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Modulation of SI and ACC response to noxious and non-noxious electrical stimuli after the spared nerve injury model of neuropathic pain

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Conflicts of interest statement

None declared

Significance

This study showed distinct cortical processing of noxious and non-noxious peripheral stimuli in SI and ACC. The processing latency in ACC and accumulated spiking activity in SI appeared to be modulated by peripheral nerve injury, which elaborated on the function of these two areas in the processing of nociception.

Abstract

Background: The current knowledge on the role of SI and ACC in acute pain processing and how these contribute to the development of chronic pain is limited. Our objective was to investigate differences in and modulation of intracortical responses from SI and ACC in response to different intensities of peripheral presumed noxious and non-noxious stimuli in the acute time frame of a peripheral nerve injury in rats.

Methods: We applied non-noxious and noxious electrical stimulation pulses through a cuff electrode placed around the sciatic nerve and measured the cortical responses (6 electrodes in each cortical area) before and after the spared nerve injury model.

Results: We found that the peak response correlated with the stimulation intensity and that SI and ACC differed in both amplitude and latency of cortical response. The cortical response to both noxious and non-noxious stimulation showed a trend towards faster processing of non-noxious stimuli in ACC and increased cortical processing of non-noxious stimuli in SI after SNI.

Conclusions: We found different response in SI and ACC to different intensity electrical stimulation based on two features and changes in these features following peripheral nerve injury. We believe that these features may be able to assist to track cortical changes during the chronification of pain in future animal studies.

Introduction

Cortical neuroplasticity is believed to be one of the keys needed to unlock our understanding of chronic pain and pave the way for novel treatments in the future. Despite many years of research, there are still many unanswered questions, including why acute pain in some cases develops into chronic pain. The use of intracortical recordings has been suggested as a method for providing unique information about cortical neuroplastic mechanisms (Shyu et al., 2008; Wang et al., 2003; Yang et al., 2006) and as a possible route for the development of non-subjective and non-behavioural measurements of chronic pain (Zhuo, 2008, 2011).

The key brain areas believed to be involved in pain processing comprising the primary (SI) and secondary (SII) somatosensory cortices, the anterior cingulate cortex (ACC), the prefrontal cortex (PFC), and the insular cortex (Thompson and Bushnell, 2012). Across different rodent models of pain and noxious stimulation, the cingulate cortex, thalamus and SI are the most consistently activated areas (Thompson and Bushnell, 2012). SI and ACC have also been shown to be activated in human neuropathic pain patients (Apkarian et al., 2005; Seifert and Maihöfner, 2009). SI and ACC are believed to be involved in very different aspects of pain processing. SI is believed to be involved in the sensory-discriminative part of the processing such as localization of stimuli whereas ACC is involved in the affective-emotional part such as the reaction to pain (DosSantos et al., 2017; Seifert and Maihöfner, 2011; Treede et al., 1999). Both may encode the intensity of the pain or nociception (Treede et al., 1999).

Several studies using complete Freund's adjuvant (CFA) showed modulated activity in SI to non-noxious stimuli (Tan et al., 2019) and noxious stimuli (Singh et al., 2020) and in ACC to noxious stimuli (Singh et al., 2020; Zhang et al., 2018). The processing in ACC of electrical stimuli in Onishi et al. (2018) was also modulated by a nerve crush injury. On the other hand, in Chang et al. (2014), the response to non-noxious stimuli in SI - among other areas - was unchanged 5 days following the spared nerve injury model. Although cortical alterations occur within the first days after an irreversible injury (Chao et al., 2018; Han et al., 2015; Thibault et al., 2012), previous animal models of neuropathic pain have mainly investigated the cortical reaction days or weeks after an injury (Chang et al., 2014; Chao et al., 2018; Onishi et al., 2018). Besides Chao et al. (2018) who investigated the cortical response in the spared nerve injury model in the acute time frame, previous investigations in acute responses have been limited to noxious stimuli in the literature. As such, the current knowledge of the immediate responses to an acute injury are sparse.

The cortical reaction to peripheral noxious stimuli has been studied previously using both fMRI and electrophysiology (Chang and Shyu, 2001; Shyu et al., 2008; Yang et al., 2006; Zhao et al., 2012).

These studies show that SI and ACC are both activated by noxious stimuli, but it takes a tenfold stimulation intensity to evoke ACC responses in comparison to SI. Using the electrical stimuli, the same type of stimuli can be both noxious and non-noxious dependent on the intensity.

The present study aimed (i) to investigate if we could detect differences in the intracortical activity of SI and ACC in response to peripheral noxious and non-noxious electrical stimuli in rats and (ii) to investigate how the intracortical responses of SI and ACC are modulated by the spared nerve injury model (Decosterd and Woolf, 2000) in the first hours. SI and ACC were chosen both based on their involvement in pain processing but also because they are easily accessible in both animal studies and human EEG studies.

If distinct cortical responses can be found, these measures can pave the way to understanding the role of SI an ACC in nociceptive and non-nociceptive processing and how a peripheral nerve injury alters this processing. This could potentially be used as an objective measure of nociception.

Methods

The procedures were approved by the Danish Animal Experiment Inspectorate (J. no.: 2016-15-0201-00884). Eighteen male Sprague Dawley rats (age: 9-11 weeks, weight: 332-417 g, Taconics Europe) were used for the experiment. Ten animals were randomly allocated to the intervention group and eight to the control (i.e. sham) group. The animals were housed in cages 2-3 rats together in a room with a 12:12 dark/light cycle and had access to food and water ad libitum. Before the experiment started, the rats were housed for two weeks to allow acclimatization. This was followed by 1-2 weeks of training, where the animals were removed from their home cages for 10 min/day, to minimize the recordings being impacted by elevated stress levels on the experiment day. The training consisted of the animal being gently held and being in the anesthesia induction chamber.

Pain model

The intervention group was defined as the group of rats subjected to a spared nerve injury (SNI) model of neuropathic pain as previously proposed by Decosterd (Decosterd and Woolf, 2000) because of its robustness and reliability (Baliki et al., 2005; Chao et al., 2018). In this model, the tibial and common peroneal branches of the sciatic nerve are ligated and transected, leaving the sural branch intact. Leaving one branch intact results in less suffering for the animal as they do not try to self-mutilate in the case of a survival study (Devor and Raber, 1983) and enables stimulation of the nerve even after the injury.

Surgery and preparation

All animals were anesthetized in an induction chamber with 4 % isoflurane at a flow rate of 2 l/min. After the initial anesthetic induction, the animals were placed in a mask in a stereotaxic frame (model 900, small animal instrument, KOPF®) and the isoflurane was kept at 1-2.5 % with a flow rate of 0.5 l/min throughout the entire experiment (no recovery). The isoflurane was regulated based on physiological parameters (heart rate and breath rate). Because isoflurane lowers the breath rate, medical-grade oxygen (100 %) was supplied continuously to maintain a good oxygen saturation (<97 %). The temperature of the animal was kept at 38 ^{o}C and automatically regulated through a closed-loop system (ATC-2000, World Precision Instruments).

The surgery started by making an incision through the skin and biceps femoris in the right hind limb. After careful freeing of the sciatic nerve, an in-house manufactured cuff electrode (bipolar, length approx. 10 mm, inner diameter approx. 2 mm, (Haugland, 1996)) was placed around the nerve and secured with a suture for stability. Also, sutures (4-0, non-absorbable silk, Ethicon) were placed loosely around the tibial and common peroneal nerve branches as preparation for inducing the spared nerve injury later in the experiment. All procedures before recordings were performed in all rats.

A craniotomy was then performed creating a 6 mm x 4 mm hole (3 mm frontal to 3 mm caudal to Bregma, and 0.5 mm to 4.5 mm lateral to the midline) in the skull on the left hemisphere. The dura was carefully removed and a multi-electrode array (MEA, tungsten pins, 75 µm shank diameter, 0.5 mm distance between electrode pins, AlphaOmega) was inserted in the cortex with six electrodes in the primary somatosensory cortex (SI, depth: 1.4 mm) and six in the anterior cingulate cortex (ACC, depth: 2.6mm) for the recording of multiunit activities. Based on previous research using local field potentials (LFP) activity, the most significant change in SI following forepaw denervation happens in layer 5 (1.05-1.5 mm) (Han et al., 2013) within which the six pins in SI were placed. The depth of the pins in ACC was chosen based on a previous study investigating the acute phase following the SNI model (Chao et al., 2018). The array was inserted 0.6 mm further down than the desired depth and then retracted to ensure penetration of the wires and avoid dimpling of the brain surface. Placement of the electrode was according to Paxinos and Watson (2007) for both SI (location: 1.5 to 2 mm posterior and 1 to 3 mm lateral to bregma) and ACC (location: 0.5 to 2 mm anterior and 0.5 to 1 mm lateral to bregma) (Paxinos and Watson, 2007). After completion of the surgery, two hours were given for cortical acclimatization to the implant before the recordings began. An intramuscular needle electrode (bipolar, two standard hypodermic needles, custom-made at our facilities) was inserted into the right biceps femoris for recording the intramuscular motor activity and to allow identification of motor threshold. During the cortical electrode acclimatization period, the rat's individual movement thresholds of the hind limb biceps were assessed.

Experimental design

The rats were anesthetized during the experiment. For both the intervention and control group, a baseline was recorded where the rats were subjected to both noxious and non-noxious electrical stimulation. After the baseline, the intervention group was subjected to the SNI and the control group to a 15 min waiting period, followed by recordings where both groups were subjected to noxious and non-noxious stimulation similar to the baseline recordings. All preparation surgery was done prior to the baseline to both intervention and control, and the only surgical procedure done after baseline recordings was the ligation and transection of the nerve branches in the intervention group. After all data for each rat were recorded, the rats were euthanized by a lethal intracardial injection of pentobarbital.

Peripheral stimuli for investigating the cortical responses

Electrical noxious stimuli can be used to evoke and thereby evaluate how nociceptive pathways change following the SNI model. The peripheral stimulation was performed through a cuff electrode (Haugland, 1996) using a Multi-channel Systems STG2008 stimulator. The stimulation used was 2 Hz 0.1 ms square pulses. The stimulation intensities were adjusted to each individual rat based on the movement threshold. To identity the movement threshold, stimulation was applied with increasing stimulation amplitude. Each increment was 0.04 mA until muscle activity was visible in a real-time recording of motor activity with the intramuscular needle electrode (resulting in 0.24±0.05 mA threshold).

In Chang and Shyu, (2001), it was shown that peripheral electrical stimulation at a level of two times the muscle activation threshold resulted in SI activity and recruitment of 50 % of the maximum Aβ motor fiber activity and a level of ten times threshold resulted in the activation of both Aβ fibers and around 70 % Aδ nociceptive fibers. With a stimulation intensity below 5 times the muscle activation, very few nociceptive fibers are belived to be activated. Activation of nociceptive fibers does not necessary equal the perception of pain which has to be validated through e.g. behavioral studies. Thus the stimulation levels of the electrical stimulation is based on the assumptions that an intensity of less than 5 times muscle activation is non-noxious, whereas 10 times muscle activation (or more) is noxious.

During the intracortical recordings, three stimulation intensities were used. For evaluation of the cortical response to activation non-nociceptive sensory pathways, two assumed non-noxious electrical stimulation intensities were used. It was of interest to investigate the cortical response to different types of fibers being activated and the stimulus intensities were chosen based on an

assumption about fiber type activation. Based on previous results (Chang and Shyu, 2001), this study investigated the following three stimulation intensities:

- Low: Two times the muscle activation threshold. This level is just above movement threshold and is believed to mostly activate the sensory fibers
- Medium: Four times the muscle activation threshold. This level is clearly above movement threshold and is believed to activate both the sensory and motor fibers
- High: Ten times the muscle activation threshold. This level is believed to activate the nociceptive A-δ and potentially C fibers in addition to sensory and motor fibers.

Data collection

The experiment consisted of four recording cycles. In the intervention group, we recorded one cycle before and three after the nerve injury. In the control group, we recorded one cycle followed by a 15 min wait (approximately the duration of performing the intervention) and three cycles after the waiting period. Each recording cycle consisted of the three electrical stimulation intensities applied through the peripheral nerve cuff individualized to each rat and simultaneously recording of the cortical signals. Low, high, and medium intensity electrical stimuli were delivered sequentially in that order. The order of stimuli was not randomized to have the possibility of comparing stimuli across time with an equal amount of time between recordings and secondly to ensure the longest possible time between high intensity stimuli. The interval between recordings was 30 min except for the SNI/waiting period because it was important to start recording immediately after the procedure. Each recording consisted of a 30-s resting state and 1 min evoked activity during electrical stimulation (resulting in a total of 120 stimuli in each recording).

[figure 1: Before the recordings, the multi-electrode array was implanted, the cuff placed around the sciatic nerve, and the nerve was prepared for SNI. One cycle of recordings with three stimulation intensities of electrical stimulation comprised the baseline and three cycles followed the SNI procedure.]

Data processing

The intracortical signals and electromyography (EMG) signals were recorded with a sampling frequency of 24,414 Hz and 4,882 Hz respectively (PZ5 neuroDigitizer and PZ2 BioAmp Processor, Tucker-Davis Technologies) and analyzed offline. The recorded intramuscular EMG signal was only used for motor detection threshold and therefore not processed further. Since small amplitude signals are sensitive to noise, the recorded intracortical data were denoised as follows. The signals

were first pre-processed with a bandpass filter from 300-3000 Hz (2^{nd} order high and lowpass Butterworth IIR filter). A notch filter for 50 Hz and harmonics (2^{nd} order bandstop Butterworth IIR order) was subsequently applied.

In a post-stimulus time histogram (PSTH) analysis, spikes above a certain threshold are traditionally counted based on peak detection. In this case, a threshold of 3.5 times root-mean-square (RMS) of the 30 s baseline prior to each recording was used, and all spikes above were marked. For each recording, the z-score was calculated by subtracting average resting-state activity from each bin and dividing with resting-state standard deviation. Data were pooled by calculating the mean of the six electrodes for each area (SI and ACC respectively). As such, our PSTH responses represent accumulated activity relative to the peripheral stimuli (-50 ms to 450 ms after stimuli) in 5 ms bins.

Data analysis

The analysis consisted of two parts: Firstly, an investigation of SI and ACC processing of the three stimulation intensities, and whether this differs between areas and secondly, whether the activity during any of the three stimulation intensities in SI and/or ACC is modulated by SNI.

In both parts, the changes in cortical activity were tracked by quantifying the amplitude and latency of the highest peak in the PSTH. To ensure that the peak found was related to the stimuli but not the stimulation artefact, the peak had to be between 0.1 and 100 ms for SI and 0.1 and 200 ms for ACC. For clarity reasons, each recoding cycle is characterized using the cycle number it belongs to, both in the following and in the result section. Thus, the first three recordings following SNI are mentioned as "1^{st} cycle".

To investigate the effect of the three stimulation intensities, all baseline activities (peak amplitude and latency) from both groups were pooled and used to identify characteristics for ACC and SI activation of the three stimulation intensities. As neither group had been subjected to any intervention at baseline these were pooled. A two-way repeated-measures ANOVA with intensity (3 levels, low, medium, and high) and area (2 levels, SI and ACC) as within-subject factors was used. A posthoc test was performed in case of a statistically significant difference between stimulation intensities, using a Bonferroni-corrected multiple comparisons.

To investigate the effect of SNI, separate analyses were done for SI and ACC. A three-way repeated-measures ANOVA with intensity (3 levels, low, medium, and high) and time (4 levels, baseline, 1^{st}, 2^{nd}, and 3^{rd} cycle) as within-subject factors and group (2 levels, intervention and control) as between-subject factor was used. A Posthoc test was not performed as there was no statistically significant difference.

Results

Part 1: Activation of SI and ACC by noxious and non-noxious stimuli

Before the introduction of the spared nerve injury, the cortical response–measured in PSTH peak amplitude and latency–was significantly different between SI and ACC (Figure 2, see result of ANOVA in Table 1). The average PSTH peak was at 77.4 (95 % CI: 68.9-86.5) ms in SI and 192.6 (95 % CI: 144.1-241.1) ms in ACC. The peak response was significantly faster in SI. The accumulated activity in SI was significantly greater (71.4, 95% CI: 35.2-107.7) than in ACC.

Also, the two areas differed in the processing of the three intensity stimuli. To explore the differences in the two areas, post hoc comparison was made for the three stimulation intensities in each area.

[figure 2: Post-stimuli time histogram (PSTH) of the cortical response to three intensity levels of electrical stimuli in primary sensory cortex (top) and anterior cingulate cortex (middle) for all rats. The PSTH is the mean and shaded standard deviation. Boxplots (bottom) of peak amplitude and latency from the PTSH's. The boxplots represent the median (red line) and 25^{th} and 75^{th} percentile (outer lines of the box). The whiskers represent the highest and lowest data points, and '+' indicates outliers (1.5 times $25^{th}/75^{th}$ quantile). '*' indicate significant ($p<0.05$) difference.]

The result of the post hoc test showed that the peak amplitude in SI was significantly higher when using the high intensity stimuli compared to medium intensity ($p=0.01$, 95 % CI: 3.80-44.86, $d=3.10$). In ACC, the peak amplitude to high intensity stimuli differed from both low ($p=0.001$, 95 % CI: 8.24-34.64, $d=4.08$), and medium ($p=0.003$, 95 % CI:7.00-36.33, $d=4.12$) intensity stimuli. It is notable that the latency of the peak response in SI deviated very little between rats, as seen as a narrow box plot, compared to ACC where the peak response differed more between rats and possibly also between trials.

Part 2A: Modulation of cortical response by spared nerve injury in SI

The peak cortical activity in SI was significantly higher for higher intensity stimulation (Figure 3, see result of ANOVA in Table 2). The average z-scored activity was 99.8 (95% CI: 69.6-130.0), 111.4 (95% CI: 87.0-135.9), and 130.9 (95% CI: 103.4-158.5) for the low, medium and high intensity stimulus, respectively. The trend in peak latency was opposite that of peak amplitude, as higher intensity stimulation resulted in a faster response. The average latency in SI was 93.2 (95% CI: 64.5-121.9), 76.5 (95% CI: 68.4-84.6), and 74.5 (95% CI: 69.6-79.4) for the low, medium and high-intensity

stimulation, respectively. The peak amplitude but not latency differed between the two groups. To explore which intensities differed significantly in peak amplitude and latency, we made a post hoc comparison.

The result of the post hoc comparison was that the high-intensity stimuli resulted in a significantly higher peak activity than the other two intensities (p=0.001, 95 % CI: 12.28-49.94, d=4.78 and p=0.034, 95 % CI: 1.30-37.75, d=3.00). The peak latency for the three stimulation intensities was not significantly different (p=0.54-0.74, d=0.26-2.39).

[figure 3: Boxplots of the modulation of the cortical processing of three intensities of electrical stimuli to low (L), medium (M), and high (H) intensity stimulation, quantified as peak amplitude and latency from the post-stimuli time histogram in the primary sensory cortex.]

Given that there was no time*group*intensity interaction for the cortical processing in SI, the following is a description of trends in the data.

Alteration of cortical processing of non-noxious stimuli in SI.

An alteration of cortical processing of non-noxious stimuli after SNI towards processing similar to that of noxious stimuli may be a sign of mechanisms similar to the allodynia response seen in human neuropathic pain patients.

The z-scored peak in cortical response to low-intensity stimulation decreased initially following SNI, followed by an increase over time from 44.25 (95 % CI: 0.57-87.93) at baseline to 33.59 (95% CI: -6.46-73.64) after 1 hour and 59.13 (95% CI: 11.79-106.47) and 84.53 (95% CI: 28.07-140.98) after 2.5 and 5 hours (Figure 4). The same initial decrease and the following increase were not seen for the control group with an average peak response in SI at 140.69 (95% CI: 91.86-189.53) at baseline, and 152.75 (95% CI: 107.97-197.53), 137.48 (95% CI: 84.55-190.87), and 146.20 (95% CI: 83.08-209.32) at the following recordings.

[figure 4: Post-stimuli time histogram in the primary sensory cortex of a mean of all rats in each of the two groups before and after SNI/wait using the three electrical stimulation intensities for both the control (black shade) and intervention (red shades) group.]

The cortical processing in the two groups was similar for medium intensity stimulation. The z-scored peak response increased from 54.47 (95% CI: 7.55-101.39) at baseline to 101.51 (95% CI: 67.23-

135.79) at the last recording for the SNI group and from 128.46 (95% CI: 76.00-180.92) to 148.76 (95% CI: 110.43-187.08) for the control group.

Alteration of cortical processing of noxious stimuli in SI.

Similar to the possible development of an allodynia-like response after SNI, an increased response to noxious stimuli after SNI may be a result of mechanisms similar to those seen in hyperalgesia in human neuropathic pain patients.

The same trend as was seen for medium intensity stimulation was seen for the high-intensity stimulation. The z-scored peak response increased from 72.91 (95% CI: 25.50-120.33) at baseline to 122.11 (95% CI: 74.60-169.61) at the last recording for the SNI group and from 160.15 (95% CI: 107.14-213.61) to 177.87 (95% CI: 124.76-230.98) for the control group.

Part 2B: Modulation of cortical response by spared nerve injury in ACC

The peak cortical activity in ACC was significantly higher for higher intensity stimulation (Figure 5, see result of ANOVA in Table 3). The average z-scored activity was 15.70 (95% CI: 12.11-19.28), 18.08 (95% CI: 13.67-22.49), and 35.81 (95% CI: 25.73-45.90) for the low, medium and high intensity stimulus respectively. The peak latency for the low and medium intensity stimulation was similar at 192.56 (95% CI: 159.68-225.45) and 194.95 (95% CI: 157.43-232.48) ms, where as for high intensity stimuli it was 126.44 (95% CI: 95.01-157.87) ms. The peak amplitude but not latency differed between the two groups. To explore which intensities differed significantly in peak amplitude and latency, we made a post hoc comparison.

The post hoc comparison showed that the high-intensity stimuli resulted in a significantly higher peak activity than the other two intensities ($p=0.001$, 95 % CI: 9.04-31.18, $d=4.35$ and $p=0.001$, 95 % CI: 7.70-27.76, $d=3.84$). Low and medium intensity peak latency were significantly higher than the peak latency using the high stimulation intensity ($p<0.001$, 95 % CI: 30.25-102.0, $d=6.45$ and $p=0.009$, 95 % CI: 16.45-102.59, $d=6.68$).

[figure 5: Boxplots of the modulation of the cortical processing of three intensities of electrical stimuli of low (L), medium (M), and high (H) intensity, quantified as peak amplitude and latency from the PSTH's in anterior cingulate cortex.]

As the cortical processing in SI in the two groups was not different over time dependent on stimulation intensity (time*group*intensity interaction, see results of ANOVA in Table 3), the following is a description of trends in the data.

Alteration of cortical processing of non-noxious stimuli in ACC.

Similar to SI processing, the trend in cortical processing of low-intensity stimulation in ACC was different between the groups. In the SNI group, the peak amplitude decreased initially from 11.29 (95% CI: 2.71-19.88) at baseline to 9.18 (95% CI: 2.51-15.86) 1 hour following SNI, followed by an increase to 10.69 (95% CI: 1.31-20.08) 2.5 hours after SNI and 12.65 (95% CI: 9.80-15.49) 4 hour after SNI (Figure 6). In the control group, the trend was opposite as the average peak amplitude decrease from 23.87 (95% CI: 14.27-33.46) at baseline to 23.63 (95% CI: 16.17-31.09), 21.76 (95% CI: 11.26-32.25), and 12.50 (95% CI: 9.32-15.68) at 1, 2.5 and 4 hours, respectively. In addition to the trend in peak amplitude response, the peak latency evolvement differed between the groups. Whereas the peak latency decreased between baseline and last recording from 266.00 (95% CI: 177.85-354.15) to 183.00 (95% CI: 101.76-264.24) ms in the SNI group, the same parameter increased from 155.63 (95% CI: 57.07-254.18) to 205.00 (95% CI: 114.17-295.83) ms in the control group. Thus, in both SI and ACC, the trend in processing low intensity, non-noxious stimuli is possibly modulated by SNI as it differs between the two groups

[figure 6: Post-stimuli time histogram in the anterior cingulate cortex of a mean of all rats in each of the two groups before and after using the three electrical stimulation intensities for both the control (black shade) and intervention (red shades) group.]

The average z-scored peak amplitude response to medium intensity stimulation increased for both groups. In the SNI group, the response increased from 12.77 (95% CI: 6.82-18.72) at baseline to 14.67 (95% CI: 4.11-25.23) at the last recording and in the control group from 21.51 (95% CI: 14.86-28.17) at baseline to 25.28 (95% CI: 13.48-37.01) at the last recording.

Alteration of cortical processing of noxious stimuli in ACC.

The cortical processing of high-intensity stimulation in ACC was, as opposed to the trend in SI, different between the two groups. In the SNI group, the peak amplitude increased from 27.76 (95% CI: 9.67-45.85) at baseline to 30.02 (95% CI: 16.29-43.74) and 33.64 (95% CI: 13.10-54.19) 1 and 2.5 hours after SNI followed by a decrease to 26.38 (95% CI: 14.41-38.34) at the last recording 4 hours after SNI. In the control group, the average peak response in ACC decreased from 51.51 (95% CI: 31.29-71.74) at baseline to 37.29 (95% CI: 21.94-52.64), 49.05 (95% CI: 26.08-72.01), and 30.84 (95% CI: 17.47-44.21) at the following recordings.

Discussion

The knowledge is currently limited regarding the role of SI and ACC in acute pain processing in addition to how these contribute to the development of sustained pain. We, therefore, compared the acute (minutes/hours) intracortical responses of SI and ACC to different intensities of peripheral electrical stimuli in rats.

Part 1: Increased fiber activation leads to increased cortical activation

With increased stimulation and thereby activation of more fibers, the cortical activation of SI and ACC was increased. Consistently with previous findings (Shyu et al., 2008; Yang et al., 2006), the response to low and high-intensity stimuli was significantly different. It may, in addition to more fibers being activated also be additional types of fibers being activated, e.g. nociceptive fibers when using high stimuli (Chang and Shyu, 2001). Which fibers are being activated can be studied using peak latency from a peripheral recording electrode. It was not an option in this study to stimulate and record from the sciatic nerve medial to the branches. In ACC the increased activity is most likely because, the activation is limited when using a stimulus less than ten times the motor threshold (Chang and Shyu, 2001; Shyu et al., 2008; Yang et al., 2006). In addition, several studies confirm this finding as ACC has increased activation when using noxious stimuli compared to non-noxious stimuli (Singh et al., 2020; Zhang et al., 2018). Based on previous research, it was expected that the peak activity in ACC would be smaller (Chang and Shyu, 2001) and slower (Kuo and Yen, 2005; Wang et al., 2008a; Xiao et al., 2019) than that in SI when using noxious stimuli. This is overall in accordance with the findings in this study for both noxious and non-noxious stimuli although, for the three stimulation intensities, the latency of the response to noxious stimuli in ACC was less different from SI. The latency could also be correlated with the fibers activating the two areas. The Aβ and Aδ fibers are myelinated and thereby faster than the unmyelinated C fibers. It is, however, difficult to draw conclusions about which fibers are activated without peripheral testing and all stimulation intensities most likely activated all types of fibers to some degree. Furthermore, the presumption about the low stimuli intensities being non-noxious and the high intensity being noxious is based on the assumption of additional fiber types being activated. The longer latency of the peak in ACC could be an expression of ACC being activated by SI. In fact, a study by Singh et al. (2020) showed that ACC receives input from SI (Singh et al., 2020).

Part 2: Animal models of pain modulates cortical activation

The SNI model of pain introduced several trends in alteration of cortical processing. Even though there was no time*group*intensity interaction, possibly due to low power in the statistical analysis,

the two groups did differ in peak amplitude. The trend in the processing of assumed non-noxious stimuli was that the SNI model modulated the processing in SI, especially for the lowest stimuli. The cortical response was increased for the intervention group after SNI. In addition, the processing of noxious stimuli following SNI resulted in a larger accumulated peak amplitude in ACC compared to baseline and the processing of non-noxious stimuli resulted in decreased latency also in ACC. An increased response to stimuli could be a result of central sensitization with a lower threshold for activation (Woolf and Doubell, 1994). This is possibly mechanisms similar to allodynia and hyperalgesia as seen in human neuropathic pain patients.

Previous studies have shown an increased firing rate (Singh et al., 2020) and increased theta and gamma LFP power (Xiao et al., 2019; Zhang et al., 2018) in ACC when using noxious stimuli in CFA-rats. Spiking activity and LFP power are correlated, especially in the lower frequencies such as the theta band (LeBlanc et al., 2017; Xiao et al., 2019). In accordance with CFA-rats, LeBlanc et al. (2014, 2016) showed increased LFP power in SI in capsaicin-rats (Leblanc et al., 2014; LeBlanc et al., 2016a). The current study indicates that changes do occur shortly after SNI. Chao et al. (2018) found an increased response in SI, insular cortex, ACC during, and shortly after (5-20 min) SNI using fMRI (Chao et al., 2018). The increase in the insular cortex and ACC was confirmed by intracortical single-unit recordings. An immediate increase was seen in this study but this was not followed by a decrease, at least not in the ~3 hours of recording. In accordance with Chao et al. (2018), Chang et al. (2017) found that the SI response to tactile stimuli was not different compared to controls 5 days after SNI recorded with fMRI (Chang et al., 2017). It is notable that the study by Chang et al. (2017) differs in recording method (fMRI instead for intracortical recordings) and uses resting-state recordings, whereas electrical stimuli were used during the recordings in the present study. In human subjects, allodynia has been found to activate SI but not always ACC (Seifert and Maihöfner, 2011).

Methodological considerations

In the present study, the rats remained anesthetized for the entire experiment, as opposed to other previous studies (Chang et al., 2014; Chao et al., 2018; Decosterd and Woolf, 2000), where behavioral observations took place along the experiments; which confirmed the development of neuropathic pain signs. By having the rats anesthetized during the whole experiment, the possibility of confirming the model of pain with behavioral signs of pain is missing, on the other hand confounding factors (e.g. stress, or movement) affecting nociceptive processing are eliminated. The results and conclusion in this study are based on the assumption that the SNI caused neuropathic

pain based on previous studies (Baliki et al., 2005; Chao et al., 2018; Decosterd and Woolf, 2000) showing consistent behavioral signs of neuropathic pain following SNI. In this study, similar to most animal research, the animals were all from the same breed, age, and sex. As pain is a subjective phenomenon, only nociceptive stimuli and models of pain can be used in animals and especially anesthetized animals. Furthermore, the anesthetic agent used in this study-isoflurane-has been shown to block part of the cortical spiking activity (Wu et al., 2012) but also that it is feasible to use in studies using electrical stimuli (Sommers et al., 2009). Even though this limitation is present, conclusions can still be made on basic mechanisms in processing nociceptive stimuli due to the rats being anesthetized before and after injury and the comparison with anesthetized control rats. Even though the anesthetic agent may influence the results, both groups are anesthetized with the same type of anesthesia and for an equally long time. The anesthetic depth was regulated based on physiological parameters and it is expected that both groups would be equally affected. Thereby must differences between the intervention and control group be caused by SNI. Similar to a possible effect of anesthesia, the results may be influenced by the previous stimulation. The order of stimulation intensities was not randomized but as with the issue of anesthesia, this was the case for both groups and it is expected that both groups would be equally effected. Another complication it that the injured nerve is also the nerve that is being stimulated. Even though the nerve is ligated, the absence of a significant immediate increase in activity in this study could be due to fewer nerve fibers firing or a reaction from the CNS to a loss of fibers.

In the present study, the cortical acitivity was analysed using multi-unit activity (MUA). Some of the issues, such as difference between rats and groups, may potentially have been avoided using single-unit activity (SUA). Through, averaging trials using PSTHs and electrodes as we have done in the present work, noise issues will typically be less problematic. Additionally, Chao et al. (2018) showed immediate cortical changes following SNI using MUA. Compared to SUA, MUA is more comparable to EEG and fMRI which is the most commen approaches to studying human pain.

A study by Wang et al. (2016) showed that cortical changes due to a model of inflammation do not occur until 28 days after intervention (Wang et al., 2016). It may be that the cortical changes after a model of pain do not occur in the time frame investigated in the present study. Cortical changes in the days following nerve injury may be a result of immediate changes besides spiking activity, e.g. changes in neurotransmitters (Hung et al., 2014) or due to interactions between cortical areas (Abaei et al., 2016; LeBlanc et al., 2016a, 2016b; Wang et al., 2008b), rather than changes in a single area. If this is the case, changes in single areas, as investigated in this study, may not appear until later (days). Another explanation could be that the changes that do occur are in the form of cortical oscillations and not stimulus-locked spiking activity, as several studies have found pain related

changes in LFP power in animals (Han et al., 2013; Leblanc et al., 2014; Li et al., 2017; Tan et al., 2019) or fMRI or electrocorticography (ECoG) studies in human subjects (Gross et al., 2007; Liu et al., 2015; Schulz et al., 2015). Cortical activity recorded in humans either from MRI or ECoG is comparable with intracortical LFP, which has been shown to correlate with intracortical spiking activity (LeBlanc et al., 2017; Xiao et al., 2019). The comparison with chronic pain studies may be complicated as most studies do not investigate the time interval and the cortical processes days or weeks after injury may differ significantly from those in the acute phase. That underlines the importance of an increased understanding of the acute phase.

Even though no previous study has investigated when exactly these two areas change in response to a nerve injury, it has been shown that some plastic changes are established during the first 10 days (King et al., 2011; Xie et al., 2005). The design of this study does not allow for conclusions about where in the nociceptive pathway, between the peripheral injury and the cortical recording site, changes appear.

When using three-way repeated-measures ANOVA, the many different comparisons require a lot of power and it may, therefore, be necessary to include more animals in a similar study to be able to show significant changes. It is noteworthy though that the general trend today is moving away from relying only on p-values as they have no meaning in itself and instead look at whether changes are meaningful or clinically relevant (Wasserstein et al., 2019).

Conclusions

The objective of this study was to investigate differences in the intracortical responses of SI and ACC in response to different intensities of peripheral stimuli in the acute time frame (minutes/hours) in rats, and how the spared nerve injury modulated processing of noxious and non-noxious stimuli. We found that the cortical response to different stimulation intensities differed significantly in SI and ACC. We found decreased latency in ACC and increased accumulation peaks in SI after SNI which may indicate modulation in the hours following injury. These findings could indicate allodynia- and hyperalgesia-like response to nerve injury in rats.

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Author contributions

L.T., G.A.D.V., and W.J., designed the study and G.A.D.V. designed the cortical electrode. L.T. and G.A.D.V. acquired the data. L.T. performed data analysis and wrote the paper. All authors discussed the results, and reviewed and commented on the paper.

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Figure legends

figure 1: Before the recordings, the multi-electrode array was implanted, the cuff placed around the sciatic nerve, and the nerve was prepared for SNI. One cycle of recordings with three stimulation intensities of electrical stimulation comprised the baseline and three cycles followed the SNI procedure.

figure 2: Post-stimuli time histogram (PSTH) of the cortical response to three intensity levels of electrical stimuli in primary sensory cortex (top) and anterior cingulate cortex (middle) for all rats. The PSTH is the mean and shaded standard deviation. Boxplots (bottom) of peak amplitude and latency from the PTSH's. The boxplots represent the median (red line) and 25^{th} and 75^{th} percentile (outer lines of the box). The whiskers represent the highest and lowest data points, and '+' indicates outliers (1.5 times 25^{th}/75^{th} quantile). '*' indicate significant ($p<0.05$) difference.

figure 3: Boxplots of the modulation of the cortical processing of three intensities of electrical stimuli to low (L), medium (M), and high (H) intensity stimulation, quantified as peak amplitude and latency from the post-stimuli time histogram in the primary sensory cortex.

figure 4: Post-stimuli time histogram in the primary sensory cortex of a mean of all rats in each of the two groups before and after SNI/wait using the three electrical stimulation intensities for both the control (black shade) and intervention (red shades) group.

figure 5: Boxplots of the modulation of the cortical processing of three intensities of electrical stimuli of low (L), medium (M), and high (H) intensity, quantified as peak amplitude and latency from the PSTH's in anterior cingulate cortex.

figure 6: Post-stimuli time histogram in the anterior cingulate cortex of a mean of all rats in each of the two groups before and after using the three electrical stimulation intensities for both the control (black shade) and intervention (red shades) group.

Tables

Table 1: Result of the statistical analysis of cortical response to different intensities of electrical stimulation. F-and, p-values, and partial eta^2.

Table 2: Result of the statistical analysis of the cortical response in the primary sensory cortex over time to different intensity of electrical stimuli for the intervention and control group. F- and p-values, and partial eta^2.

Table 3: Result of the statistical analysis of the cortical response in the anterior cingulate cortex over time to different intensity of electrical stimuli for the intervention and control group. F- and p-values, and partial eta^2.

Table 1: Result of the statistical analysis of cortical response to different intensities of electrical stimulation. F-and, p-values, and partial eta^2.

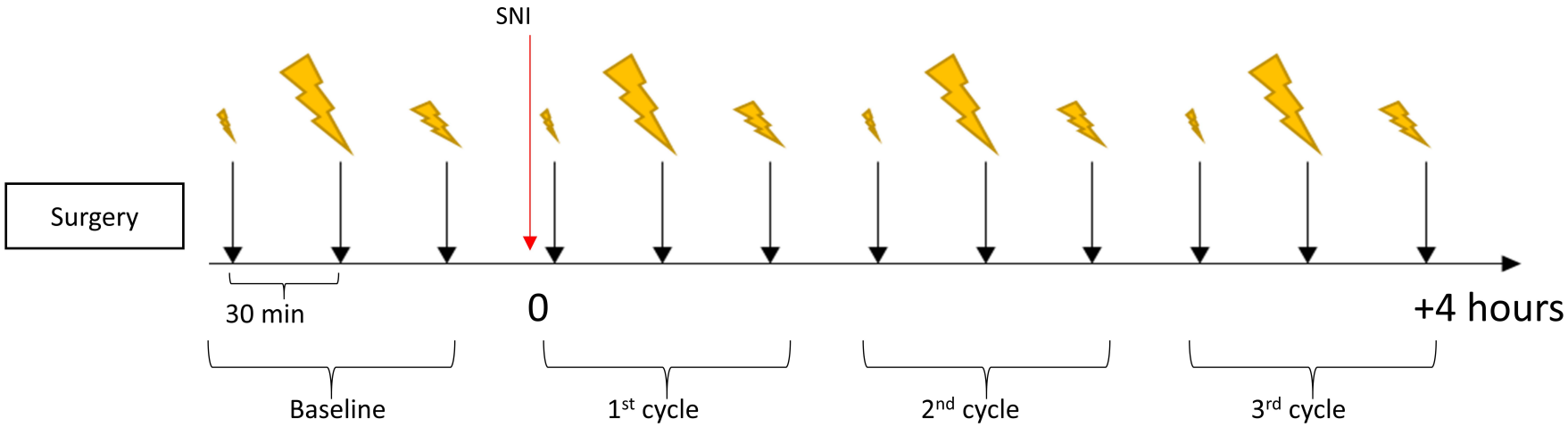
	Peak amplitude	Peak latency
Area	$F_{1,17}$=20.34, P<0.001, η_p^2=0.55	$F_{1,17}$=19.79, P<0.001, η_p^2= 0.54
Intensity	$F_{1.44,34}$=19.41, P<0.001, η_p^2= 0.53	$F_{2,34}$=5.34, P=0.01, η_p^2= 0.24
Area*intensity	$F_{2,34}$=1.38, P=0.27, η_p^2=0.08	$F_{2,34}$=3.40, P=0.045, η_p^2= 0.17

Table 2: Result of the statistical analysis of the cortical response in the primary sensory cortex over time to different intensity of electrical stimuli for the intervention and control group. F- and p-values, and partial eta^2.

	Amplitude	Latency
Time	$F_{1.75,48}$=2.11, p=0.15, η_p^2=0.12	$F_{1.93,48}$=0.53, p=0.59, η_p^2=0.03
Group	$F_{1,16}$=9.85, p=0.006, η_p^2=0.38	$F_{1,16}$=0.24, p=0.63, η_p^2=0.02
Intensity	$F_{2,32}$=11.12, p<0.001, η_p^2=0.41	$F_{1.02,32}$=1.72, p=0.21, η_p^2=0.10
Time*group	$F_{1.75,48}$=0.81, p=0.44, η_p^2=0.05	$F_{1.93,48}$=1.72, p=0.20, η_p^2=0.10
Time*Intensity	$F_{4.00,96}$=0.53, p=0.71, η_p^2=0.03	$F_{2.56,96}$=1.12, p=0.35, η_p^2=0.07
Group*intensity	$F_{2,32}$=0.05, p=0.36, η_p^2=0.06	$F_{1.02,32}$=0.02, p=0.89, η_p^2=0.001
Time*group*intensity	$F_{4.00,96}$=0.65, p=0.63, η_p^2=0.04	$F_{2.56,96}$=1.14, p=0.34, η_p^2=0.07

Table 3: Result of the statistical analysis of the cortical response in the anterior cingulate cortex over time to different intensity of electrical stimuli for the intervention and control group. F- and p-values, and partial eta^2.

	Amplitude	Latency
Time	$F_{1.87,48}$=1.46, p=0.25, η_p^2=0.08	$F_{3,48}$=0.75, p=0.53, η_p^2=0.05
Group	$F_{1,16}$=4.71, p=0.05, η_p^2=0.23	$F_{1,16}$=2.53, p=0.13, η_p^2=0.14
Intensity	$F_{1.44,32}$=20.16, p<0.001, η_p^2=0.56	$F_{2,32}$=10.86, p<0.001, η_p^2=0.40
Time*group	$F_{1.87,48}$=1.33, p=0.27, η_p^2=0.08	$F_{3,48}$=4.73, p=0.006, η_p^2=0.23
Time*Intensity	$F_{3.65,96}$=2.01, p=0.11, η_p^2=0.11	$F_{3.53,96}$=0.26, p=0.88, η_p^2=0.02
Group*intensity	$F_{1.44,32}$=0.14, p=0.80, η_p^2=0.009	$F_{2,32}$=2.77, p=0.09, η_p^2=0.15
Time*group*intensity	$F_{3.65,96}$=1.29, p=0.28, η_p^2=0.08	$F_{3.53,96}$=0.47, p=0.74, η_p^2=0.03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Surgery
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0
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1st cycle
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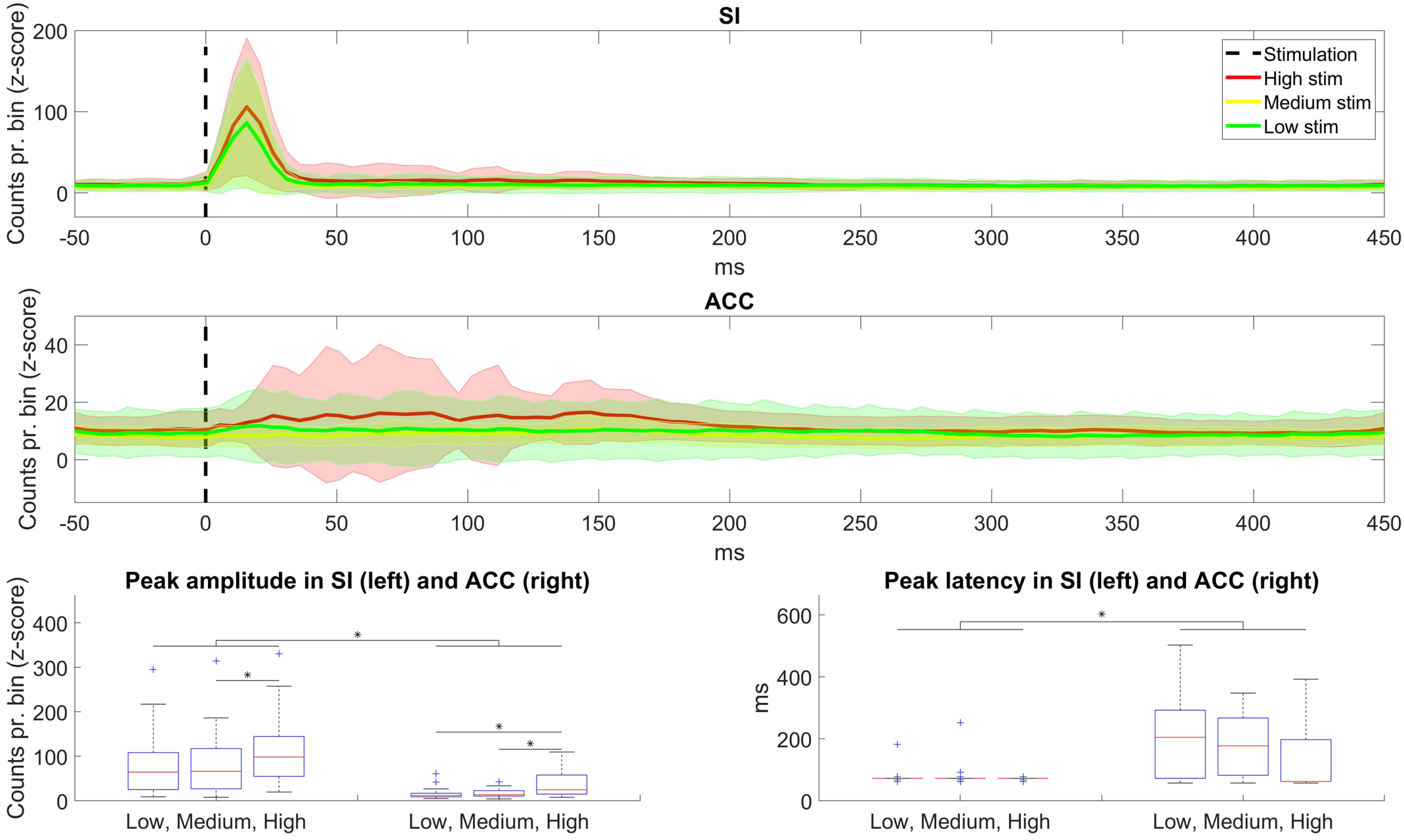
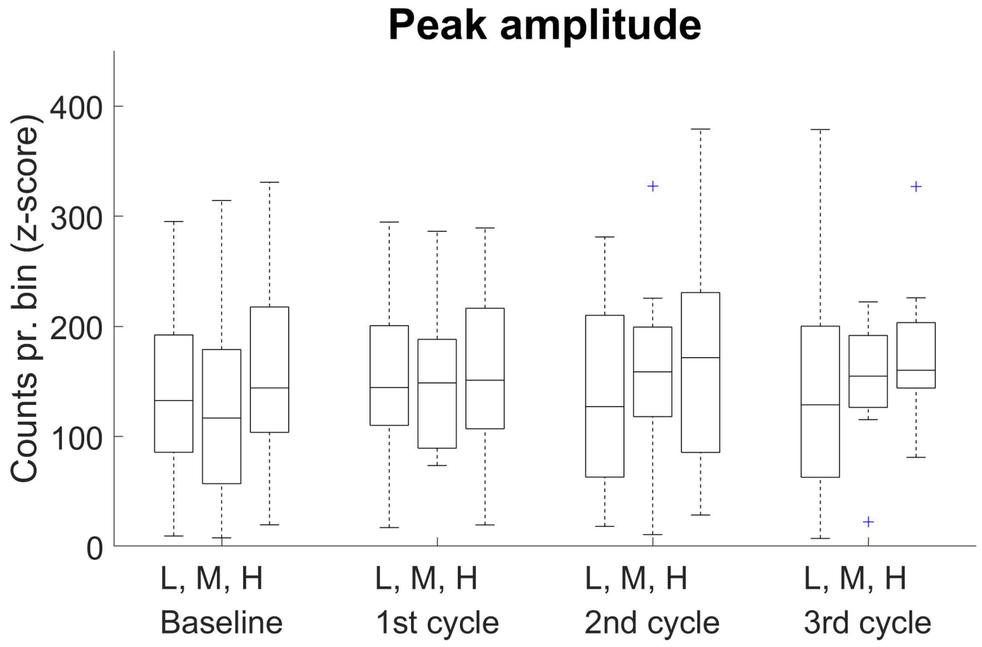
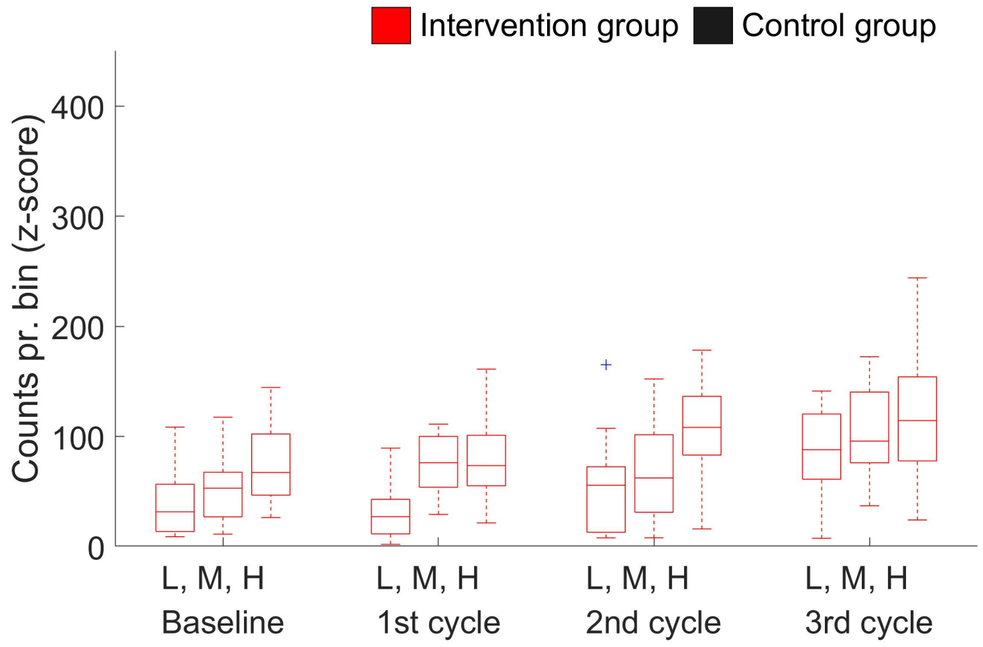
3rd cycle


SI
Counts pr. bin (z-score)
200
100
0
-50
0
50
100
150
200
250
300
350
400
450
ms
Stimulation
High stim
Medium stim
Low stim
ACC
Counts pr. bin (z-score)
40
20
0
-50
0
50
100
150
200
250
300
350
400
450
ms
Peak amplitude in SI (left) and ACC (right)
Counts pr. bin (z-score)
400
300
200
100
0
Low, Medium, High
Low, Medium, High
Peak latency in SI (left) and ACC (right)
ms
600
400
200
0
Low, Medium, High
Low, Medium, High


Peak amplitude
Counts pr. bin (z-score)
0
100
200
300
400
L, M, H
Baseline
L, M, H
1st cycle
L, M, H
2nd cycle
L, M, H
3rd cycle


Intervention group
Control group
Counts pr. bin (z-score)
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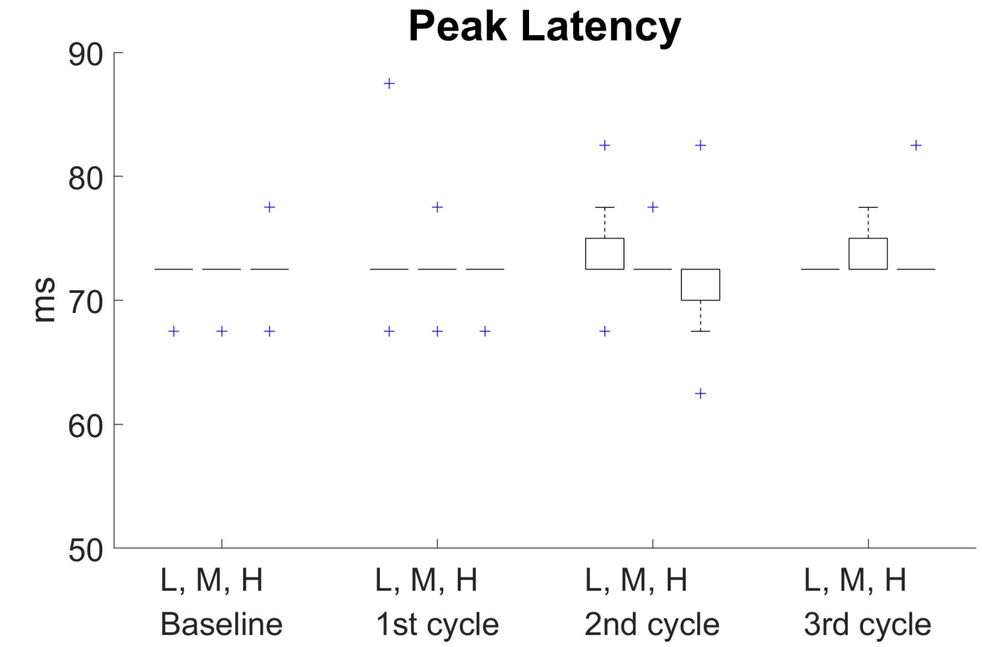
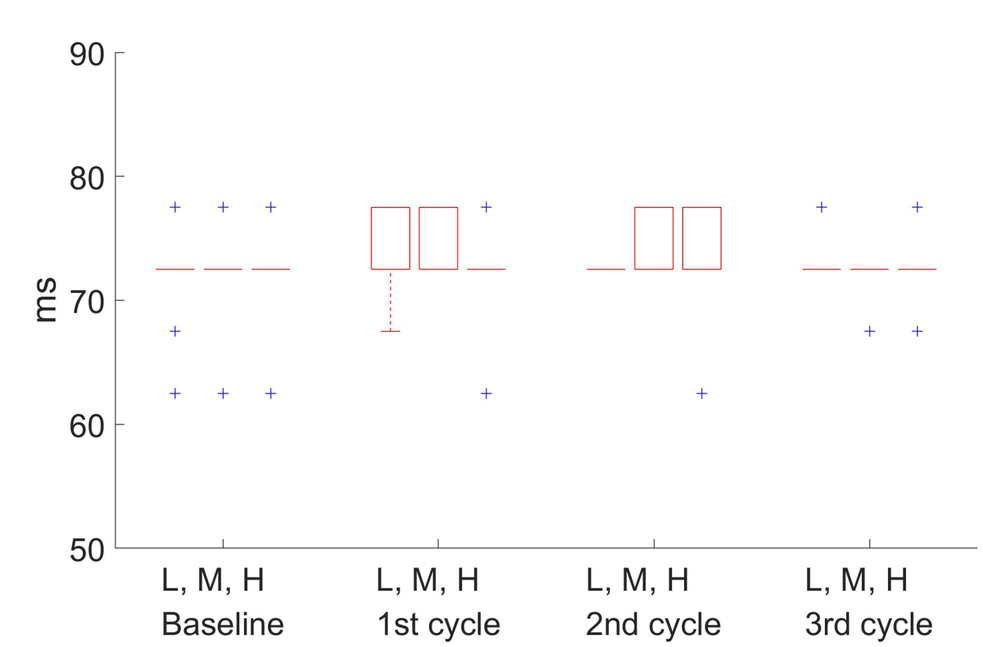
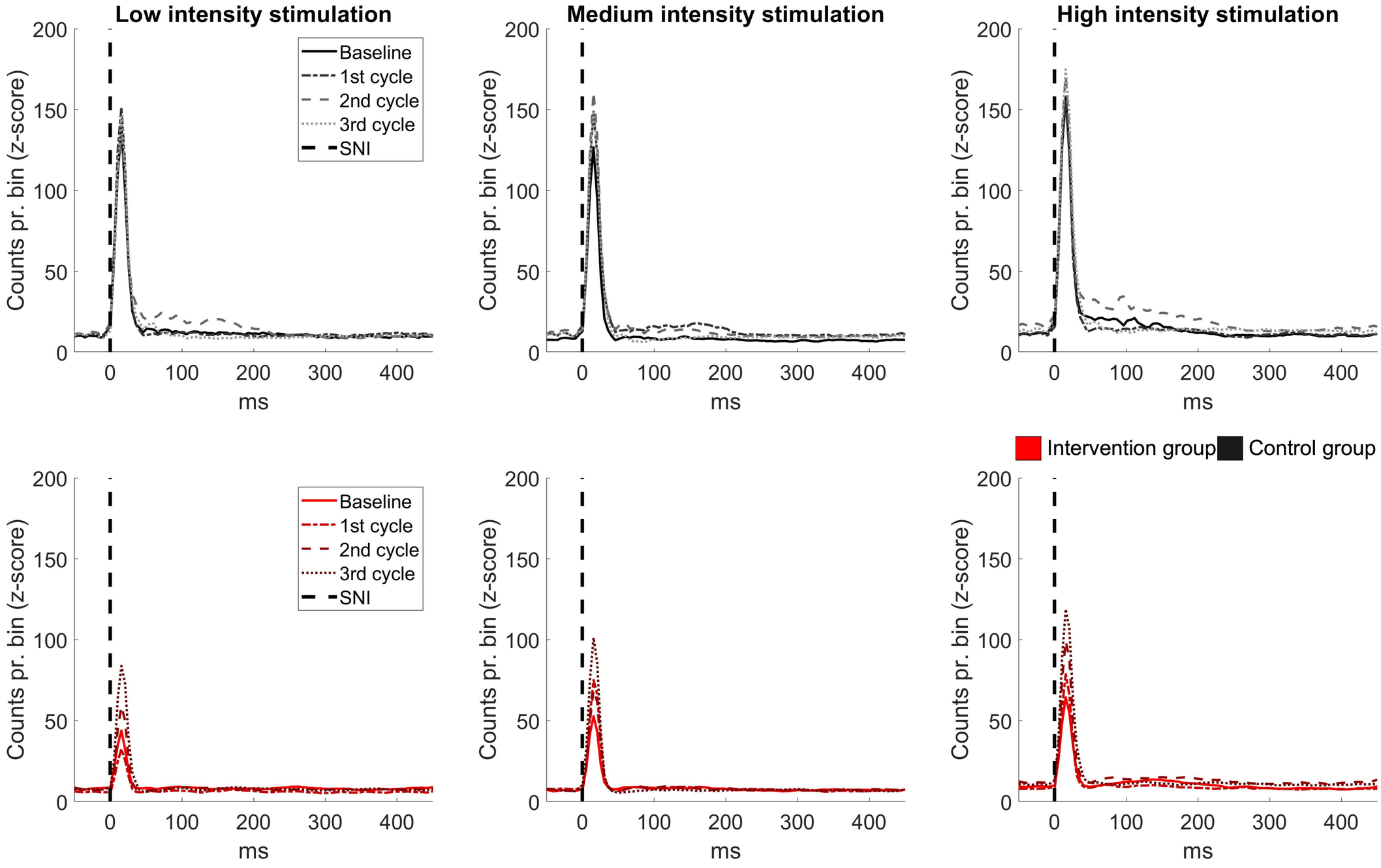
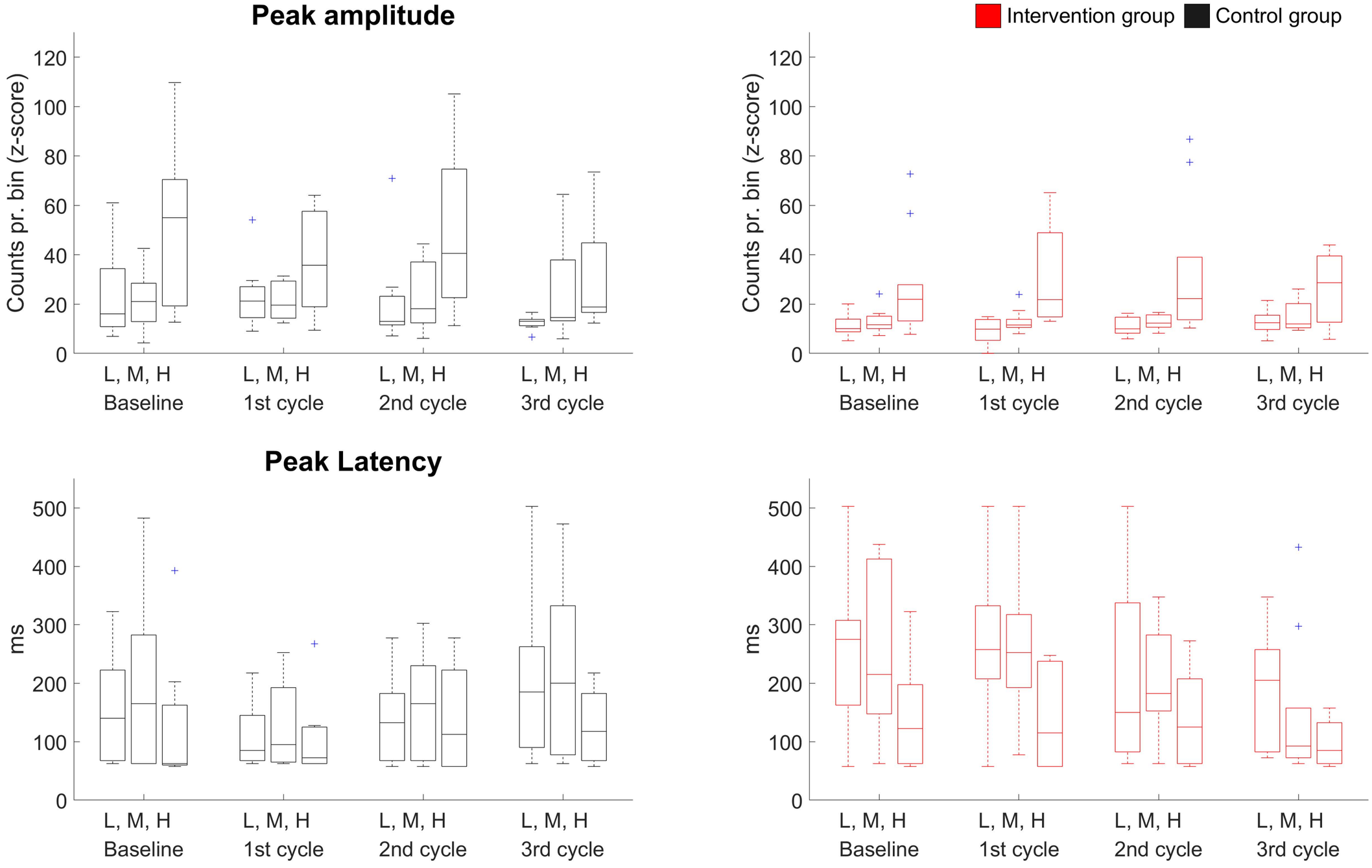
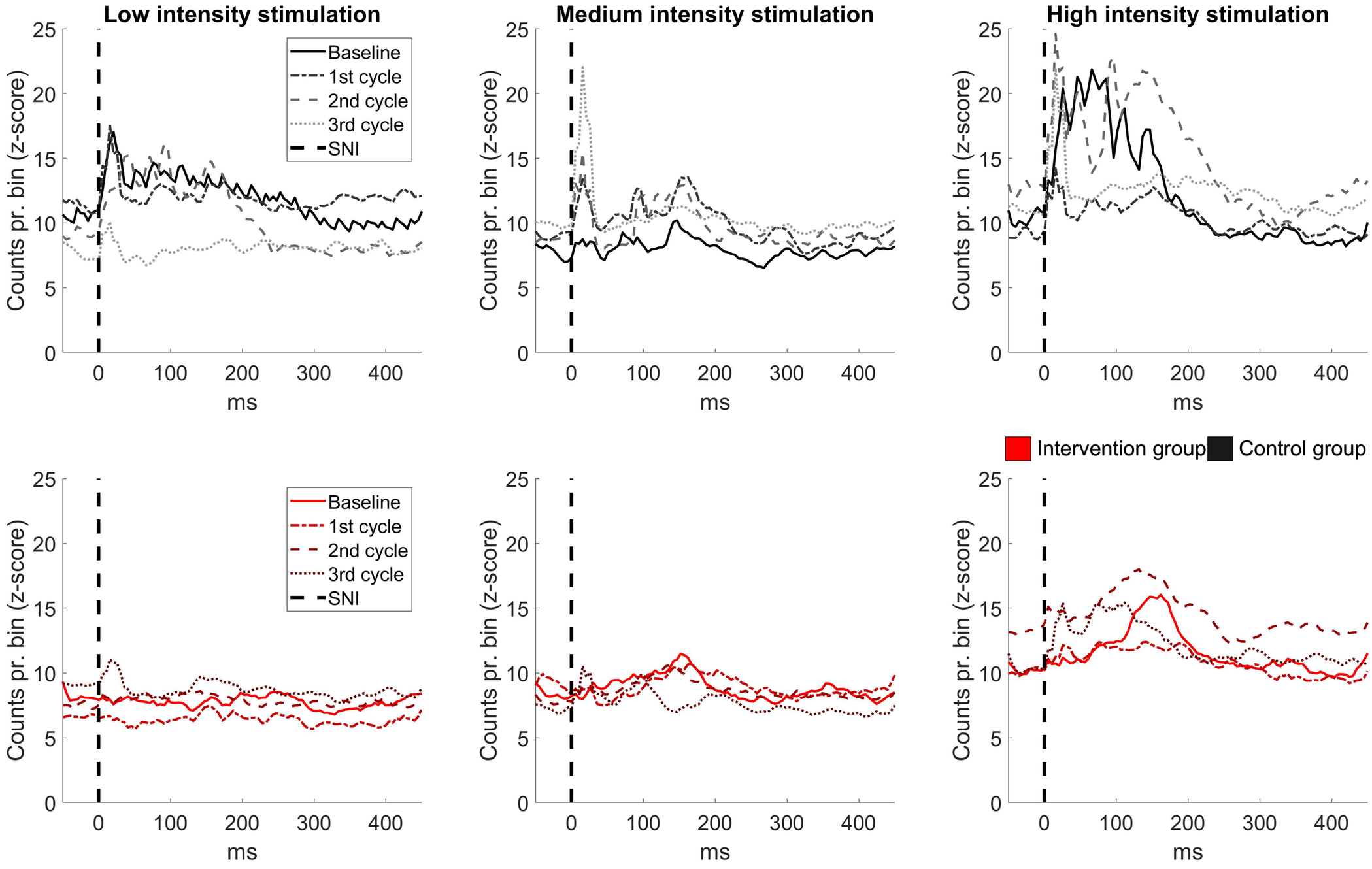
400
L, M, H
Baseline
L, M, H
1st cycle
L, M, H
2nd cycle
L, M, H
3rd cycle


Peak Latency
ms
50
60
70
80
90
L, M, H
Baseline
L, M, H
1st cycle
L, M, H
2nd cycle
L, M, H
3rd cycle


ms
50
60
70
80
90
L, M, H
Baseline
L, M, H
1st cycle
L, M, H
2nd cycle
L, M, H
3rd cycle


Low intensity stimulation
Medium intensity stimulation
High intensity stimulation
Baseline
1st cycle
2nd cycle
3rd cycle
SNI
Counts pr. bin (z-score)
ms
0
50
100
150
200
0
100
200
300
400
Intervention group
Control group


Peak amplitude
Intervention group
Control group
Counts pr. bin (z-score)
0
20
40
60
80
100
120
L, M, H
Baseline
1st cycle
2nd cycle
3rd cycle
Peak Latency
ms
0
100
200
300
400
500


Low intensity stimulation
Medium intensity stimulation
High intensity stimulation
Baseline
1st cycle
2nd cycle
3rd cycle
SNI
Counts pr. bin (z-score)
ms
0
100
200
300
400
5
10
15
20
25
Intervention group
Control group