Deliverable **D2.1**

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V1.0	First Draft	26-02-2024

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Abbreviations

WP : Work Package

T.X.X: Task X.X

LFP: Local field potential

GU: Ghent University

TUD: Technical University of Delft

UF: University of Freiburg

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Executive Summary

This document serves as a concise summary of the content within Deliverable **D2.1**, encapsulating the key findings, methodologies, and advancements achieved in Work Package 2 (WP2). It aims to provide stakeholders with an accessible overview of the project's technological strides, and the implications of these developments for future research and applications.

The problem/objective: The primary objective of WP2 is to enhance the resolution and efficacy of neurophysiological data acquisition. Traditional methods face challenges in spatial resolution and signal quality, which can impede the identification of precise neural biomarkers. The goal is to develop conformable neural probes with a high electrode density that can record local field potentials (LFPs) with greater accuracy and less invasive procedures.

The solution: In response to this challenge, we have successfully designed and fabricated two generations of neural probes both employing polymer based MEAs. The first generation was established with a robust quality control process, and its in vivo testing has successfully recorded high-resolution LFP data. The second-generation probes built on know-how from its processor and offer an increased electrode density (>2000 electrodes/cm^2) for even higher resolution recordings.

In vivo experiments performed with the first-generation probes at GU and second-generation probes at UF have provided high-quality LFP data, demonstrating the probes' capacity for precise neural recordings. This advancement represents a significant step in our project.

The deliverable consolidates the efforts of developing a scalable interconnection strategy, potentially translatable to other technologies, and instrumental for integrating with different interfaces. The data gathered from the experiments have laid the groundwork for further analysis and subsequent experiments that are expected to reveal relevant neural biomarkers.

The high-density probes designed, and the quality of data recorded (*in vivo*) denote the successful completion of **Deliverable D2.1**.

1. Introduction

Work Package 2 (WP2) has set forth the following objectives:

1. To develop polymer-based passive microelectrode arrays (MEAs) that are conformable and have ultra-low impedance.
2. To create IGT-arrays for the immediate amplification of neurophysiological signals.

To accomplish these objectives, WP2 has been structured into three distinct tasks:

- Task 2.1: Development of passive MEAs
- Task 2.2: Development of IGT-arrays
- Task 2.3: Intra-operative neurophysiological recordings using an animal model

Deliverable 2.1 (**D2.1**), titled "In vivo LFP Detection with Conformable Passive MEA (Density > 1500 electrodes/cm²)," is linked to both Task 2.1 and Task 2.3. This deliverable is of paramount importance as it constitutes an important step for the front-end neural array with wich eREC will be connected. The quality of these arrays will determine the spatiotemporal resolution with which LFP data are recorded. The specific actions undertaken within these tasks to achieve D2.1 are outlined subsequently.

2. Development of passive MEAs

In this task GU received input from TUD and UF to design and fabricate conformable passive neural arrays employing conductive polymer poly(3,4 ethylenedioxythiophene)–poly(styrenesulfonate) (PEDOT:PSS) on parylene substrates. The optimization process of these arrays was divided in several subtasks that reveal the best strategies for interconnections, size and shape specifications. The progress in this task in relation to the current reporting period and the corresponding deliverable is described in.

Table 1 Planning and progress for task 2.1

Sub-task	M3	M6	M9	M12	M15	M18	M21
2.1.1 – Interconnections and connection strategies		✓					
2.1.2 – Array design, size and shape			✓				
2.1.3 – Next design				✓		D2.1	

2.1 Interconnections and connection strategies

To meaningfully investigate, in parallel to other work packages, our neural probes, we had to connect them to a commercially available electrophysiology acquisition system. Due to ease of use, the ability to trace impedance at 1KHz, reliability, and price, we have chosen an open ephys acquisition system. Such a system consists of an acquisition board that can stream up to 512 channels of neural data to a computer via USB, and a low-profile 64 channel headstage with a 3-axis accelerometer, based on an INTAN RHD2164 chip. The upper side of the headstage accommodates the SPI connector for connection of the SPI cable

with the acquisition board. The lower side of the headstage accommodates the RHD2164 chip and a HIROSE EIB connector (male) for connection with the neural probe (Figure 1A). To connect to this headstage we have designed several PCB board adaptors in which one side connects to our neural probe and the other side accommodates a HIROSE EIB (female) connector for connection with the headstage (Figure 1C). In such a way, we could design several neural probes attach them to our adaptors and plugging them to the headstage for several tests. To connect our neural probe with our PCB board adaptor we decided to utilize BGA package-like contact format since they are commonly used on INTAN RHD2164 and other chips, and could facilitate future connection with our customized chip (Figure 1**Error! Reference source not found.**B). Therefore, we designed both the back side of our PCB adaptor and our microfabricated neural probes with BGA contacts to attach them together. The PCB adaptor and microfabricated probes were connected via flip chip bonding which is a method that will later be translated for connection with our customized chip (Figure 1D). The final connection is show in Figure 1E

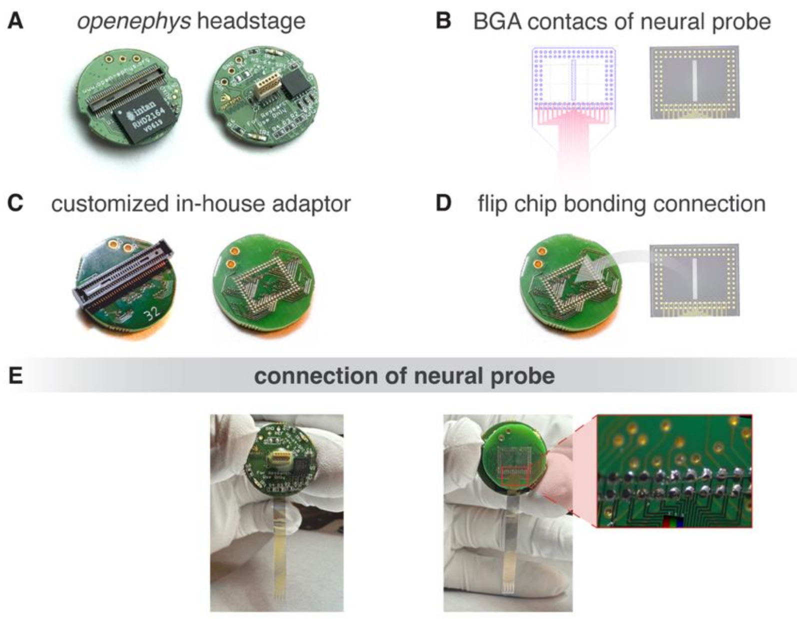

Figure 1. Connectors and connection strategy

2.2 Array design, size and shape

To facilitate description, we can separate the microfabricated neural probes into three different areas: i) the back-end (BGA contacts), ii) the interconnection ribbons and iii) the front end (arrays) (Figure 2). As we referred to the previous section for back-end we have chosen BGA-like contacts to be flip chip bonded with our adaptors. The interconnection ribbons have been designed to maximize workability without sacrificing impedance, according to experience and after discussions with UF, with a length of around 3cm and a width of ~50um with a gradual decreasing footprint per interconnection.

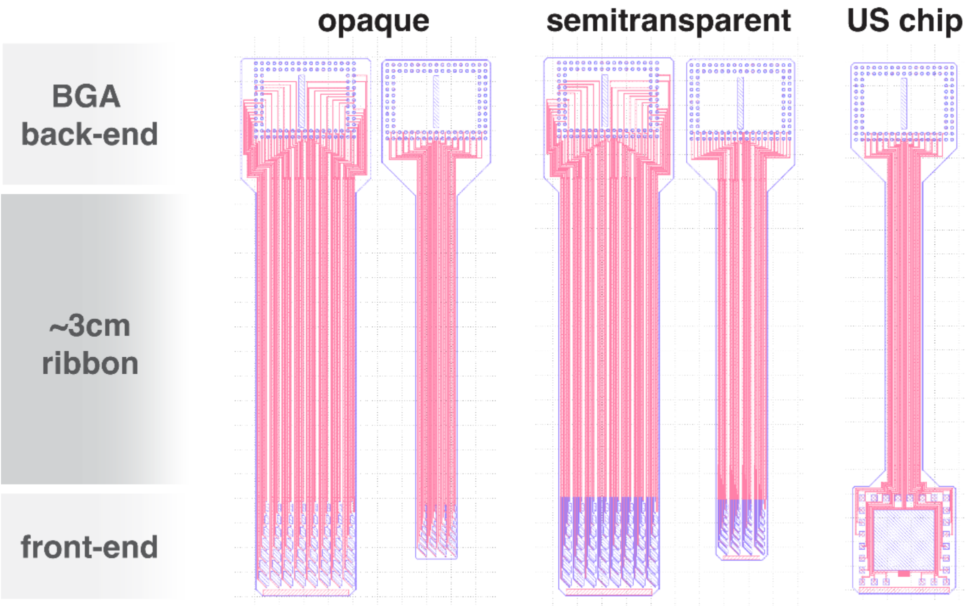

Figure 2. Design of 1st generation neural arrays

We have designed and microfabricated three different designs for the front-end area: i) An opaque array to minimize impedances with 64 and 32 electrodes. After discussions with UF we have opted for an overall area that can enables craniotomy without sacrificing maximum coverage of the neural tissue. The corresponding dimensions for 64 channels are 8mm x 8mm and for 32 channels 4.5 mm x 3.3 mm. In this 1st generation probes the electrode area was 50 µm x 50 µm. ii) A semitransparent array that can be used for further imaging application of neural tissue (e.g. calcium imaging). Similar logic has been followed for the dimensions of the overall array with an electrode area of 40 µm x 40 µm (Figure 3). iii) A probe with a 5 mm middle square gap to accommodate our customized ultrasound chip. After discussions with TUD we have opted for an overall dimension of 8.5 mm by 8.9 mm with 28 500µm x 500µm electrodes for this 1st generation. All probes have perforation holes to facilitate liquid flow from tissue, microinjections or placement of additional depth electrodes. All probes have versions that employ a temperature sensor to trace relative temperature change during electrical or ultrasound stimulation. The logic behind our three different designs is to evaluate the effect that ultrasound stimulation (at this stage with an external system) has on tissue and different electrodes, collect neural data, optimize microfabrication, optimize placement techniques and surgery protocols and test a chip incorporated architecture.

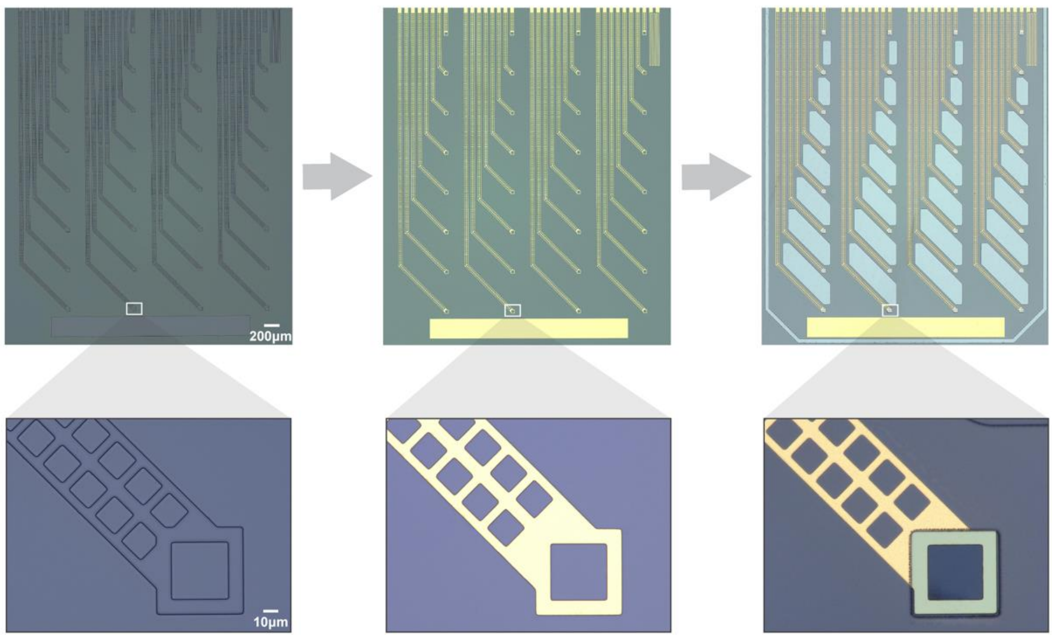

Figure 3. Example of fabrication steps. Microscope images of a semitransparent PEDOT:PSS based neural array

2.3 Next generation design

At every stage of development, data analysis and feedback are diligently gathered to inform the initiation of activities aimed at designing the second-generation models. These new designs feature electrodes with a reduced footprint and a higher density, as illustrated in Figure 4 and Figure 5. We have successfully demonstrated in vivo LFP detection using conformable passive MEAs with an initial density of approximately 2000 electrodes/cm². The 2nd generation probes have been meticulously designed to incorporate smaller electrode footprints, measuring 40 µm x 40 µm and 25 µm x 25 µm, thus achieving a density greater than 1500 electrodes/cm². Following consultations with the University of Freiburg (UF) we have optimized the total area of the array, resulting in dimensions of 1.5mm x 2.5mm for the 32-electrode arrays and 3mm x 2.5mm for the 64-electrode variants. Additionally, we have engineered and produced shank probes with the intent to enable recordings at various depths and to support comparative analyses of electrophysiological data. These second-generation probes have been actively employed to capture in vivo LFP activity in a collaborative effort with UF.

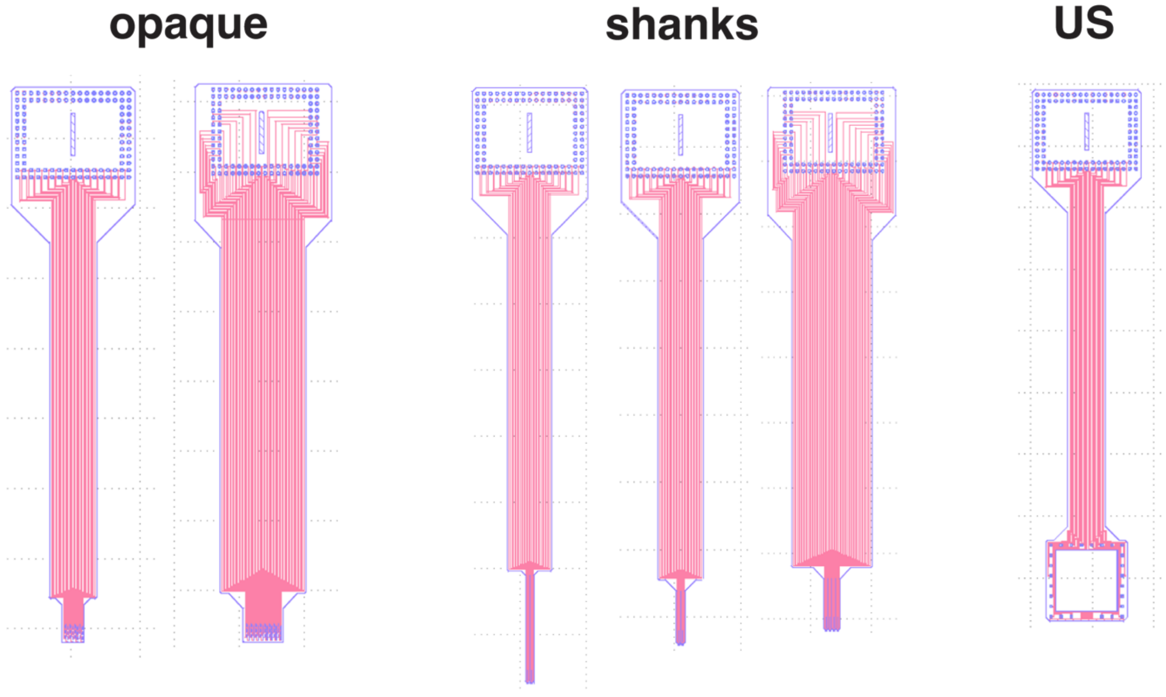

Figure 4. Design of 2nd generation neural arrays.

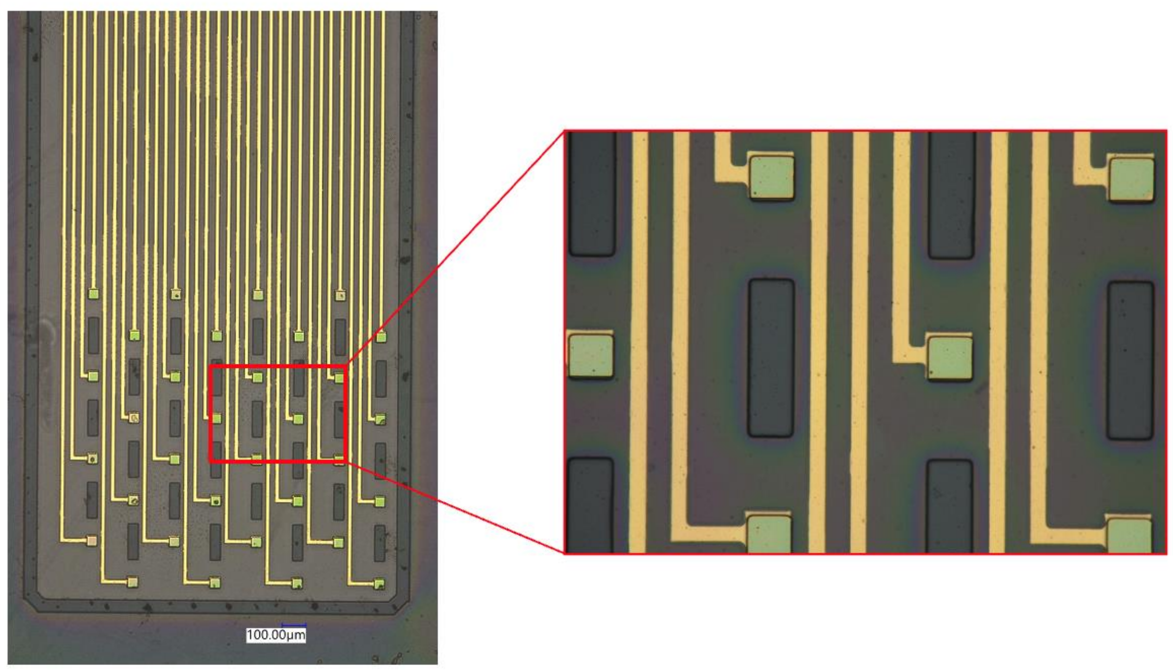

Figure 5. Microscope images of a 2nd generation opaque high density PEDOT:PSS based neural array.

3. Intra-operative animal-model neurophysiological recordings

In this task we designed and microfabricate passive polymer-based arrays (T2.1) with inputs from TUD and UF. We performed independently in vivo characterization with the purpose to correct potential design flaws that appear during surgery. All design corrections were then applied to achieve 2nd generation probes (T.2.1.3). The progress in this task in relation to the current reporting period and the corresponding deliverable is described in.

Table 2 Planning and progress for task 2.3

Sub-task	M3	M6	M9	M12	M15	M18	M21
2.3.1 – *In vivo* validation of passive MEAs				✓		**D2.1**	

3.1 *In vivo* validation of passive MEAs

After microfabrication the conformable probe bonded on the adaptor via flip chip bonding and sealed with medical grade silicon glue. The HIROSE EIB (female) side of our adaptor was then connected with the HIROSE EIB (male) of the open ephys headstage (Figure 1D,E). To evaluate/optimize the connection strategy, the quality of our microfabricated probes, and the communication between open ephys GUI we performed electrochemical impedance analysis for each electrode. Beforehand, we have designed our PCB adaptor so that each contact corresponds to an electrode on the front-end and a channel at the open ephys GUI. We therefore assigned each of the microfabricated electrodes to a channel at the open ephys GUI and created a spatial map of our array (Figure 6).

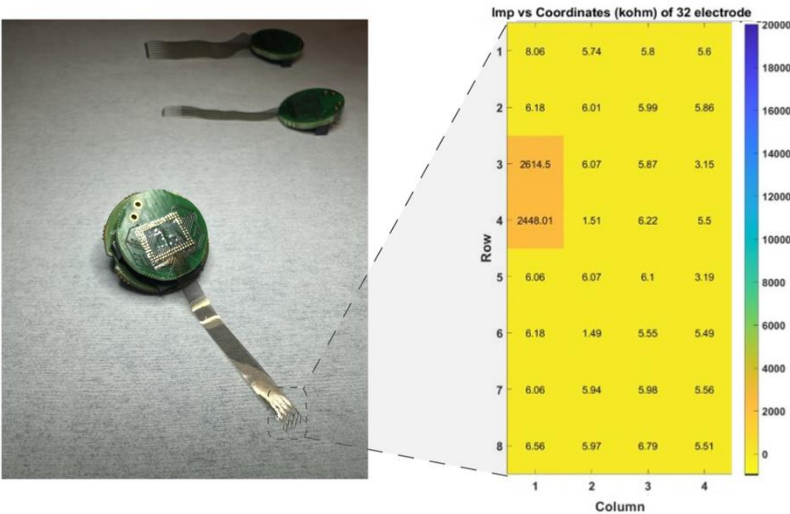

Figure 6. Quality control and spatial map of electrodes. The impedance values at 1KHz are shown for each electrode.

We then proceeded with the in vivo validation of our probes. The goal of these first experiments was the successful acquisition and analysis of neural data from Sprague Dawley rat model. To test the efficacy of LFP acquisition we have recorded from before and after 4-aminopyridine(4AP)-induced seizures. All animal experiments were approved by the Ethical Committee. The implantations were carried out epidurally on female Sprague Dawley rats that had no previous experimentation. Animals were anesthetized with 2% (0.6L/min) isoflurane during the intracranial implantation surgery, recording and induction. Two #000-gauge stainless-steel screws were implanted in the frontal bone and used as ground (GND) and reference (REF) electrodes. A craniotomy (4.5 x 6.5 mm^2) was performed (anteroposterior = from 1.5 mm to 6mm, mediolateral = from -1.5mm to +5.00mm), to expose the dura mater. After the placement of the probes, we have collected neural data for several minutes (Figure 7). We then inject via a micro injection fixed on a stereotactic hand 4AP on specific locations through the perforation holes of the probe. We recorded the signal through openephys GUI and import data to a custom MATLAB software to analyze them and create spatial maps (Figure 8 & Figure 9). Spectrograms for tracing specific frequency responses were generating using wavelet transformation (Gabor).

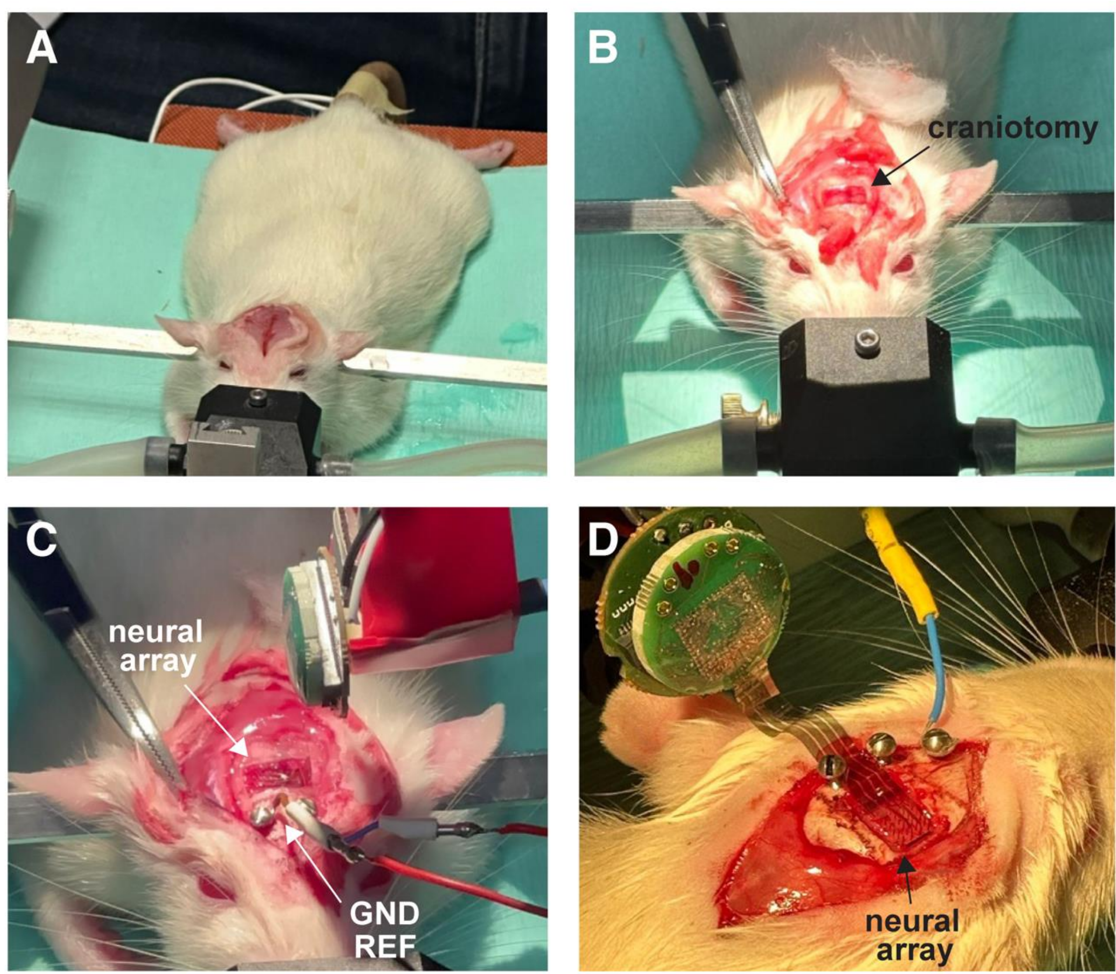

Figure 7. In vivo validation of neural arrays at GU. A) Incision, B) Craniotomy, C) Placement of neural array on dura, D) different perspective of a neural array placed on dura.

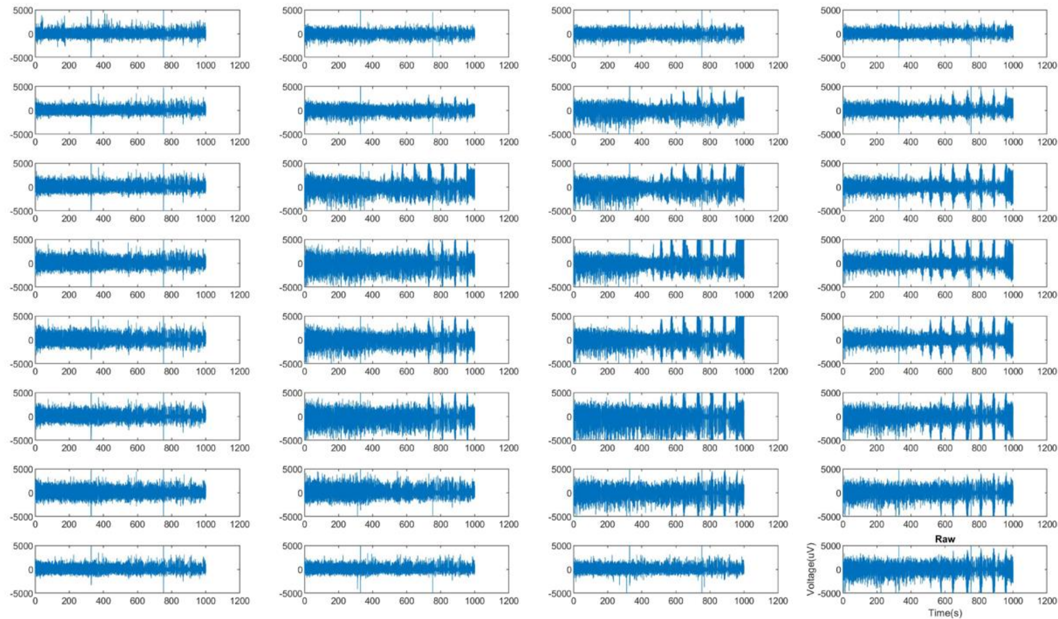

Figure 8. Electrophysiological signal acquisition with spatial resolution. Sample time trace of signals recorded from polymer-based neural arrays. 4AP was microinjected on the middle up right side of the array at ~450s. Local spontaneous epileptic activity can be seen after that time point.

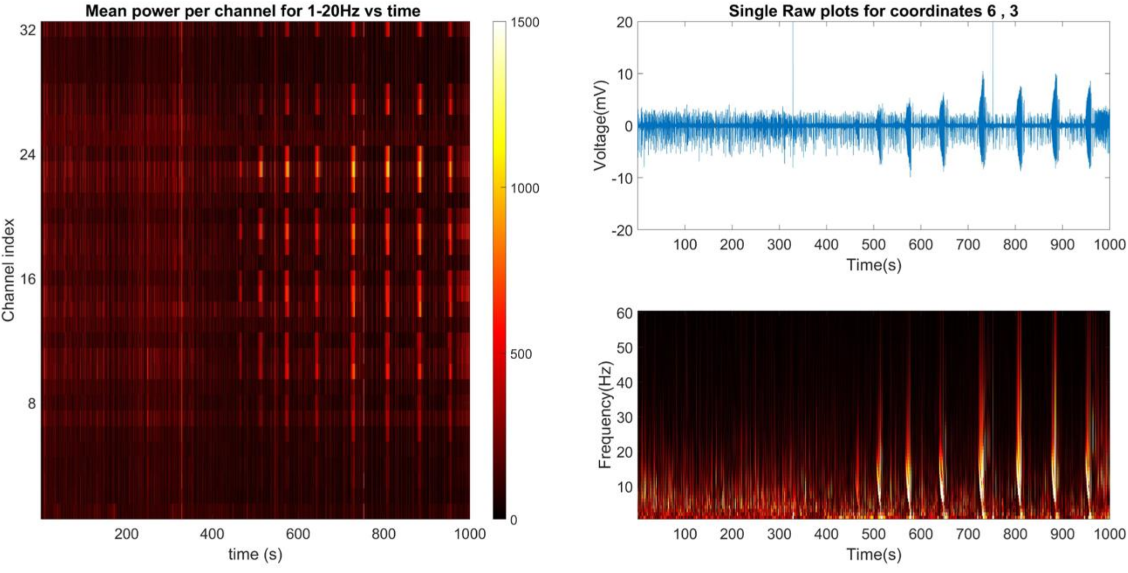

Figure 9. Samples of time-frequency spectrograms. Mean power per channel (1-20 Hz) for a time window of 1000 seconds (left). Sample time trace of signal from one channel (upper right) and its corresponding time frequency spectrogram (right lower). 4AP was microinjected at ~450s. The increasing intensity of mean power for 1-20Hz denotes the local spontaneous epileptic activity after that time point.

Following the successful validation of our initial designs, we have progressed to integrating the high-density 2nd generation models (approximately 2000 electrodes/cm²) into our in vivo experiments. These pivotal experiments, depicted in Figure 10, were conducted at the University of Freiburg (UF) and played a crucial role in advancing our efforts towards recording and identification of relevant biomarkers.

At UF, our objective was to proficiently record and analyse neural data from the Flinders Sensitive Line (FSL) albino Sprague Dawley rat model. We have ensured that all animal experiments received approval from the appropriate Ethical Committee. The implantation procedures were performed epidurally on female FSL albino Sprague Dawley rats with no prior history of experimentation. For these protocols, the animals were anesthetized using an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (8 mg/kg), which marked a deviation from the 2% isoflurane used during earlier experiments at GU. Throughout the recording and stimulation phases, we maintained anaesthesia by administering ketamine (1/3 of the dose) and xylazine (1/3 of the dose) alternately every 30 minutes. This variation in anaesthesia methods was employed to glean critical insights into the impact of different anaesthetics on neural data and LFPs. For the grounding (GND) and reference (REF) points, two #000-gauge stainless steel screws were placed in the frontal bone. A craniotomy measuring 3.5 x 3 mm² was carefully executed on the prefrontal cortex to access the dura mater. Following the probe placement, we systematically collected neural data over several minutes.

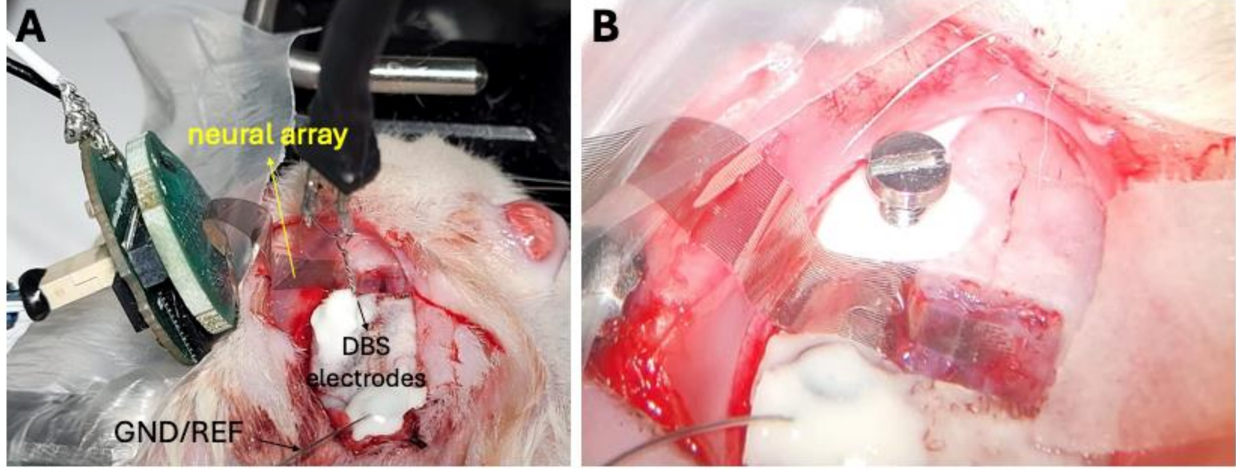

Figure 10. In vivo LFP recordings with high density neural array in UF. Neural array was placed on the prefrontal cortex.

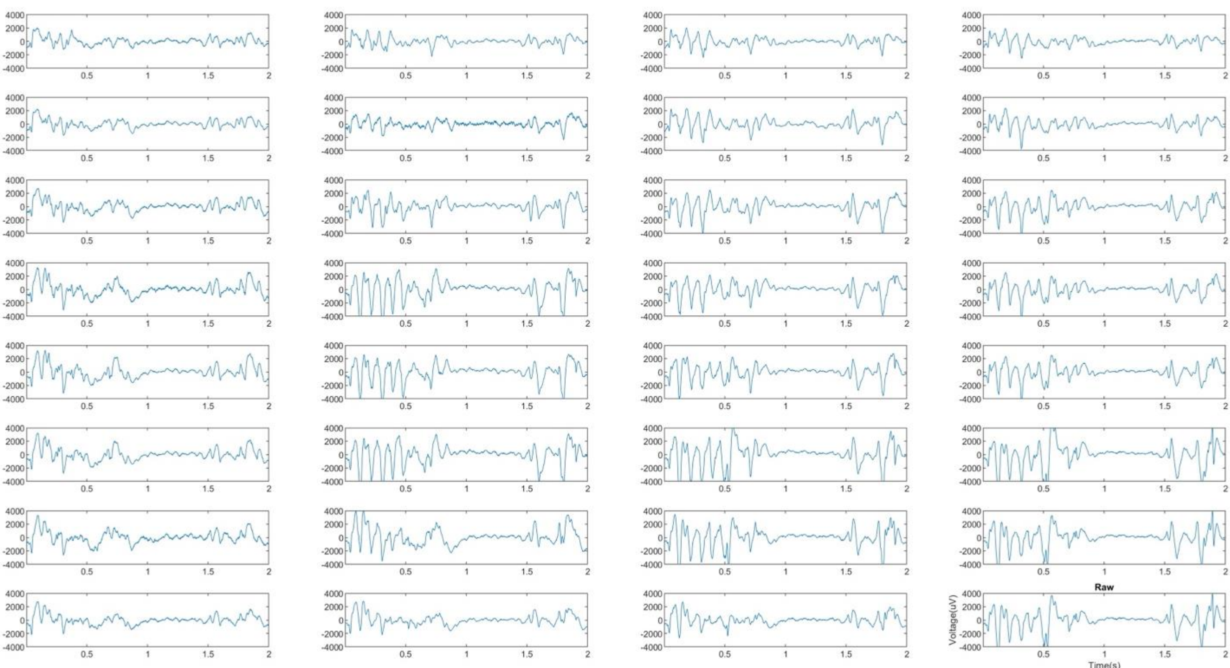

Figure 11. High spatial resolution acquisition of electrophysiological signals. Samples of time traces show signals recorded epidurally using polymer-based neural arrays, with each plot representing recordings from various electrodes that map different areas of the exposed brain.

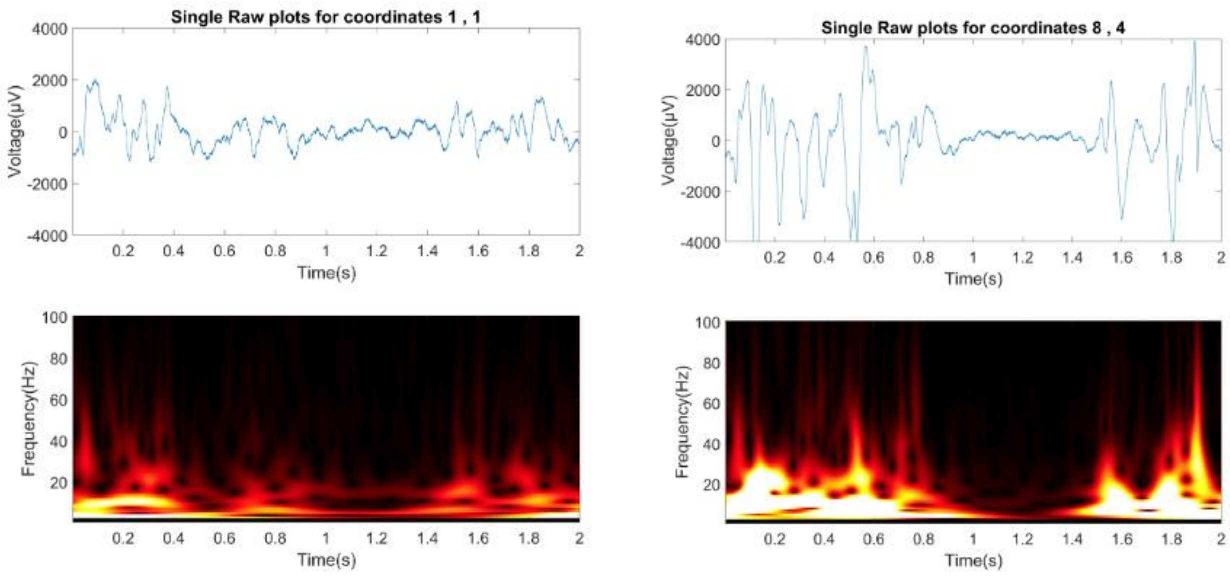

Figure 12. Samples of time-frequency spectrograms. Sample time trace of signal from channel 1.1 (upper left) and its corresponding time frequency spectrogram (lower left). Sample time trace of signal from channel 8.4 (upper right) and its corresponding time frequency spectrogram (lower right). The significant differences of the two signals demonstrate high spatial resolution.

4. Conclusions

The progress of Work Package 2 (WP2) aligns closely with our projections, featuring only minor deviations in planning and yielding promising early results. We have successfully demonstrated an interconnection strategy that not only meets our current needs but also shows potential applicability in other technological areas and compatibility with various interfaces.

Our team has designed and microfabricated a first-generation conformable neural probe utilizing a polymer-based microelectrode array (MEA). We have implemented a rigorous quality control process, and the neural probe has proven successful in in vivo trials by recording local field potential (LFP) data with high spatial resolution. Following this, a second-generation probe was also designed and microfabricated to enable recordings with higher density.

Conducting in vivo experiments using the first-generation probes at GU and the second-generation probes at UF has resulted in high-quality LFP data acquisition at both institutions. Our current focus is on the further analysis of this data and conducting additional experiments to identify pertinent biomarkers.

The activities described above and the data collected thus far signify the fulfilment of Deliverable **D2.1**.