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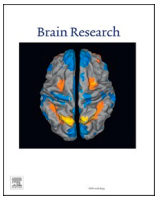

Differences in intracortical responses following non-noxious and noxious stimulation in anaesthetized rats

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ABSTRACT

Cortical responses have been proposed as a source for the extraction of unique and non-subjective sensory information. The present study aimed to investigate if it is possible to distinguish between non-noxious and noxious cortical responses with two different types of anesthesia. Sixteen rats were randomly allocated to receive either Hypnorm/Dormicum (HD) or isoflurane (ISO) anesthesia. Each animal had a custom-made microelectrode array implanted in the primary somatosensory cortex to record the local field potentials and a cuff electrode implanted around the sciatic nerve to deliver electrical stimulations. Three stimulation intensities were applied: 1x movement threshold (MT) (i.e., non-noxious activation), 5x MT (low intensity noxious activation), and 10x MT (high intensity noxious activation). The evoked potentials were assessed by extracting three features: 1) the negative peak (NP), 2) the positive peak (PP), and 3) the peak-to-peak (PtP) amplitudes. Our results showed that it was possible to distinguish between three levels of stimulation intensities based on the NP, PP, and PtP features for the HD group, whereas it was only possible to make the same differentiation with the use of PP and PtP when applying ISO. This work is believed to contribute to a basic understanding of how the cortical responses change in the hyperacute phase of pain and which cortical features may be suitable as objective measures of nociception.

1. Introduction

PAIN is influenced by the sensory, affective, and cognitive systems and can thereby be difficult to examine. Today pain is typically assessed by verbal communication in humans and behavioral measurements in animals. As such, the assessments are often based on subjective rather than objective measurements (Delgado et al., 2018; Deuis et al., 2017). Therefore, the use of information extracted directly from the brain has been hypothesized to provide unique and non-subjective, non-behavioral information of cortical mechanisms of pain processing (Zhuo, 2011; Zhuo, 2008).

Pain processing involves several brain areas, including the primary somatosensory cortex (SI), the secondary somatosensory cortex (SII), the anterior cingulate cortex (ACC), and the insula cortex. However, none of these areas are exclusively related to pain processing. The main roles of pain processing in these four areas are believed to be: SI is mainly involved in the sensory discriminative aspects of pain; SII is mainly involved in the recognition, learning, and memory of pain; ACC is mainly involved in the unpleasantness and response choice; and insula is mainly involved in the reaction, learning, and memory of pain. Therefore, the SI is a target of interest since it is thought to mainly transmit information about sensory features of noxious stimuli and not as much about the affective-motivational aspects of pain as other areas. (Li et al., 2017; Xiao et al., 2019; Su et al., 2019; Shaw et al., 2001; Wang et al., 2003; Hudson, 2000; Schnitzler and Ploner, 2000; Van Oostrom et al., 2007; Schouenborg et al., 1986).

Using intracortical (IC) recordings in animal models to assess the cortical function may pave the way for a more intricate and detailed understanding of neural pain processing in acute and chronic timeframes.

It has previously been attempted to identify objective measures of pain and nociception by using sensory evoked potentials (SEPs) following high-intensity peripheral laser or electrical stimulation. SEPs have been identified as a promising target feature since they have shown to correlate with the pain intensity in humans and with freezing behavior in rats (Van Oostrom et al., 2007; Kakigi et al., 1989; Van Oostrom et al., 2005; Murrell and Johnson, 2006; Stienen et al., 2004). Kakigi et al. (1989) showed that when decreasing the stimulation intensity in humans, the subjective perception of pain decreased along with a reduction of the SEP amplitude (Kakigi et al., 1989).

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Several studies have investigated the change in SEPs in rats following peripheral electrical stimulation applied at different intensities. For example, a study by Zhang et al. (2018) investigated how non-noxious, low intensity noxious, and high-intensity noxious stimulation affected the sensory evoked local field potentials (LFPs). They analyzed the power, amplitude, and latency of the LFPs, which showed a relatively high specificity and sensitivity in decoding the onset and intensity of pain (Zhang et al., 2018). In addition, a study by van Oostrom et al. (2007) showed that an increase in SEP amplitude correlated with increased fear-induced behavior in rats when the stimulation intensity was increased (Van Oostrom et al., 2007). The tests and recordings for both these studies were carried out while the animals were awake and moved around freely. Oppositely, a study by Chang et al. (2001) investigated the changes in brain activation following non-noxious and noxious electrical stimulation of the sciatic nerve with the use of fMRI while the rats were anesthetized. They showed increased activation of the somatosensory cortex during noxious stimulation in comparison with non-noxious stimulation. The same study also recorded the compound action potentials of the dorsal root for analyzing Aα, Aβ, Aδ, and C fiber activation. They showed that at higher stimulation intensities the dorsal root activity increased and C fibers started to be activated (Chang and Shyu, 2001).

Instead, Li et al. (2017) investigated laser-evoked cortical neural oscillations of the LFPs ranging from non-noxious to noxious stimulation intensity in rats (Li et al., 2017). The results showed that it could be identified when the noxious stimulation was present based on the neural oscillation features.

Electrical stimulation is an easy and safe method to elicit SEPs in both human and animal studies. However, SEPs elicited by non-noxious, low intensity noxious, and high intensity noxious electrical stimulation have not been studied in anesthetized animals. In anesthetized animals, it is important that the anesthesia does not block or suppress the cortical signals or nociceptive responses. The two main types of anesthesias are injection anesthesia and inhalant anesthesia, of which the injection anesthesia is more difficult to regulate. However, there are different active components in the two types of anesthesia as most inhalant anesthesias contain components with a brain suppressing effect, which most injection anesthesias do not (Flecknell, 2009; Antognini and Carstens, 1999). Therefore, the present study aimed to investigate if it was possible to distinguish between intracortical responses evoked by non-noxious and noxious stimulations (i.e., three intensities) with two types of anesthesia (inhalant and injection). The work is believed to contribute to a basic understanding of how the cortical responses change in the hyperacute phase of pain and which cortical features may be suitable as objective measures of nociception.

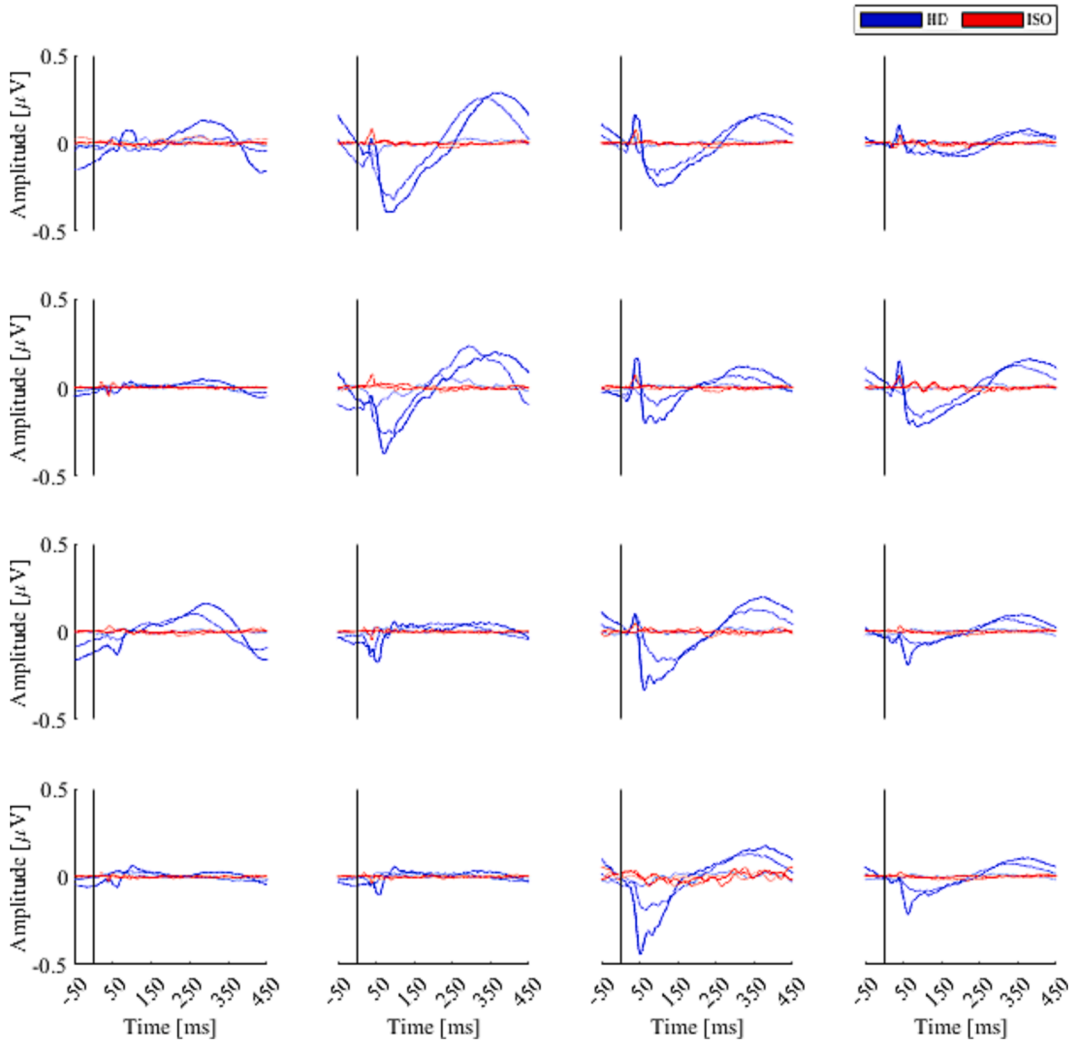

Fig. 1. Shows the responses for both HD (blue, the darker blue line, the higher the stimulation intensity) and ISO (red, the darker red line, the higher the stimulation intensity) with the three intensities for all 16 channels shown visually as placed in the cortex. it is seen that the responses correlate with the increased intensity. this is valid for both HD and ISO but is more prominent for HD than ISO. the vertical black line is the stim onset. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Results

2.1. Local field potential ERPs

The ERPs (event-related potentials) obtained from the LFPs for the three intensities and both the HD and ISO group are plotted in Fig. 1. It is seen that the HD group had a higher response than the ISO group based on the visual inspection. The ISO group also had ERP responses. However, the magnitude of these responses was not as prominent as for the HD group, see Fig. 2. Both groups revealed a positive correlation between stimulation and the amplitude of the response.

2.2. Negative peak amplitude (NP)

The change in amplitude represented by the grand mean of channels and rats is plotted in Fig. 3(A). The difference in NP was higher with the use of HD than with ISO. A significant increase ($p < 0.001$) between Non-Nox and Low-Nox and between Non-Nox and High-Nox stimulation for both the HD and ISO groups was found. Furthermore, a significant increase ($p < 0.001$) was found between Low-Nox and High-Nox for the HD group, but not the ISO groups. Our results indicated that it is only possible to distinguish the Non-Nox from the two noxious intensities (Low-Nox and High-Nox) for both types of anesthesia. However, for HD it is also possible to distinguish between Low-Nox and High-Nox stimulation. Secondly, the difference in absolute amplitude is more prominent for the HD group than for the ISO group.

2.3. Positive peak amplitude (PP)

The change in the PP amplitude as a grand mean of channels and rats is visualized in Fig. 3(B). A statistically significant ($p < 0.001$) increase was found between Non-Nox and Low-Nox as well as between Non-Nox and High-Nox stimulation for both the HD and ISO group. Statistical significance was also found for both HD ($p < 0.05$) and ISO ($p < 0.001$) between Low-Nox and High-Nox stimulation intensities. Our results indicate that this feature can be used for distinguishing between all three stimulation intensities.

2.4. Peak-To-Peak amplitude (PtP)

The change in PtP amplitude as a grand mean of channels and rats is visualized in Fig. 3(C). A significant increase ($p < 0.001$) was found between Non-Nox and Low-Nox and between Non-Nox and High-Nox stimulation intensities. Additionally, a significant increase was found for both HD ($p < 0.001$) and ISO ($p < 0.005$) between Low-Nox and High-Nox stimulation intensities. These results indicate that this feature can be used for distinguishing between all three stimulation intensities.

2.5. Negative peak latency

The change in NP latency is visualized in Fig. 3(D) as a grand mean of all channels and rats. Here, a significant increase ($p < 0.05$) was seen for HD between the Non-Nox and High-Nox stimulation intensities. Furthermore, a significant decrease ($p < 0.001$) was seen for ISO between Non-Nox and Low-Nox as well as between Non-Nox and High-Nox. These results may indicate that for HD, in which there was an increase in the latency, the slower fibers were more likely to be activated at the High-Nox stimulation intensity than for the Non-Nox stimulation intensity. This indicates that not only did the responses have a greater magnitude, but they were also slower; thus, pointing towards slower fibers being activated.

3. Discussion

In the present study, the response of the SI following Non-Nox, Low-Nox, and High-Nox stimulation intensities was investigated in anesthetized rats. We found that a distinction could be made between the Non-Nox and the two noxious stimulation intensities: Low-Nox and High-Nox in both the HD and ISO groups for the three features: NP, PP, and PtP. In addition, for the HD group a statistical significance was observed between Low-Nox and High-Nox for the same three features (NP, PP, and PtP). For ISO, this was only found for two features: PP and PtP. Furthermore, statistical significances were found in the NP latency feature. Here, an increase in latency was observed between Non-Nox and High-Nox for HD, whereas for the ISO a significant decrease was found in the latency between the non-noxious and the two noxious stimulation intensities. A difference was also observed in the magnitude of the IC (intracortical) responses in which the responses were larger in the HD group than in the ISO group. This indicates that it is possible to distinguish between the three intensities while the rats are anesthetized for the three features NP, PP, and PtP with the use of HD, whereas with the use of ISO it is only possible to distinguish between the three intensities with two of the features: PP and PtP. Therefore, HD seems to be the most suitable method for recording of cortical responses to noxious

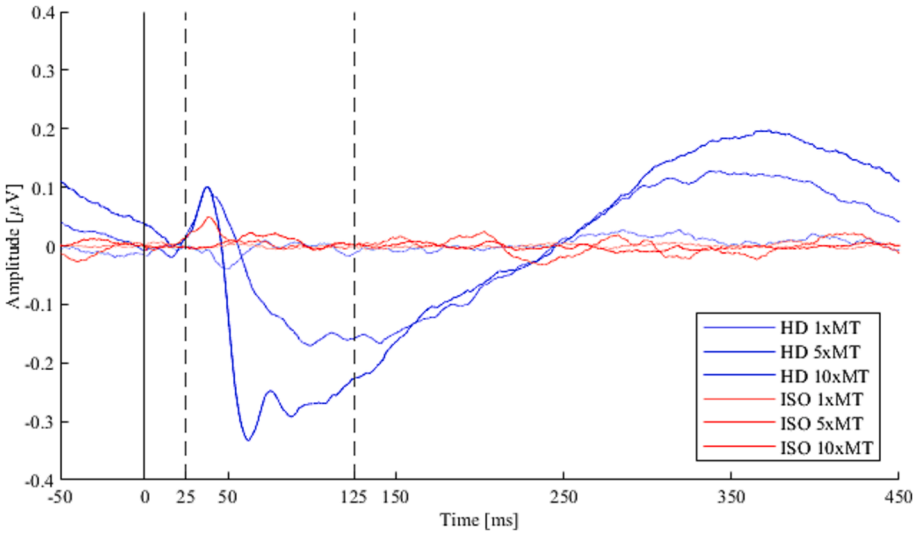

Fig. 2. Shows the same channel from one rat anaesthetized with HD and one rat with ISO. the vertical solid line represents stimulation onset. the two vertical dashed lines represent the time window used for identification of the N1 and P1. It is seen that both HD and ISO have responses, although the response magnitude is higher for HD. this channel and these rats are representative of all rats and channels.

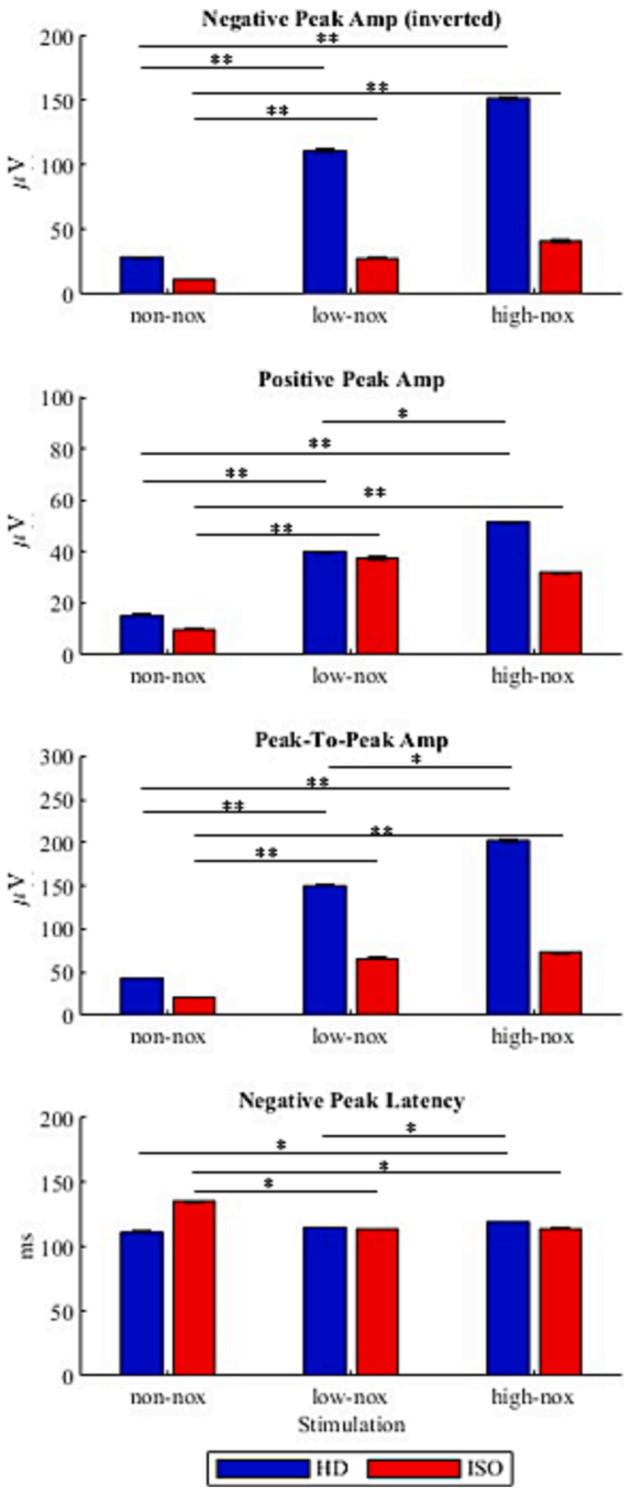

Fig. 3. Shows the NP, PP, and PtP amplitudes as well as the latency of N1. All calculated as a grand mean of all channels and rats (mean ± SEM). (A) shows the NP amplitude. It is seen that both the HD and ISO group have a significant change from Non-Nox to both Low-Nox and High-Nox. The HD also shows a significant difference between Lox-Nox and High-Nox. (B) shows the PP amplitude. For both HD and ISO a significant difference is seen between all three stimulation intensities. (C) shows the PtP amplitude. Here, a difference is also seen between all three stimulation intensities for both HD and ISO. (D) shows the latency change of the negative peak. For ISO, a significant decrease is seen in the latency for both Low-Nox and High-Nox to Non-Nox. For HD, a significant increase is seen in the latency for High-Nox compared with Non-Nox.

stimulation in anesthetized rats.

3.1. Local field potentials as a measure of nociception

LFPs have previously been used as a measure of pain intensity and as a measure of the depth of anesthesia (Xiao et al., 2019; Su et al., 2019; Zhang et al., 2018; Antunes et al., 2003; Silva et al., 2010). Upon inspection of the signals in the present work, a more pronounced variance and fluctuation were observed in the signals when using ISO compared with HD. This finding may indicate that with the use of ISO, the brain signals might be suppressed even though the level of anesthesia was kept as low as possible throughout the experiment. However, it was still possible for the PP and PtP features to distinguish between all three intensities.

The amplitude of NP, PP, and PtP provides information about how much the IC signals change according to the stimulation intensities used (Xiao et al., 2019; Su et al., 2019; Zhang et al., 2018; Antunes et al., 2003). In the current study, the NP, PP, and PtP amplitudes all showed a difference between all three intensities with the use of HD, whereas with the use of ISO, a difference between all three intensities could only be found in the PP and PtP amplitudes. This is in line with the studies by van Oostrom et al. (2007) and Zhang et al. (2018). They found that with noxious stimulation the amplitude of the signals increased compared with the non-noxious stimulation intensities (Van Oostrom et al., 2007; Zhang et al., 2018). Overall, our results indicate that it should be possible to differentiate between non-noxious and noxious stimulation when using either HD or ISO. In addition, with HD it is also possible to differentiate between Low-Nox and High-Nox. As such, the ISO anesthesia might have a suppressing effect on the cortical response. This neural suppression caused by ISO has previously been reported by, e.g., Antunes et al. (2003), who found that ISO caused greater depression of the central nervous system than halothane at the same levels of anesthesia (Fontanini and Katz, 2008).

3.2. Distinction between stimulation intensities

The difference between the cortical responses to Low-Nox and High-Nox was not as pronounced as it was between Non-Nox and the two noxious stimulation intensities. A probable reason may be that the cortical response reached a plateau before the Low-Nox stimulation intensity (5x MT). In the study by Stienen et al. (2004), in which they investigated the response to different stimulation intensities from 0.1 to 5 mA, it was seen that the response did not change significantly between going from 2 mA up to 5 mA. This also indicates that the response might plateau in this range (Stienen et al., 2004). However, it should be remembered that the results cannot be directly compared with the difference in the choice of stimulation intensities.

3.3. The latency of the negative peak

The latency of the NP may be related to the type of fibers recruited. Slower Aδ and C fibers are more likely to be recruited at higher stimulation intensities than at lower stimulation intensities. In the current study, there was a significant decrease in the NP latency with ISO between the Non-Nox and the two noxious stimulation intensities, whereas with HD, the NP latency increased from Non-Nox to High-Nox stimulation. This could indicate that with the use of HD as the anesthetic agent the fibers with lower conduction velocities are responding with the High-Nox stimulation intensity, whereas for the ISO the conduction velocity decreased from Non-Nox to the two noxious stimulation intensities. Therefore, this could indicate that when using HD as anesthesia, it is possible to obtain responses to fibers with lower conduction velocities than when using ISO (Martini and Nath, 2009; Kandel et al., 2000).

3.4. Considerations regarding anesthesia

When using HD as anesthesia, bolus injections are normally given every 30–40 min. However, in the current study an infusion pump was chosen for continuous administration of HD to have the same conditions for both types of anesthesia. The amount of HD anesthesia administered with the pump was equal to bolus injections given over the same period (Nielsen and Jensen, 2017).

When using a bolus injection every 30–40 min, there would likely be periods when the anesthesia would be deeper due to an uncontrolled uptake of the substances in the body, which may influence the IC signals.

The cases in which it was needed to add additional anesthesia could potentially influence the data in that some data points were made when anesthetic depth was higher than others. However, this does not seem to be the case in the current study since the modulation was still present and it was evaluated with stimulation intensity in random order for each of the rats used.

4. Conclusion

In the present work, we compared IC responses to three stimulation intensity levels: Non-Nox (1x MT), Low-Nox (5x MT), and High-Nox (10x MT) in two groups of rats anesthetized by HD or ISO, respectively. The IC response could differentiate between the three stimulation intensities for PP and PtP with both types of anesthesia (HD and ISO). In addition, with HD a differentiation could also be made between all three intensities for the NP. This suggests that with the use of HD it is possible to differentiate between non-noxious and noxious stimulation intensities regardless of the three features. This makes it interesting to investigate if it is possible to identify when there is a shift between the non-noxious and noxious stimulation and possibly when the stimulation shifts from low noxious to high noxious fibers being activated.

5. Methods

5.1. Experiment preparation

The Animal Experiment Inspectorate under the Danish Veterinary and Food Administration (application: 2016–15-0201–00884/MABJE) approved all experimental procedures. Sixteen male Sprague-Dawley rats (age 10–12 weeks, weight (mean ± SD): 357 g ± 27.8 g, Taconics Europe) were included in the study. All cages had soft bedding, animals had access to food ad libitum, and a 12 h/12 h day/night cycle. Rats were never housed alone in a cage for more than two days. On arrival, the rats were quarantined for 14 days, followed by one week of daily hand training to familiarize the rats with handling by the experimenter. Hand training was performed to avoid stress while inducing the initial anesthesia.

5.2. Animal preparation

The rats were randomly divided into two groups for the administration of anesthesia using either Hypnorm Dormicum (HD) (injection anesthesia) or Isoflurane (ISO) (gas anesthesia). Both types of anesthesias was chosen because they are some of the most commonly used types of anesthesia, that do not contain potent analgesics, which is desirable to avoid when investigating nociception. The only difference between the two groups was the anesthesia; the remaining procedures were identical for both groups.

HD group (9 rats): For the induction of anesthesia with HD (a mix of fentanyl 0.315 mg/ml, fluanisone 20 mg/ml and midazolam 5 mg/ml), the rats were first given a 0.3 ml/100 g body weight bolus injection. For continuous administration of anesthesia throughout the experiment, the rats were then connected to a subcutaneous catheter with a micropump (CMA 402 by Harvard Apparatus). The micropump had a flow rate corresponding to 0.0033 ml/100 g body weight/min. This dose corresponded to anesthesia levels administered in previous studies, see, e.g., (Nielsen and Jensen, 2017). A supplementary dose of 0.025 ml/100 g body weight was given subcutaneously if needed, e.g., if tail stiffening, whisker movement, or a response to pinching of the paw was observed.

ISO group (7 rats): For the induction of anesthesia with ISO, the rats were placed in an induction chamber with an ISO level of 4 L/min and an oxygen level of 2 L/min. Then, the rats were placed in an anesthesia mask fitted on the stereotaxic frame to maintain the anesthesia throughout the experiment (ISO = 2 L/min, oxygen = 0.5 L/min). The level of anesthesia was carefully monitored throughout the experiment, and if any regulations were needed, the anesthesia was changed up or down in steps of 0.25 L/min. The ideal level of anesthesia was defined as the amount of anesthesia needed to abolish the tail and paw-pinching reflexes.

For both groups the heart rate, respiration rate, and oxygen saturation were monitored throughout the experiment (MouseOx by Life-Sciences Corp). An automatic temperature controller (ATC2000 by World Precision Instruments) was used to keep the animals at a temperature of 36.5–38 °C.

5.3. Recording procedures

All cortical recordings were obtained with a custom-made IC MEA from Microprobes (16 channels, platinum/iridium 70/30%, electrode diameter = 75 μm, recording area = 3 mm × 3 mm with an interelectrode spacing of 1 mm). A stereotaxic frame with a micromanipulator was used for the insertion of the electrode into the SI cortex (location: 0.5–3.5 mm caudally to bregma and 0.5–3.5 mm laterally from bregma, depth = 1.4 mm). A TDT system was used for data acquisition. The TDT system (Tucker-Davis Technologies, Alachua, FL, USA) consisted of an RZ2-4 amplifier and a PZ5-32 digitizer preamplifier for the signal. The sampling frequency was 24.414 kHz.

5.4. Peripheral simulation

For delivering the peripheral stimulation, an in-house fabricated bipolar cuff electrode was used (length = 10 mm, inner diameter = 2.4 mm). The cuff electrode was placed around the sciatic nerve on the right hindlimb proximal to the level of the sural, common peroneal, and tibial nerve branches.

For the stimulation of the sciatic nerve, three stimulation intensities were used: 1x movement threshold (MT), 5x MT, and 10x MT. These three stimulation intensities were regarded as: 1x MT = non-noxious (Non-Nox) stimulation, 5x MT = low-intensity noxious (Low-Nox) stimulation, and 10x MT = high-intensity noxious (High-Nox) stimulation. The assumption that the 5x MT and 10x MT stimulation would be noxious was based on a previous study by Chang et. al. (2001). This study used 0–20 × movement threshold and recorded the compound action potentials at the dorsal root. They found that from 3 × movement threshold Aδ was activated and from 7 × movement threshold the C fibers were activated (Chang and Shyu, 2001).

The MT was determined at the beginning of the experiment. Subsequently, the stimulation intensity was increased from 0 mA with increments of 0.25 mA until a twitch in the right foot was visually identified by the experimenter (MT range = 1–2.75 mA, mean ± SD = 1.42 ± 0.57 mA).

The three different stimulation intensities were randomly applied to avoid inducing a wind-up effect or bias during the experiments. For each stimulation intensity, 240 stimulations were delivered through the cuff electrode (fs = 2 Hz, pulse width = 0.1 ms, square biphasic pulse, Grass SD9 Stimulator). Between application of each stimulation intensity, there was at least an 8 min resting period.

5.5. Data analysis

The data analysis focused on the LFPs and on determining the

negative peak (NP) amplitude, positive peak (PP) amplitude, peak-to-peak (PtP) amplitude, and latency of NP.

Preprocessing. First, the data were filtered to obtain the LFPs (Butterworth filter, 1. Order, bandpass 0.1–100 Hz). Secondly, a filter was used to remove the 50 Hz noise (zero-phase IIR filter, 20. Order, stop-band 46–51 Hz, with a ripple around 100 Hz). Following filtering, the mean response was calculated for each of the three different intensities over 240 repetitions for each channel and rat. Following this, a visual inspection of the signals was performed to identify and remove noisy recordings.

Feature extraction. A time window from 25 ms to 125 ms after stimulation onset was used for the identification of the NP and PP. The PtP was then calculated, and the NP latency was identified. The features were determined and calculated for each channel for each of the three intensities.

5.6. Statistical analysis

The Statistical analysis was conducted in IBM SPSS. Friedman's test was used for each of the four features and separately for HD and ISO (all channels were used separately for the analysis) followed by a Bonferonni corrected Wilcoxon ranked test as a posthoc. P-values<0.05 were considered significant and annotated '*' in the figures. P-values<0.001 were annotated by '**'.

CRediT authorship contribution statement

L.E.D. Lykholt: Conceptualization, Methodology, Formal analysis, Investigation, Visualization. **C.D. Mørch:** Conceptualization, Writing – review & editing. **W. Jensen:** Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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