati, 2014); Petzold et al., 2015). In this regard, different mesencephalic structures as pedunculopontine tegmental nucleus, laterodorsal termental nucleus, locus coeruleus among others, which receives sensory information from periphery in order to regulates the context of brain during sensory perception, can be affected in function when somatosensory inputs are reduced due to SCI, therefore same general effects over entire somatosensory cortex (including CxFP and CxHP) must be expected.

4.4. Experimental restrictions

Our experimental approach has some limitations that must be considered. Due to the interventions required to study cortical changes immediately after SCI, animals were maintained under anesthesia during electrophysiological recording, both in control conditions and when the acute affects of spinal cord lesions were studied (2–5 h after SCI). This approach is well established in our laboratory (Aguilar et al., 2010; Yagüe et al., 2011, 2014; Humanes-Valera et al., 2013, 2017; Alonso-Calviño et al., 2016) and it allows us to establish the same cortical conditions in all different groups. Once under the same cortical state of slow-wave activity, the isolation of neuronal activity during up-states in all different conditions facilitates the experimental comparison of neuronal dynamics exclusively during the up-states (Humanes-Valera et al., 2013). Therefore, while assuming the limitation imposed regarding the use of anesthesia to induce a stable slow-wave activity in all experiments, we consider that slow-wave activity is a powerful tool to study cortical activity after SCI. In addition, neural activity during up-states is thought to be mechanistically related to the vigilant state (Destexhe et al., 2007; Castro-Alamancos, 2009).

There was also a difference between the acute and chronic experimental groups regarding the sites of recording in the CxFP and CxHP. In acute animals, the correct location was ensured by identifying the optimal responses to peripheral stimulation of the forepaw and hindpaw before the lesion. However, in chronic animals, it was impossible to locate the CxHP through peripheral stimulation because the SCI impeded this and we were therefore limited to record these animals (control group, 1 week and 1–3 months) based on stereotaxic coordinates to guarantee that consistent data was obtained in the chronic experiments.

5. Conclusions

The data presented here demonstrate that spontaneous slow-wave activity of cortical neuronal populations is directly affected by SCI, yet in a different manner depending on the time after lesion: immediately, at one week or in the chronic phases after SCI. Immediately after SCI there is a reduction in neuronal excitability, while one week after SCI some homeostatic recovery has taken place. Finally, in chronic phases cortical neuronal populations display hyperexcitability during up-states that is compensated by a lower up-state frequency. Therefore, our work shows that homeostatic processes in slow-wave activity contribute to the cortical reorganization after SCI. Future experiments will be necessary to decipher the physiological implications of each of these states.

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References

Aguilar, J., Humanes-Valera, D., Alonso-Calviño, E., Yagüe, J.G., Moxon, K., Oliviero, A., Foffani, G., 2010. Spinal cord injury immediately changes the state of the brain. J. Neurosci. 30, 7528–7537. https://doi.org/10.1523/JNEUROSCI.0379-10.2010.

Alonso-Calviño, E., Martínez-Camero, I., Fernández-López, F., Humanes-Valera, D., Foffani, G., Aguilar, J., 2016. Increased responses in the somatosensory thalamus immediately after spinal cord injury. Neurobiol. Dis. 87, 39–49. https://doi.org/10.1016/j.nbd.2015.12.003.

Bishop, H.I., Zito, K., 2013. The downs and ups of sensory deprivation: evidence for firing rate homeostasis in vivo. Neuron 80, 247–249. https://doi.org/10.1016/j.neuron.2013.10.011.

Castro-Alamancos, M.A., 2009. Cortical up and activates states: implications for sensory information processing. Neuroscientist 15 (6), 625–634. https://doi.org/10.1177/1073858409333074.

Castro-Alamancos, M.A., Gulati, T., 2014. Neuromodulators produce distinct activated states in neocortex. J. Neurosci. 34 (37), 12353–12367. https://doi.org/10.1523/JNEUROSCI.1858-14.2014.

Chapin, J.K., Lin, C.S., 1984. Mapping the body representation in the SI cortex of anesthetized and awake rats. J. Comp. Neurol. 229, 199–213. https://doi.org/10.1002/cne.902290206.

David, F., Schmiedt, J.T., Taylor, H.L., Oban, G., Di Giovanni, G., Uebele, V.N., Renger, J.J., Lambert, Leresche N., Crunelli, V., 2013. Essential thalamic contribution to slow waves of natural sleep. J. Neurosci. 33 (50), 19599–19610. https://doi.org/10.1523/JNEUROSCI.3169-13.2013.

Destexhe, A., Hughes, S.W., Rudolph, M., Crunelli, V., 2007. Are corticothalamic 'up' states fragments of wakefulness? Trends Neurosci. 30 (7), 334–342. https://doi.org/10.1016/j.tins.2007.04.006.

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 (Pt 11), 2951–2961. https://doi.org/10.1093/brain/awm237.

Favero, M., Varghese, G., Castro-Alamancos, M.A., 2012. The state of somatosensory cortex during neuromodulation. J. Neurophysiol. 108, 1010–1024. https://doi.org/10.1152/jn.00256.2012.

Ghosh, A., Sydekum, E., Haiss, F., Peduzzi, S., Zörner, B., Schneider, R., Baltes, C., Rudin, M., Weber, B., Schwab, M.E., 2009. Functional and anatomical reorganization of the sensory-motor cortex after incomplete spinal cord injury in adult rats. J. Neurosci. 29 (39), 12210–12219. https://doi.org/10.1523/JNEUROSCI.1828-09.2009.

Goloshevsky, A.G., Wu, C.W., Dodd, S.J., Koretsky, A.P., 2011. Mapping cortical representation of the forepaw and hindpaw with BOLD fMRI reveals two spatial boudaries. Neuroimage 57 (2), 526–538. https://doi.org/10.1016/j.neuroimage.2011.04.002.

Hains, B.C., Saab, C.Y., Waxman, S.G., 2006. Alterations in burst firing of thalamic VPL neurons and reversal by Na(v)1.3 antisense after spinal cord injury. J. Neurophysiol. 95 (6), 3343–3352. https://doi.org/10.1152/jn.01009.2005.

Hengen, K.B., Lambo, M.E., Van Hooser, S.D., Katz, D.B., Turrigiano, G.G., 2013. Firing rate homeostasis in visual cortex of freely behaving rodents. Neuron 80, 335–342. https://doi.org/10.1016/j.neuron.2013.08.038.

Herbert, D., Tran, Y., Craig, A., Boord, P., Middleton, J., Siddall, P., 2007. Altered brain wave activity in persons with chronic spinal cord injury. Int. J. Neurosci. 117 (12), 1731–1746. https://doi.org/10.1080/00207450701242826.

Hou, J.M., SunTS, Xiang Z.M., Zhang, J.Z., Zhang, Z.C., Zhao, M., Zhong, J.F., Liu, J., Zhang, H., Liu, H.L., Yan, R.B., Li, H.T., 2014. Alterations of resting-state regional and network-level neural function after acute spinal cord injury. Neuroscience 277, 446–454. https://doi.org/10.1016/j.neuroscience.2014.07.045.

Humanes-Valera, D., Aguilar, J., Foffani, G., 2013. Reorganization of the intact somatosensory cortex immediately after spinal cord injury. PlosOne 8 (7), e69655. https://doi.org/10.1371/journal.pone.0069655.

Humanes-Valera, D., Foffani, G., Alonso-Calviño, E., Fenández-López, E., Aguilar, J., 2017. Dual cortical plasticity after spinal cord injury. Cereb. Cortex 27 (5), 2926–2940. https://doi.org/10.1093/cercor/bhw142.

Jutzeler, C.R., Freund, P., Huber, E., Curt, A., Kramer, J.L.K., 2015. Neuropathic pain and functional reorganization in the primary somatosensory cortex after spinal cord injury. J. Pain 16 (12), 1256–1267. https://doi.org/10.1016/j.jpain.2015.08.008.

Kaas, J.H., Qi, H.X., Burish, M.J., Garbawie, O.A., Onifer, S.M., Massey, J.M., 2007. Cortical and subcortical plasticity in the brains of humans, primates and rats after damage to sensory afferents in the dorsal columns of the spinal cord. Exp. Neurol. 209 (2), 407–416. https://doi.org/10.1016/j.expneurol.2007.06.014.

Kao, T., Shumsky, J.S., Murray, M., Moxon, K.A., 2009. Exercise induces cortical plasticity after neonatal spinal cord injury in the rat. J. Neurosci. 29 (23), 7549–7557. https://doi.org/10.1523/JNEUROSCI.2474-08.2009.

Keck, T., Keller, G.B., Jacobsen, R.I., Eysel, U.T., Bonhoeffer, T., Hübener, M., 2013. Synaptic scaling and homeostatic plasticity in the mouse visual cortex in vivo. Neuron 80, 327–334. https://doi.org/10.1016/j.neuron.2013.08.018.

Lee, S.H., Dan, Y., 2012. Neuromodulation of brain states. Neuron 76 (1), 209–222. https://doi.org/10.1016/j.neuron.2012.09.012.

Lemieux, M., Chen, J.Y., Lonjers, P., Bazhenov, M., Timofeev, I., 2014. The impacto of cortical deafferentation on the neocortical slow oscillation. J. Neurosci. 34 (16), 5689–5703. https://doi.org/10.1523/JNEUROSCI.1156-13.2014.

Masri, R., Quiton, R.L., Lucas, J.M., Murray, P.D., Thompson, S.M., Keller, A., 2009. Zona Incerta: a role in Central Pain. J. Neurophysiol. 102, 181–191. https://doi.org/10.1152/jn.00152.2009.

Mena-Segovia, J., Sims, H.M., Magill, P.J., Bolam, J.P., 2008. Cholinergic brainstem neurons modulate activity during slow oscillations. J. Physiol. 586 (12), 2947–2960. https://doi.org/10.1113/jphysiol.2008.153874.

Morales-Botello, M.L., Aguilar, J., Foffani, G., 2012. Imaging the spatio-temporal dynamics of supragranular activity in the rat somatosensory cortex in response to stimulation of the paws. PLoS One 7 (7), e40174. https://doi.org/10.1371/journal.pone.0040174.

Moxon, K.A., Oliviero, A., Aguilar, J., Foffani, G., 2014. Cortical reorganization after spinal cord injury: always for good? Neurosience 283, 78–94. https://doi.org/10.1016/j.neuroscience.2014.06.056.

Paxinos and Watson, 2007. The Rat Brain in Estereotaxic Coordinates. Academic Press, Amsterdam.

Petzold, A., Valencia, M., Pál, B., Mena-Segovia, J., 2015. Decoding brain state transitions in the pedunculopontine nucleus: cooperative phasic and tonic mechanisms. Front. Neural Circuits. 9, 68. https://doi.org/10.3389/fncir.2015.00068.

Quiton, R.L., Masri, R., Thompson, S.M., Keller, A., 2010. Abnormal activity of primary somatosensory cortex in central pain syndrome. J. Neurophysiol. 104, 1717–1725. https://doi.org/10.1152/jn.00161.2010.

Rey, H.G., Pedreira, C., Quian Quiroga, R., 2015. Past, present and future of spike sorting techniques. Brain Res. Bull. 119, 106–117. https://doi.org/10.1016/j.brainresbull.2015.05.007.

Reyes-Puerta, V., Yang, J.W., Siwek, M.E., Kilb, W., Sun, J.J., Luhmann, H.J., 2016. Propagation of spontaneous slow-wave activity across columns and layers of the adult rat barrel cortex in vivo. Brain Struct. Funct. 221 (9), 4429–4449. https://doi.org/10.1007/s00429-015-1173-x.

Rigas, P., Castro-Alamancos, M.A., 2007. Thalamocortical up-states: differential effects of intrinsic and extrinsic cortical inputs on persistent activity. J. Neurosci. 27 (16), 4261–4272. https://doi.org/10.1523/JNEUROSCI.0003-07.2007.

Steriade, M., 2000. Corticothalamic resonance, states of vigilance and mentation. Neuroscience 101 (2), 243–276. https://doi.org/10.1016/s0306-4522(00)00353-5.

Steriade, M., Nuñez, A., Amzica, F., 1993. A novel (< 1Hz) oscillation of neocortical neurons in vivo. J. Neurosci. 13, 3253–3265. https://doi.org/10.1523/JNEUROSCI.13-08-03252.1993.

Teichert, M., Liebmann, L., Hübner, C.A., Bolz, J., 2017. Homeostatic plasticity and synaptic scaling in the adult mouse auditory cortex. Sci. Rep. 7 (1), 17423. https://doi.org/10.1038/s41598-017-17711-5.

Timofeev, I., Chauvette, S., 2017. Sleep slow oscillation and plasticity. Curr. Opin. Neurobiol. 44, 116–126. https://doi.org/10.1016/j.conb.2017.03.019.

Tran, Y., Boord, P., Middleton, J., Craig, A., 2004. Levels of brain wave activity(8–13 Hz) in persons with spinal cord injury. Spinal Cord 42, 73–79. https://doi.org/10.1038/sj.sc.3101543.

Volgushev, M., Chauvette, S., Mukovski, M., Timofeev, I., 2006. Precise long-range synchronization of activity and silence in neocortical neurons during slow-wave oscillations. J. Neurosci. 26 (21), 5665–5672. https://doi.org/10.1523/JNEUROSCI.0279-06.2006.

Whitt, J.L., Masri, R., Pulimood, N.S., Keller, A., 2013. Pathological activity in mediodorsal thalamus of rats with spinal cord injury pain. J. Neurosci. 33 (9), 3915–3926. https://doi.org/10.1523/JNEUROSCI.2639-12.2013.

Wilhelm, I., Kurt, S., Ringli, M., Mouthon, A.L., Buchmann, A., Geiger, A., Jenni, O.G., Huber, R., 2014. Sleep slow-wave activity reveals developmental changes in experience-dependent plasticity. J. Neurosci. 34 (37), 12568–12575. https://doi.org/10.1523/JNEUROSCI.0962-14.2014.

Wydenkeller, S., Maurizio, S., Dietz, V., Halder, P., 2009. Neuropathic pain in spinal cord injury: significance of clinical and electrophysiological measures. Eur. J. Neurosci. 30, 91–99. https://doi.org/10.1111/j.1460-9568.2009.06801.x.

Yagüe, J.G., Foffani, G., Aguilar, J., 2011. Cortical hyperexcitability in response to preserved spinothalamic inputs immediately after spinal cord hemisection. Exp. Neurol. 227, 2014. https://doi.org/10.1016/j.expneurol.2010.11.011. *252-263* Yague et al.

Yagüe, J.G., Humanes-Valera, D., Aguilar, J., Foffani, G., 2014. Functional reorganization of the forepaw cortical representation immediately after thoracic spinal cord hemisection in rats. Exp. Neurol. 257, 19–24. https://doi.org/10.1016/j.expneurol.2014.03.015.

Zhu, L., Wu, G., Zhou, X., Li, J., Wen, Z., Lin, F., 2015. Altered spontaneous brain activity in patients with acute spinal cord injury revealed by resting-state functional MRI. PLoS One 10 (3), e0118816 10.1371/journal.pone.0118816. eCollection 2015.