Can artificial intelligence help to decode human pain signaling?

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Project partners and principle investigators

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Summary

Neuropathic pain is a debilitating condition affecting millions of patients. No sufficient mechanism-based treatment for neuropathic pain exists and in the last 10 years no new treatment was developed, because the underlying mechanisms of neuropathic pain are poorly understood. The technique of **microneurography (MNG)** can record single C-nociceptors' (type of peripheral nerve fibers, major players in signaling pain) activity directly in patients with neuropathic pain. MNG is the only method to explore pathological discharges in C-nociceptors and the underlying mechanisms. In this technique, a very thin electrode placed within a peripheral nerve is used to record actions potentials from several neighboring fibers. Before the neural coding can be understood, **sorting** those action potentials to single nerve fiber is necessary. Unfortunately, the standard shape-based sorting algorithms, successfully used in in-vitro data, cannot be reliably used in microneurography due to low signal-to-noise ratio and high variability in spike shapes. Currently the only tool to understand the fiber composition is the **marking method.** This method tracks the changes of conduction speed linked to preceding activity of single C-nociceptors. Electrical stimuli are applied in regular time intervals and any additional action potentials decrease the conduction speed, which means an **increase in response latency** of the electrically induced action potential.

Our idea, based on preliminary results, is that a smart combination of waveform features and the latencies tracked by the marking method can be combined for complete **spike clustering** of human microneurography recordings. That will, in turn, allow for scrutinizing different physiological and pathophysiological discharge patterns in healthy subjects and neuropathic patients, leading to new hypotheses about molecular targets and cellular mechanisms responsible for neuropathic pain.

Our previous work with MNG data indicated the difficulties of manual feature selection for clustering. In this project, we will tackle the problem from a different perspective: we will test **AI solutions for spike sorting** on the data prepared via our open-source platform for microneurography data analysis. We will **combine autoencoder, wavelet-based features and clustering algorithms,** optimize the parameters based on existing golden standard for spike clustering in animal single nerve fiber recordings and **validate** our AI solution on increasingly complex human data sets. Finally, we will control for **generalizability** of the developed methods in an international collaboration with Dunham's lab in Bristol.

The project will be part of our **open science** initiative and an important building block in our planned open-source analytic software project for the first automatic pipeline of analyzing microneurographic data.

State-of-the-art

Neuropathic pain has been estimated to affect between about 7% and 10% of the general population [1]. In Germany about 6% of the population suffer from neuropathic pain [2] and most patients are not sufficiently treated. **Peripheral neuropathy (PN)** affects peripheral nerve fibers, which bring information from the periphery of the body to the spinal cord. A subpopulation of the very thin and slow nerve fibers, so called C-nociceptors, are responsible for transmission of information about potentially tissue-damaging (nociceptive) stimuli to the spinal cord. These signals may be perceived as pain. Indication of such stimuli is vital but in peripheral neuropathy pain turns into a disease itself making the ongoing pain the most distressing symptom reported by the patients. In the last decade no new treatment with a low number needed to treat and acceptable balance between side-effects and pain reduction was developed. One cause might be that the underlying mechanisms of ongoing neuropathic pain are poorly understood since this symptom is difficult to examine in animal in addition to substantial species differences in pain signaling neuronal structures. Solely the technique of **microneurography** [3] can assess peripheral nerve activity of single C-nociceptors directly in awake human patients. Microneurography can thus explore the pathological discharges in C-nociceptors and their underlying mechanisms.

In microneurography a very fine needle electrode is inserted into a peripheral nerve fascicle containing C-nociceptors for recording their signals, so called action potentials.

In neuropathy patients [4, 5] C-nociceptors become **spontaneously active** and their signaling is changed. Since number of spontaneously active C-nociceptors correlates to ongoing pain and reduced ongoing pain under treatment, these nerve fibers are major players in neuropathic pain [6]. Specific discharge patterns in spontaneous activity being different from stimulus-evoked patterns are likely to contribute to increased spinal synaptic transmission and changed central signal processing leading to increased painfulness and unpleasantness of neuropathic pain [7]. In animal experiments, differences in discharge patterns could be correlated to the painfulness of a chemical stimulus [8].

Specific neuropathic discharge patterns in human e.g., bursting or chaotic firing have not been assessed before due to technical challenges: it is currently not possible to scrutinize the pathological discharge patterns directly in microneurography data. Therefore, an indirect method, the so-called "**marking method**" [9] is used. Electrical test pulses with fixed frequency (typically 0.125 to 0.5 Hz) are used to activate the nerve fibers under observation. As long as the nerve fiber is solely activated by the test pulses, their **response latency** remains stable. Figure 1 uses a waterfall plot to illustrate the marking method for the case of two nerve fibers contributing to the signal. Lines 1-5 show the stable reaction (green and red) at individual latency. Please note the different shapes and sizes of action potentials belonging to the same nerve fiber and the similarity between action potentials of the red and green marked fibers. When the nerve fiber discharges **additional action potentials** (line 6), the response latency to the regular electrical pulse is suddenly increased. The magnitude of increase (lines 6) correlates roughly to the magnitude of the preceding activity (in this case each fiber fires twice in response to the extra pulses). These relationships can be studied via applying additional electrical stimulations of controlled frequency, number and timestamp.

Spontaneous firing in neuropathy (line 10), is similarly causing a latency increase, making it possible to be localized with the precision of the regular stimulation interval length (here 4 s). This is not sufficient for the analysis of the spiking patterns (e.g., number spikes, their frequencies, bursting characteristics), for which we need to separate the individual fiber spontaneous activity or equivalently, **sorting of action potentials** according to their source fibers. Such sorting is successfully performed e.g., in animal in-vitro recordings where similar stimulation protocols are studied [10]. Typically, clustering algorithms are based on the features extracted from the **spike shape** (maximum, minimum, width, etc.).

Unfortunately, for microneurography data those algorithms fail due to low signal-to-noise ratio, high variability of spike shapes caused by overlying signals from other nerve fibers.

Technical specifics of the setup, such as drifts of the electrode position during the experiment and electrical phenomena affecting particularly action potentials from different fibers but close in time of recording contribute to the failing of currently known algorithms.

Thus, in order to study different physiological and pathophysiological firing patterns in healthy subjects and neuropathic patients we need new analyzing methods, which will utilize the time-domain knowledge provided by the “marking method” and combine it with waveform-domain features to support the sorting process.

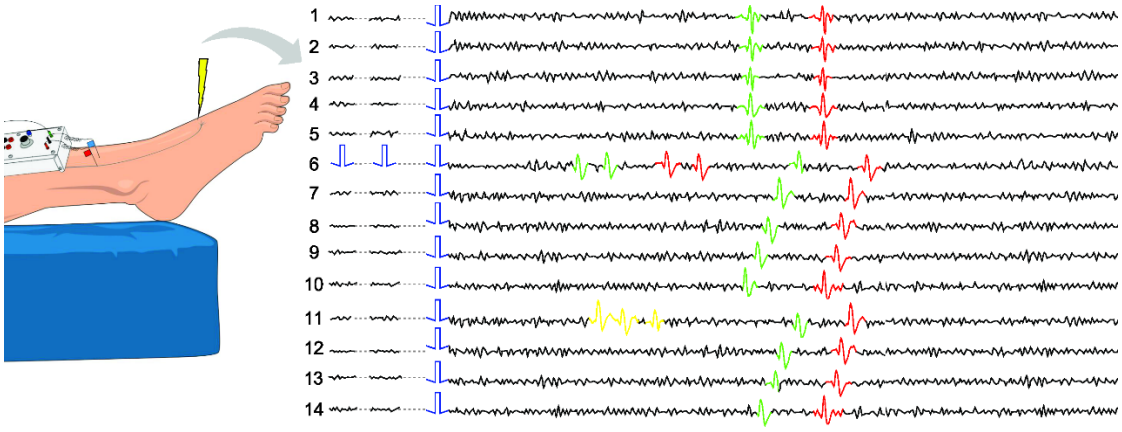

Figure 1. Example of the marking method in action potentials from two different nerve fibers. The electrically induced action potentials of two different nerve fibers are shown in green and red with slightly different latency. Please note the inconsistent spike shapes. The action potentials can be sorted to the respective fiber by their stable latency (conduction velocity). When two extra electrical pulses are applied (line 6) and the fibers discharge each two action potentials, the response to the regular electrically stimulus is delayed. If there is spontaneous discharge (line 11), it is currently not possible to sort the exact spikes to the respective fiber. It can only be deducted that the fiber with the greater latency shift (green) has fired more action potentials before the latency shift than the other nerve fiber (red).

Relevant preliminary work of the applicants

Barbara Namer is junior research group leader of the IZKF funded group Neuroscience: “Translation pain research” at the university hospital Aachen. She is successfully working on microneurography in healthy subjects and neuropathy patients since 2005 in her own lab at University of Erlangen and since 2019 in Aachen [11]. Microneurography of nociceptors in neuropathy patients are currently only performed in c.a. **5 laboratories world-wide**. She showed that spontaneous activity in patients correlates to painfulness and can be reduced by effective pharmacological treatment in an example of personalized medicine [4, 6]. Ekaterina Kutafina is a mathematician, leader of Biosignals group within the Department of Medical Informatics and she is working on data analysis of medical data and machine learning since 2014 [12,13,14].

In 2020, BN and EK started collaborating on microneurography data analysis, with the following main goals in mind:

- Build a FAIR (**f**indable, **a**ccessible, **i**nteroperable, **r**eusable [15]) data collection from the already existing and collected in the future experimental files.
- Initiate international network of microneurography researchers to develop compatible approach to data organization and starting the shift to open science in this research domain.
- Develop automatic analytic software, which will allow bringing the research to its full potential, by enabling efficient work with large searchable datasets, but also by applying completely novel algorithms.

We plan to develop an open-source platform for automatic MNG analysis (openMNGlab [16], see figure 2). The current version works with three popular MNG recording software systems (Spike2, Dapsys, OpenEphys), which are currently unified to a common structure based on Neo solution for electrophysiological data [17]. Together with collaborators from Bristol microneurography lab of James Dunham, we adapted also odML tables [18] for metadata storing, laying the basis for searchable collaborative data collection.

Peter Rossmanith (PI) has broad expertise in theoretical aspects of artificial intelligence and machine learning algorithms [19, 20] and has been working previously in cooperation with EK on machine learning applications to EEG (electroencephalography) signals [21, 22]. Together we are co-supervising the master thesis of a computer scientist student, Alina Troglio, who is studying the dependencies between action potential latencies and preceding activity from animal recordings of peripheral nerve fibers with machine learning [23].

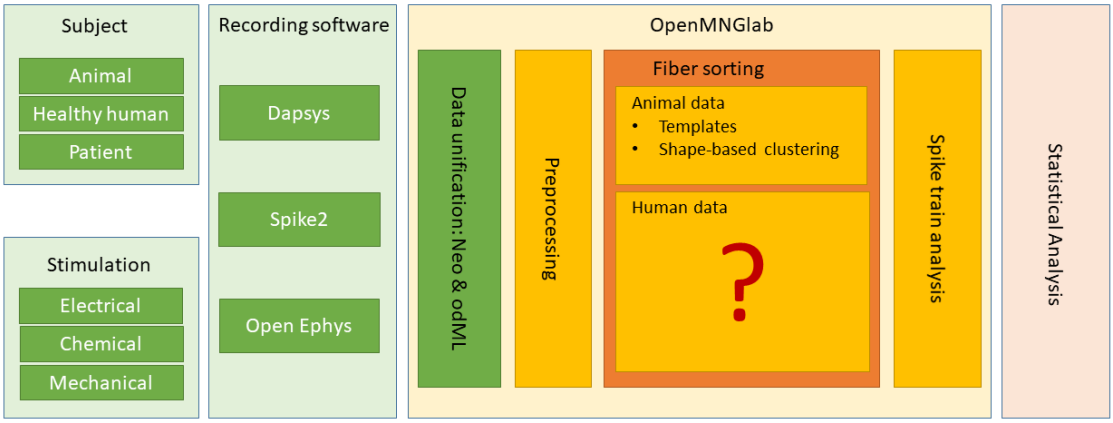

Figure 2. Complete workflow between the data collection and the statistical analysis of spontaneous activity of C-nociceptors in patients with different neuropathies. OpenMNGlab [16] takes care of most computational parts with fiber sorting in human data remaining the main challenge.

Goals and methodology

This project's goal is to make a first crucial step on the way to decoding the peripheral pain signaling in human subjects and therefore to better understanding the mechanisms of neuropathic pain: **spike sorting for human C-nociceptor microneurography data** (see figure 2). The background work on openMNGlab framework and complementary expertise areas of the PIs allow us to employ AI technologies on a large and unique medical data collection, for development of an efficient data-driven algorithm, directly guided by expert feedback.

The first step for spike sorting is to extract suitable data for our approach. We will take the advantage of existing modules of openMNGlab and the possibility to read and align large data collections as long as an expert adds the annotations. We will start with a readily available data collection which will be organized in compatible searchable way including metadata structuring in odML format as first part of the project.

The main pipeline of the algorithm is planned as follows:

1. Divide raw data by intervals between the regular electrical stimuli. Typically, it would mean c.a. 80 000 datapoints per interval.
2. Get number of fibers available in the data (contained in metadata, obtained via semi-manual clustering of the spikes associated to the "regular" electrical stimulation, as on fig.1) and magnitudes of those tracked delays.
3. Get the timestamps for the spikes in the intervals via dynamical thresholding.
4. Reduce the dimension of the raw data input. We will put main focus on **autoencoder**, but additionally **wavelet decomposition** would be used for comparison purposes.
5. Apply several clustering algorithms (K-medoids, ISO-SPLIT and others tested in [24]) to the compressed raw data, combined with the spike timestamp and the latencies associated with the given interval.

We expect this pipeline to be successful, because the data prepared as described above carries a) information about the shape of the spike, b) information about which fiber discharges at what approximate time point as read from the latencies, c) information about additional activity from nearby nerve fibers influencing latencies.

We will test autoencoders with different architectures first for their efficiency for purely compression purposes and then we will optimize the most successful models in combination with the clustering algorithms. Recent publications [25, 26] suggest that autoencoder can become a very efficient tool for biosignals analysis. Wavelet decompositions are commonly used in electrophysiological data, like EEG (electroencephalography, [16]) as feature extraction algorithms. We will use them for the baseline comparison.

Training and validation of the results remains a challenge for microneurography data, as there is no “ground truth” available. Experienced researchers can only manually check a subset of electrically evokes spikes, as they can be distinguished by their temporal localization. Therefore, the validation will be based on the available “proxy-ground-truth” labels as follows:

A) We will use animal in-vitro data (mice) for the feasibility check. The spiking activity in this data is very well clustered by the existing software (e.g., Spike2). Additionally, we will use data with chemically and mechanically evoked activity which is resembling spontaneous neuropathic firing better than electrical stimulation since the timing of action potentials is less controlled for.

B) In the next step we will take human data with excellent recording conditions and high signal-to-noise ratio. For example, the microneurography experts in the group will label recordings containing only one large and one small nerve fiber action potentials.

C) As last step of complexity we will analyze normal quality human data. Our focus will be here on the activity evoked by the additional electrical stimulation, providing us with the knowledge of the expected spike number and locations.

D) We will prepare test data set with labelled spontaneous activity from chosen recordings which allow manual labelling.

E) Finally, we will test the generalizability of our algorithm for data from different microneurography laboratories and recording hard-and software by using human data from JD’s lab in Bristol (UK).

With the results from this project, we will be able to use the existing huge data collection as well as new data, not only from our own lab, but from the developing international community supporting open science principle in microneurography data. In future, with fully developed OpenMNGlab including this crucial step of spike sorting, it will be possible to search through the metadata, open necessary files, unify the data to the same structure, extract the spiking activity belonging to single neural fibers and statistically analyze the complex relationships between experimental conditions, diagnosis, medical parameters and demographics.

Working plan

WP 1. Data preparation (BN, EK 1-12 m)

Expert work on the data preparation will be needed across the whole project duration and is a cornerstone of the project. OpenMNGlab is ready to handle large amounts of data, but first the preparation of the data, such as filling the odML metadata templates is needed. Mining our database, which is for now only human-readable, for the appropriate subsets will require work of a microneurography experienced postdoc. The database contains recordings from the past 17 years, with experiments using a variety of different hardware, software and experimental setups.

Student researchers (computer scientists or mathematicians) will support this work by updating the current openMNGlab code to the needs of previously unseen configurations, and working on automatization of the processes.

WP 2. Autoencoder for microneurography data encoding (PR, EK, 2-7 m)

In this WP, we will explore autoencoder as AI approach to the data encoding/feature extraction in MNG. Different types (denoising, sparse, LSTM) and architectures will be tested for encoding and further reconstruction. Successful models will be further proposed as dimension reduction of the data for the next WP. The work will require efficient computational approaches. EK and PR will be primary responsible for the supervision of model development and efficient computational implementation done by student researchers.

WP 3. Clustering (PR, BN, EK, 4-9 m)

This package will focus on experimenting with different types of clustering algorithms on the data compressed via autoencoders and additional information (latencies, spike timestamps, fiber number). We will start from animal in-vitro data, where conditional ground truth is defined and proceed further increasing the data complexity. Wavelet decomposition will be used as an alternative feature-extraction approach for comparison. This package will require a close interaction between the interdisciplinary participants, due to the challenges of labelling quality of train/validation data and need for expert-based supervision of the results. Programming will be done by the students supervised by EK and PR, and the expert support by BN and the postdoc.

WP 4. Generalize on data from JD's lab and adapt openMNGlab (BN, EK, JD, 9-12 m)

The development of feature extraction via autoencoder and wavelets is an important part of openMNGlab, the platform that aims at open science and FAIR data handling in the MNG community. Together with our collaborators from Microneurography lab in Bristol (JD), we will work together to ensure that the developed methodology generalizes well to the data recorded with different hardware and software system. OpenMNGlab is already compatible with the data format used by JD's group.

WP 5. Dissemination (BN, PR, EK, JD, 10-12 m)

The achieved results will be summarized in scientific papers and presented on conferences. The development of the open-source software will be documented and added to the public Git repository. We will further prepare for long term funding proposals of the project.

Financial plan

Applicant	Personnel costs	Amount (€)
B. Namer	50% (TVL) postdoc position for 9 months	30 750
E. Kutafina	Student assistant, 14h/week for 12 months	10 570
P. Rossmanith	Student assistant, 14h/week for 12 months	10 570
	Equipment	
B. Namer	Hard drives	1 000
	Conferences	
B. Namer		2 000
E. Kutafina		2 000
P. Rossmanith		2 000
		59 250

Expected long-term impact

The proposed project bridges AI research with the high-priority medical problem of neuropathic pain. The result of this project is a crucial **building stone of openMNGlab**: our open-source framework for massive inter-lab data handling and analysis. With this framework, large quantities of already available data and in future recorded data of neuropathic patients with different diagnosis and genetic variations as well as healthy controls will be ready for efficient quantification and subsequent statistical analysis. That, in turn, will be a revolutionary step towards **understanding mechanisms of neuropathic pain** and therefore towards **personalized treatment of peripheral neuropathy.**

Our further steps will be to apply for funding to support on one hand the further research steps regarding data analyses (e.g., preparation and analysis with specific data subsets) and on the other hand the development of the framework openMNGlab towards a **medical product for PN research and diagnostics**. We will approach BMBF and Else-Kröner-Fresenius-Stiftung "Schlüsselprojekt" for national as well as Horizon2020 and EITHealth for our international collaborations.

Research data management plan

One of the major project goals is to support **open science** idea and FAIR data management. No new data will be collected in the project, but a large subset of the existing collection will be re-organized via transition to uniform structure (Neo) and addition of the metadata (odML). In this form it will become compatible with the standards set by **NFDI, Die nationale Forschungsdateninfrastruktur** (DFG initiative), which we plan to join in the upcoming months. Within the project, only pseudo-anonymized electrophysiological data will be shared with the partners.

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