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Nerve injury decreases hyperacute resting-state connectivity between the anterior cingulate and primary somatosensory cortex in anesthetized rats

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***Abstract*— A better understanding of neural pain processing and of the development of pain over time, is critical to identify objective measures of pain and to evaluate the effect of pain alleviation therapies. One issue is, that the brain areas known to be related to pain processing are not exclusively responding to painful stimuli, and the neuronal activity is also influenced by other brain areas. Functional connectivity reflects synchrony or covariation of activation between groups of neurons. Previous studies found changes in connectivity days or weeks after pain induction. However, less in known on the temporal development of pain. Our objective was therefore to investigate the interaction between the anterior cingulate cortex (ACC) and primary somatosensory cortex (SI) in the hyperacute (minute) and sustained (hours) response in an animal model of neuropathic pain. Intra-cortical local field potentials (LFP) were recorded in 18 rats. In 10 rats the spared nerve injury model was used as an intervention. The intra-cortical activity was recorded before, immediately after, and three hours after the intervention. The interaction was quantified as the calculated correlation and coherence. The results from the intervention group showed a decrease in correlation between ACC and SI activity, which was most pronounced in the hyperacute phase but a longer time frame may be required for plastic changes to occur. This indicated that both SI and ACC are involved in hyperacute pain processing.**

***Index Terms*—Coherence Analysis, Functional Connectivity, Local Field Potentials, Invasive Microelectrode Recording, Pain Neurophysiology**

I. Introduction

PAIN is mostly evaluated as a subjective phenomenon. However, several objective measures have been evaluated in human/clinical studies, such as brain imaging, reflexes, or other measures of nerve responses [1]–[3]. The primary somatosensory cortex (SI), cingulate cortex, and thalamus are the most consistently activated brain areas in animal models of pain [4]. A study has shown that the difference in the response to different stimuli may only be in synchrony between pairs of neurons and not firing rate [5] and it is believed that temporal relations between responses from different groups of neurons are as important as the amplitude of the responses [6], [7]. The cortical areas previously believed to be representing pain [10]–[12], cannot be used as signatures of pain as they are not specifically activated by nociceptive stimuli, but also by non-painful stimuli, e.g. visual stimuli [13]. The key to understanding the processing of nociceptive stimuli and pain may lie in the interaction between areas and not the activation in itself.

In human studies, gamma oscillations have been of special interest when investigating chronic pain, as they are correlated with the subjective self-rated perception of pain [2], [8]. This is supported by Schulz et al. (2015), who found that pain ratings correlate with gamma oscillations and the stimulus intensity is correlated with beta oscillations in the hemisphere contralateral to the stimuli [3]. Increased gamma oscillations have also been associated with behavioral reaction to an animal model of chronic pain, monoarthritis, and hyperalgesia in rats [9].

In addition to studying absolute changes in cortical activity from relevant areas, connectivity analysis provides information on the effect of pain on the coupling between areas. For example, functional connectivity analyses have been used to study interactions between cortical areas, as statistical dependency or models of interactions [10]. Functional connectivity reflects synchrony or covariation between groups of neurons, although it does not imply a direct connection [10]–[12].

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Several acute and chronic pain model studies have focused on SI, cingulate cortex, and thalamus and their interaction or interaction with other areas to model or investigate functional reorganization in response to pain or nociception [13]–[19]. In these studies, spontaneous resting-state responses were recorded, unaffected by type and intensity of a stimulus. Resting-state connectivity is a measure of the spontaneous pain or nociception, independent of type or intensity of stimuli, which are altered in humans suffering from neuropathic pain [20].

In a study by LeBlanc et al. (2014), 15 min after applying capsaicin the coherence between SI and thalamus in the delta, theta, and gamma-band was lower compared to baseline [15]. Resting-state functional MRI showed no change in connectivity one [21] and five [13] days after the spared nerve injury.

In the weeks following a peripheral nerve injury, fluctuations between nearby cells correlate in the anterior cingulate cortex (ACC) and gamma, theta, and delta activity increase [22]. This was shown using cross-correlation and power spectrum analysis after a chronic constriction injury in rats recorded one and two weeks after injury [22]. Zippo et al. (2015,2016) found increased resting-state phase locking value relative to control in one study using the chronic constriction injury model [23], whereas they found decreased phase locking value with several different models of neuropathic pain [19]. Supporting, a change in connectivity 28 days after peripheral nerve injury has been found [13]. It is notable that these changes were mostly seen in the limbic system and that after 5 days only minimal, non-significant changes were observed. In up to two weeks after non-reversible models of pain, awake rats showed decreased coherence between SI and thalamus after chronic constrictive injury [15], increased coherence between SI and pre-frontal cortex after chronic constrictive injury [16], and decreased theta band synchronization (measured by phase lag index) between ACC and amygdala after a model of inflammatory bowel disease [14].

The studies discussed above have found changes in functional connectivity days or weeks after the pain induction. Although these studies extensively documented cortical changes in response to pain in animal models, the temporal evolution of these changes shortly after the painful event are still unclear. Indeed there are no studies that have compared both the hyperacute (minutes) and sustained (hours) response to an injury model of pain in animals. Therefore, the temporal development of central changes with pain is unknown. Nontheless, the acute phase in the reaction to a neuropathic pain model is fundamental to assess pain development and chronification. This information may provide additional evidence for the mechanisms explaining the development of pain and propagation of neural changes. The aim of this study was therefore to compare the connectivity between SI and ACC in the hyperacute (minutes) and sustained (hours) phase in a rat model of neuropathic pain.

II. Materials and Methods

All procedures were approved by the Danish Animal Experiment inspectorate (J.no.: 2016-15-0201-00884). Eighteen Sprague Dawley rats from Taconics Europe were used in this study. The animals were housed in cages of 2-3 in a room with 12:12 light/dark cycle and controlled humidity and temperature. The rats had access to food and water ad libitum. Before the experiment, the rats were subjected to a two-week acclimatization period, followed by 1-2 weeks of training to get the rats accustomed to the investigator and limit stress. The rats were randomly assigned to a group subjected to spared nerve injury (in total 10, weight: 332-398 g, age: 9-12 weeks) or control (in total 8, weight: 333-417 g, age: 10-11 weeks).

A. Animal preparation

All rats were anesthetized with 4 % isoflurane with a 2 L/min flow rate and kept on a 1-3 % isoflurane level with a 0.5 L/min flow rate throughout the experiment. Following the initial anesthesia, the rats were placed in a stereotaxic frame, and the anesthesia was supplied through a mask. An incision was made through the biceps femoris in the right hind limb, and sutures were placed around the tibial and common peroneal branch of the sciatic. A craniotomy was performed, resulting in a 6 x 4 mm hole in the skull. The dura was then carefully removed, and a microelectrode array (multi-electrode array, AlphaOmega, tungsten needles, the distance between pins = 0.5 mm, shank diameter = 75 µm) was placed with six pins in SI and six pins in ACC. The electrode in SI was placed 1.5 to 2 mm anterior and 1 to 3 mm lateral to bregma, with a target depth of 1.4 mm (measured from the surface of the brain). The electrode in ACC was placed 2 to 0.5 mm posterior and 0.5 to 1 mm lateral to bregma, with a depth of 2.7 mm, all based on the Paxinox rat atlas [24]. The array was initially inserted 0.6 mm deeper than the desired depth into the cortex and then retracted to the correct depth to avoid dimpling of the brain. The target electrode depth was based on a similar study recording from ACC [21] and a study showing that layer 5 (1.05-1.5 mm) in SI shows the most significant change in LFP following forepaw denervation [25]. The rats were euthanized after the last recording by the intracardiac injection of pentobarbital. Although the intracortical recordings limit the number of recording sites, the temporal resolution is high and comparable with EEG,

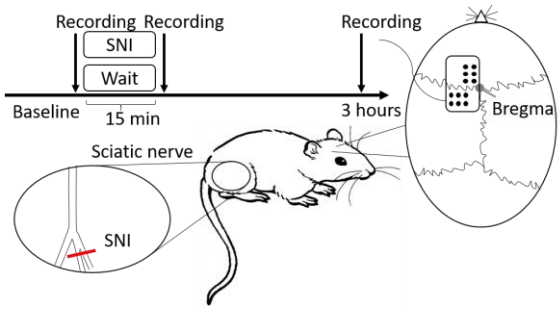

Fig. 1 Experimental setup. An electrode array with 6 pins in ACC and 6 pins in SI was placed in the cortex. After the baseline recording, two of the three branches were cut as illustrated by the red line. The timeline shows the recordings relative to either SNI (intervention group) or 15 min wait (control group). One recording was made immediately before, one immediately after, and the last recording 3 hours after SNI/wait.

providing an advantage in connectivity studies as it can detect fast changes.

B. Spared Nerve Injury

To mimic neuropathic pain in this study we chose to apply the spared nerve injury model [26]. The SNI model has shown to result in behavioral symptoms characterizing neuropathic pain, such as mechanical and thermal sensitivity [26], [27]. This model of neuropathic pain has been used previously and shown to be robust and reliable based on behavioral observations [13], [28], [29]. In this model, the tibial and common peroneal branches of the sciatic nerve are cut while leaving the sural branch intact. The procedure for the control group was the same, except that the ligation and transection of the nerve were not performed. Instead of intervention, consisting of ligation and transection of the nerve, the control group was subjected to a 15-minute wait period, as this was the approximate time to perform the injury procedure.

C. Data recording

The animals were anesthetized during the entire experiment and thus, all recordings were carried out while the animals were anesthetized. One resting-state baseline recording was initially acquired followed by either the spared nerve intervention or a 15-minute wait. Two subsequent recordings were then obtained: 1) immediately after SNI/wait (intervention/control group), to capture the acute response, and 2) three hours after SNI/wait, to capture a sustained response. The data was a part of a larger data set (manuscripts in preparation) including both resting-state recordings and recordings during peripheral nerve electrical stimuli. In this study, only resting-state data in periods that were not influenced by these electrical stimuli were used. Each recording (three for each rat) lasted 30 s. All data were recorded with a sampling frequency of 24,414 Hz (PZ5 neuroDigitizer and PZ2 BioAmp Processor, Tucker-Davis Technologies).

D. Data processing

Data analysis was performed offline in Matlab R2019b (The Mathworks, Inc., Massachusetts, USA). The data was pre-processed by filtering and calculating the double differential from the six electrodes in each area of the cortex. The connectivity between SI and ACC was calculated as coherence and correlation, which was based on the analytic signal.

To analyze the intracortical local-field potentials (LFP), data were preprocessed with a Butterworth bandpass filter (2^{nd} order Butterworth, cut off frequencies at 1 Hz and 200 Hz) and a notch filter at 50 Hz and harmonics (2^{nd} order Butterworth, cut off +/- 1 Hz). All recordings were visually inspected, and channels containing noise that could not be removed by the filters were rejected (7/648).

All functional connectivity measures were calculated at the specified time points between signals from ACC and SI in predefined frequency bands (corresponding EEG frequency bands δ: 0.5-4, θ: 4-8, α: 8-13, β: 14-40, γ: 40-49, γ+: 51-100 [30].

To decrease the complexity of the analysis and further de-noise the signal, two double differential signals were calculated using the pins as a Laplacian filter. The double differential signals were obtained in two steps for the two areas in parallel. Firstly, the difference between the two central electrodes and the related outer electrodes was found in each area. Secondly, the difference between the two signals from the first step was found for each area. These two double differential signals (one from each area) were used throughout the connectivity analysis.

To be able to extract phase from a time-series signal, a transform to an analytic signal is necessary [2], [11], [29], [31]. The analytic signal was computed by bandpass filtering the signal at the predefined frequency bands (2^{nd} order Butterworth), followed by applying the Hilbert transform [11]. The Hilbert transform is the imaginary part of the complex analytical signal, while the real part is the original signal. To ensure stationarity (constant statistical properties such as mean, variance, etc. over time), the signals were divided into 2s-long epochs, and connectivity was calculated across time for each epoch and averaged.

The two functional connectivity parameters, correlation, and coherence were calculated using custom made Matlab scripts across epochs points for each rat, and each recording [11]. The connectivity parameters were normalized by the baseline (recording before intervention) division for each rat to reduce between-subject variance.

Power-based functional connectivity reflects the number of neurons firing or the spatial extent of the neural population [11]. To investigate the power-based temporal changes, Spearman's correlation between cortical activity in SI and ACC was calculated using the Matlab function 'corr' across the 2s epochs of the analytical signal. The analytical signal was squared for calculation of correlation. The function corr ranks the signals and calculates the difference between data points. The ranked signals used in the Spearman's correlation (also called Spearman's ρ) are appropriate when working with data that do not follow a normal distribution [11], as for the current data. To visualize correlation across frequencies for the 2s epochs, the Welch transform with 0.5 s windows and 50 % overlap were used.

The degree of synchronization of the cortical signal oscillations is calculated as coherence. Coherence is phase-based but does consider power information. Phase-based connectivity reflects the timing of activity with or between neural populations [11]. Coherence is based on

$$\mathrm{Coh}=\mathrm{abs}\left(\frac{S_{xy}}{\sqrt{S_{xx}*S_{yy}}}\right)^2 \quad (1)$$

Where S_{xy} is the cross-spectral density, S_{xx} and S_{yy} is the spectral density (power) for SI and ACC. The cross-spectral density was calculated as in

$$S_{xy}(t)=\hat{X}(t)\times\hat{Y}(t)^* \quad (2)$$

Where the analytical 2s epochs signals from SI, $\hat{X}$, are multiplied with the complex conjugate from ACC, $\hat{Y}$. In the case of S_{xx} and S_{yy}, the analytical signal from each area were multiplied with its complex conjugate.

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E. Statistical analysis

The statistical analysis was performed in SPSS 26 (IBM, New York, USA). As a result of a non-normal distribution (Kolmogorov-Smirnov's test of normality), the coherence and correlation data were transformed using the log-transform and Fisher's Z-transform, respectively. The acute and sustained responses were analyzed separately applying analysis of variance (within-subject factors: [δ, θ, α, β, γ, γ+], between-subject factors: [SNI, control]). A repeated measure analysis of variance was carried out to investigate the changes for each group with time (three levels: baseline, acute, sustained) and frequency bands (six levels) as within-subject factors and group (two levels: SNI and control) as between-subject factor. Bonferroni-corrected post hoc comparisons were made for statistically significant effects at $p < 0.05$.

III. Results

A. Hyperacute reaction

The hyperacute reaction to SNI is an expression of changes in the synchronized interaction minutes after the SNI.

The correlated acute response to SNI was for most frequency bands for the control group similar to baseline (Figure 2 and 4). In the δ, α, and β band, the response for the intervention group decreased to approximately half compared to the baseline response. This was also the case for the control group, but only in the gamma band. This indicates that, in several frequency bands, there was a desynchronization or inhibition of communication following the intervention in the hyperacute phase that was not observed in the control group. The cortical correlation from the two groups differed significantly ($p = 0.019$, $F_{1,108} = 5.70$, $\eta_p^2 = 0.66$) but there was no band*group interaction ($p = 0.96$, $F_{5,108} = 0.22$, $\eta_p^2 = 0.10$) indicating that the difference between groups was similar in all frequency bands.

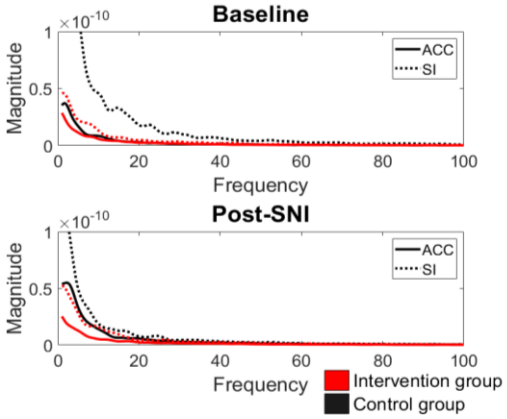

Fig. 2. Frequency distribution of the signals from ACC and SI activity from both the intervention and control group (top row) at baseline and immediately after SNI (found using Welch transform).

The post hoc test revealed that no difference was present after Bonferroni correction (p: 0.13-0.85) and thereby no strong difference in one specific frequency band was present. There was a large intra-group variance.

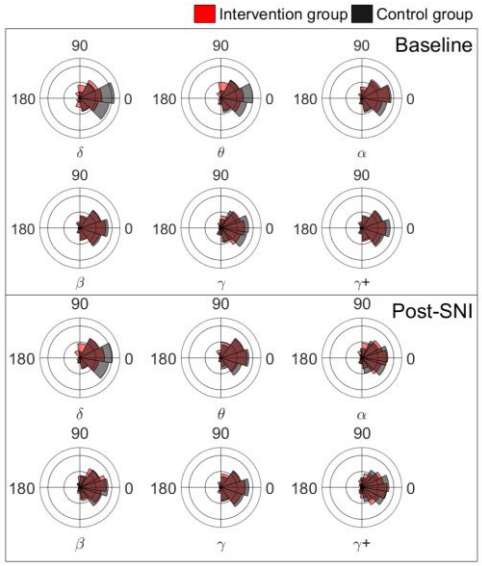

Fig. 3. Polar plots of average coherence in 2s epochs at baseline (top 2 rows) and immediately after SNI (bottom 2 rows).

The coherence, expressing of similarity of phase or synchronized oscillatory processes [11], between ACC and SI was similar to baseline for both groups (Figure 3 and 4). It is evident from the polar plot that the phase angle difference in the theta band is more clustered, indicating less difference in phase angles between SI and ACC, for the intervention group after SNI. This is probably because coherence is calculated taking the amplitude of the signal into account. Complementary to correlation, the coherence in the hyperacute phase differed between the intervention and control group ($p = 0.004$, $F_{1,108} = 0.66$, $\eta_p^2 = 0.82$) but there was no band*group interaction ($p = 1$, $F_{5,108} = 0.02$, $\eta_p^2 = 0.056$) indicating that the difference between groups did not depend on the frequency band, similarly to what was observed for the correlation. The post hoc test showed no significant difference between groups in any frequency band after Bonferroni correction (p: 0.16-0.34). The trends in coherence were not similar to correlation as it in most

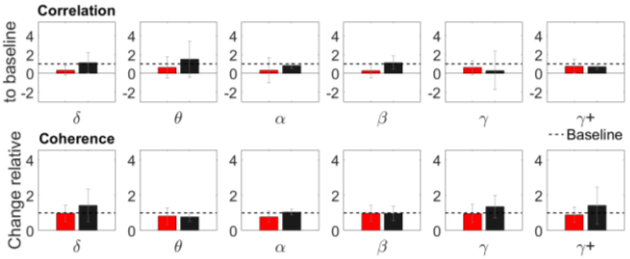

Fig. 4: Box plots showing the average correlation (top row) and coherence (bottom row) immediately after SNI for each group relative to baseline.

frequency bands did not differ much from baseline. There was a slight increase in the control group's coherence and a decrease in the intervention group's coherence (Figure 4).

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B. *Sustained reaction*

The sustained reaction is an expression of the synchronized interactivity following SNI hours after the injury. In several frequency bands, the correlation increased for one or both groups (Figure 5 and 7). That is an indication of a general facilitation of synaptic activity between ACC and SI.
In the beta band, the correlation was half compared to the baseline in the intervention group and double in the control group.

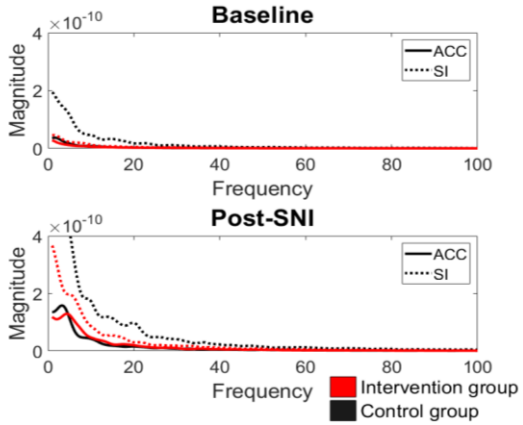

Fig. 5. Frequency distribution of the signals from ACC and SI activity from both the intervention and control group (top row) at baseline and 3 hours after SNI (found using Welch transform).

It was also observed from the frequency transform that the magnitude of activity increased at the last recording. The correlated response to SNI hours after injury and the response from the control group did not differ significantly between groups ($p = 0.79$, $F_{1,108} = 0.003$, $\eta_p^2 = 0.04$).

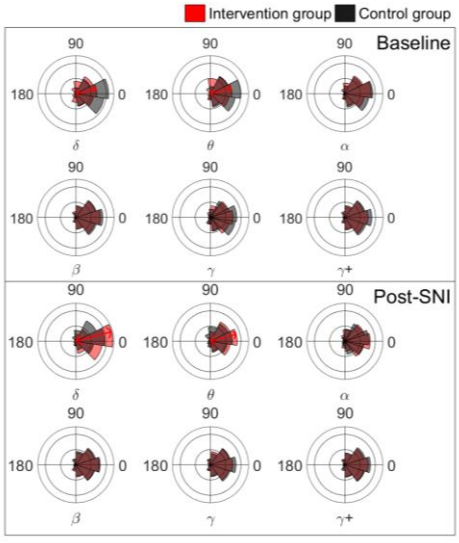

Fig. 6. Polar plots of average coherence in 2s epochs at baseline (top 2 rows) and 3 hours after SNI (bottom 2 rows).

For both groups, the response was similar or increased, and in the delta, theta, and beta it was twofold that of the baseline in the intervention group (Figure 5 and 7). From the polar plots, it is evident that the largest change in clustered phase angle difference is in the lower frequency bands for the intervention group (red arrows, Figure 5). There is not a direct comparison with coherence as this also takes signal magnitude into account. The phase-based sustained response to injury and control did not differ between groups ($p = 0.30$, $F_{5,108} = 1.08$, $\eta_p^2 = 0.01$).

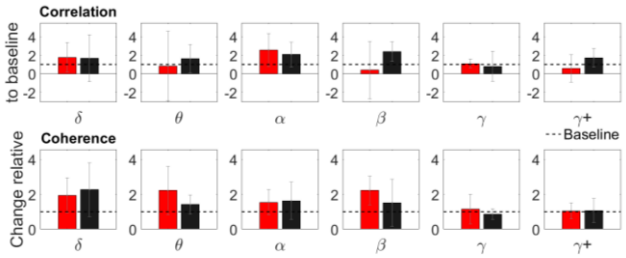

Fig. 7: Box plots showing the average correlation (top row) and coherence (bottom row) 3 hours after SNI for each group relative to baseline.

C. *Change in functional connectivity over time*

The development in functional connectivity over time was quantified as the difference in response from baseline to the hyperacute (minutes) and sustained (hours) response for each of the six frequency bands. When comparing the control and intervention groups, lower connectivity in the intervention group than control may be interpreted as an inhibition of synchronized activity whereas higher connectivity can be seen as facilitation of synchronized activity.

In the lower frequencies, immediate inhibition of activity in the intervention group (more activity in the control group compared to intervention) was seen, followed by excitation in

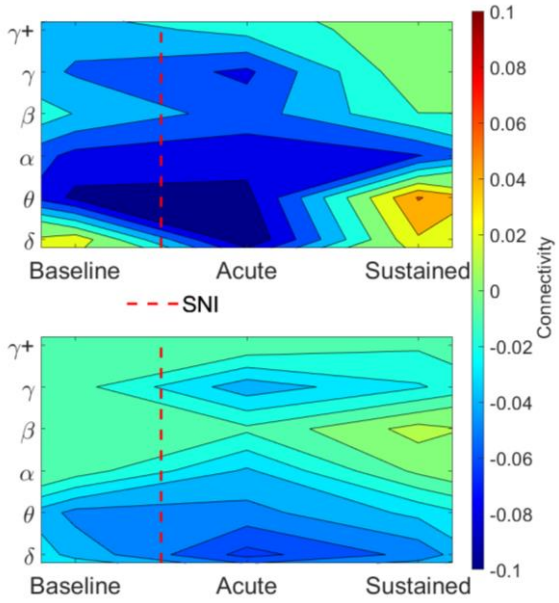

Fig. 8. Difference in correlation (top) and coherence (bottom) between SI and ACC between the intervention and control group. Yellow/red colors indicate more activity in the intervention group (excitation), and blue colors indicate more activity in the control group (inhibited activity in the intervention group). The data points are interpolated both in the x-axis (baseline to acute to sustained activity) and y-axis (delta, theta, alpha, beta, gamma and gamma+ frequency band).

the sustained phase (Figure 8). The cortical response differed between frequency bands ($p < 0.001$, $F_{1.66,0.14} = 12.20$, $\eta_p^2 = 0.43$) and changed over time ($p = 0.009$, $F_{2,32} = 0.06$, $\eta_p^2 = 0.26$). The cortical response in the two groups did not change significantly different over time (group*band*time interaction, $p = 0.54$, $F_{4.29,68.61} = 0.80$, $\eta_p^2 = 0.047$).

The coherence was similar for both groups in all frequency bands (Figure 8). There was an increase in the activity in the control group in the delta band, shown as less activity in the intervention group compared to controls. The cortical response differed between frequency bands ($p < 0.001$, $F_{5,80} = 43.45$, $\eta_p^2 = 0.73$) and changed over time ($p < 0.001$, $F_{2,32} = 146.29$, $\eta_p^2 = 0.90$). Similar to the correlation, the cortical coherence did not change differently for the two groups (group*band*time interaction, $p = 0.98$, $F_{10,160} = 0.30$, $\eta_p^2 = 0.018$).

IV. Discussion

The hyperacute and sustained reaction to a peripheral nerve injury was investigated in relation to functional connectivity.

We observed a decrease in the cortical correlation for the intervention group in the hyperacute phase. Additionally, there was a trend, although non-significant, in the intervention group for an increased coherence three hours after SNI. There was, however, no statistically significant effect of the nerve injury for neither the correlation in the time domain nor for the phase-based coherence when investigating specific frequency bands. This indicates an effect of acute nerve injury that is not related to one specific frequency band .

Several previous studies have investigated resting-state connectivity following a model of pain in rats either in a short (minutes) or long (days) time frame (Table 1). The most frequently used functional connectivity measures in resting-state studies are correlation and coherence. These two analyses contribute with different aspects of the interpretation of cortical changes. Correlation is the temporal synchronization of fluctuations of power while coherence is the clustering of difference in phase angles between the two signals modulated by signal amplitude [11]. For correlation to change, either an activation of silent synapses, formation of new or active synapses to become silent is needed. Changes in coherence can be seen as neurons having more or less synchronized.

Communication between two groups of neurons is more effective if it is coordinated [12] and an inhibited coherence indicates a disrupted communication, as shown in Zippo et al. (2016) where complex network theory and phase-locking value was used to differentiate nociceptive interventions [19].

A. Acute response

Cortical connectivity, quantified either as coherence or correlation, was inhibited in the intervention group. However, contrary to findings from previous research [15], [16], we observed that the acute response for the intervention and control group did not correspond to a significant change in coherence in specific frequency bands.

Only one previous study has investigated functional connectivity between SI and ACC in the acute phase of a model of pain [21], and the current study differs on several aspects, both in terms of recording method (fMRI vs. intracortical) and because they did not base their analysis dividing signals into the traditional frequency bands. The response in SI and ACC was found to correlate in the gamma and beta frequency bands during spontaneous pain behavior after repeated noxious laser stimuli in awake rats [17].

From acute capsaicin studies, it was shown that there was no significant change when measuring from outside the cortex [16] but a decrease in low (delta and theta) and high-frequency coherence (gamma) when measuring from inside the cortex [15]. In addition to the different models of pain, the interacting areas investigated differed from the two areas investigated in this study. Given that this was between SI and the pre-frontal

TABLE I
SUMMARY OF PREVIOUS RESEARCH USING RESTING-STATE FUNCTIONAL CONNECTIVITY ANALYSIS TO INVESTIGATE THE RESPONSE TO A MODEL OF PAIN OR NOXIOUS STIMULI, INCLUDING AT LEAST ONE OF THE AREAS INCLUDED IN THIS STUDY (SI AND ACC).

Study	Pain model	Connectivity measure	Cortical areas	Change
Chao et al. (2018)	SNI (immediate and after 1 and 8 days)	Correlation	SI, ACC, Insular-, pre-frontal cortex (fMRI)	N.S. (SI-ACC)
Baliki et al. (2014)	SNI	Correlation	All (fMRI)	N.S. (SI-ACC)
Song et al. (2019)	Repeated laser stimuli	Correlation	ACC and SI	↑Beta, Gamma
Xiao et al. (2019)	Repeated laser stimuli	Coherence	ACC and SI	N.S. 1-80 Hz
Cao et al. (2016)	IBS (after 7-14 days)	Phase lag index	ACC and amygdala	↓Theta
LeBlanc et al. (2014)	Capsaicin (15 min)	Coherence	SI and thalamus	↓ Delta, Theta, Gamma N.S. Alpha, Beta
LeBlanc et al. (2014)	CCI (after 3 days)	Coherence	SI and thalamus	↓ Delta, Beta, Gamma N.S. Theta, Alpha
LeBlanc et al. (2016)	Capsaicin (30 min.)	Coherence	SI and pre-frontal cortex	N.S. 1-30 Hz
LeBlanc et al. (2016)	CFA (after 2 days)	Coherence	SI and pre-frontal cortex	N.S. 1-30 Hz
LeBlanc et al. (2016)	CCI (after 7/14 days days)	Coherence	SI and pre-frontal cortex	↑ 1-30 Hz

N.S. = no statistical significance, SNI = spared nerve injury, IBS = inflammatory bowel syndrome, CCI = chronic constriction injury, CFA = Complete freund's adjuvant

cortex, and SI and thalamus, it is uncertain whether it would also be the case for coherence between ACC and SI, indicating very different results depending on which areas are being investigated, even within the areas traditionally related to pain processing.

Moreover, a similar study found a too large variance in coherence to draw a conclusion [18]. Similar to the study by Xiao et al. (2019), a large intra-group variance was found in this study, which is a likely explanation for the lack of significant results.

B. Sustained response

The SNI model did result in a non-statistically significant increase in low frequency and a decrease in high-frequency synchronization between SI and ACC for the intervention group. Lower frequency activity implies slower communication, in this case, a possible result of the peripheral injury. This was not significant, indicating that the change was small or that it was only the case in some rats.

The findings in the sustained phase of this study were exploratory, as it was not known what changes to expect in the hours following nerve injury. Most studies investigating the response to nerve injury recorded the response several days or weeks after injury. Thus, the sustained or sub-acute response has rarely been investigated. In the study by LeBlanc et al. (2014), no further investigation of functional connectivity was performed two hours after injection of capsaicin because the mean power had returned to baseline [15]. In anesthetized rats, the low-frequency potentials between areas immediately after a peripheral injury can be used to differentiate between injury and non-injury states in rats [19]. In general, previous studies [13]–[16], [22] have shown both increases and decreases in functional connectivity depending on the pain model and data analysis methods. A significant difference between these studies and the present study is the use of awake rats. With awake animals, the effect of anesthesia does not influence the results; on the other hand, many other factors (e.g. cognitive) may influence the outcome.

C. Development in functional connectivity

Mixed results occurred when investigating the correlation and coherence and how these parameters develop from the baseline into the acute and sustained phase. The change over time did not differ significantly between the groups. In the acute phase, an inhibited correlation and coherence were observed for the intervention rats, and in the sustained phase, an increased correlation for the intervention rats. To the authors' knowledge, only one other study has investigated the connectivity both in the acute and sustained phase. In a study by Chao et al. (2018), no change was found between baseline, the acute state, and the sub-acute state [21]. It is notable, however, that the sub-acute phase in this study was one day after injury and thereby the response in the period between minutes and a full day is still to be outlined. Since several studies have shown a change in connectivity days after injury between either SI or ACC and other cortical areas [14]–[16], [21], cortical reorganization must happen post-injury, possibly as a result of an increased synaptic transmission [14]. These cortical changes may not occur in the period investigated in this study. Following an animal pain model, Zippo et al. (2016) found collapsed functional connectivity (phase-locking value) between SI and thalamus after injury [19]. A human study with different groups of chronic pain patients found a stronger correlation between ACC, insula, and prefrontal cortex for the control group compared to the chronic pain patient groups [32]. In another study with pain patients, increased functional connectivity (phase lag index) was found when provoking the pain compared to a resting state [33]. However, there are no human studies investigating the acute (hours or days) pain phase.

D. Methodological considerations

One explanation for the observed changes in both the intervention and control group may be the use of an anesthetic agent. Isoflurane or similar anesthesia has been shown to induce slow cortical oscillations [34] and to break down interactions [34]–[37]. In fact, the isoflurane level was regulated to keep the physiological parameters stable and not to ensure the same level between rats and groups for ethical reasons. Isoflurane anesthesia has been shown to weaken the correlation in resting-state connectivity (fMRI) in mice [38]. Other types of anesthesia do not have the same effect on resting-state connectivity [38] and may, therefore, be recommended for future studies. Connectivity, measured as cross-correlation, in SI has shown to be preserved with low dose (1%) isoflurane although decreased compared to awake mice in fMRI – and isoflurane is better than other anesthetic agents in regards to depression of spiking activity [39]. It is therefore not known how the functional connectivity between ACC and SI is affected by the long-term administration. One study on monkeys, however, found decreased connectivity due to the long-term administration of isoflurane [40]. This could be a contributing factor to the non-significant results of this study. The effect of isoflurane on the results was checked using isoflurane level at the time of recording as a co-variant in the statistical analysis and it was found that there was no significant effect on any of the phases.

V. Conclusion

Using intracortical recordings of resting-state LFP's before and after the intervention, correlation and coherence were calculated to quantify the interaction between ACC and SI. Relative to baseline, the correlated time-frequency power decreased in the intervention group following peripheral nerve injury in the acute phase. Three hours after injury, the cortical synchronized activity, measured both as correlation and coherence, increased in the lower frequency bands and decreased in the higher frequency bands. This study shows that there are no significant cortical changes in resting-state functional connectivity in the hyperacute and sustained phase following a peripheral nerve injury. However, the connectivity between SI and ACC is altered in the hyperacute state. This observation indicates changes in the communication between SI and ACC with fewer neurons active after a nerve injury. These adaptions were not observed following the hyperacute

state. We conclude that the cortical activation induced by an animal model of neuropathic pain, as evidenced by coherence analysis, is immediately affected but a time frame longer than a few hours is required for the development of cortical plastic changes.

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