

UPSIDE

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Abbreviations

EIC : European Innovation Council

eFUS : epidural focused ultrasound

IP: Intellectual Property

MDR : Medical Device Regulations

MRI : Magnetic Resonance Imaging

PO : Project Officer

PM : Programme Manager

WP : Work Package

Executive Summary

The objectives of WP2 were as follows: (i) Development of conformable polymer-based passive MEAs with ultra-low impedance. (ii) Development of IGT-arrays for on-spot amplification of neurophysiological signals.

To achieve these objectives, WP2 was divided into three tasks:

- **T2.1:** Development of passive MEAs
- **T2.2:** Development of IGT-arrays
- **T2.3:** Intra-operative animal-model neurophysiological recordings

The work performed during the period M24-M30 towards these goals is described below.

1. Intra-operative animal-model neurophysiological recordings

The primary objective of Task 2.3 is the chronic acquisition of high-quality EEG signals (>3 weeks) from an in vivo animal model to extract relevant biomarkers, which will be used to develop biomarker detection algorithms. We successfully achieved high-quality EEG signal acquisition from chronic implantations performed on rats using high-density passive microelectrode arrays with very low impedance. However, there was a necessary deviation from the initial plan to use IGT arrays due to the following reasons:

1. Challenges arose in designing a custom PCB adapter that connects the conformable IGT probes on a common source amplifier mode with the the low-profile 64-channel headstage (based on the INTAN RHD2164 chip) from the commercially available Open Ephys system.
2. A customized CMOS chip for direct current recording from the high-density IGT arrays (developed in Task 2.2) is not yet available.

The progress made during this reporting period and related deliverables are detailed in this report.

1.1 Design and development of custom PCB to interface IGTs probes with the Open Ephys acquisition system

To sustain progress in this task, we developed a custom PCB to interface the IGT probes with our existing commercially purchased neural signal acquisition system (Open Ephys). This allows us to characterize the IGTs in a controlled bench-top setup and mimic their behavior as implants, without the need for the custom CMOS chip. The PCB serves as a bridge, translating the IGTs' current output into voltage output that can be read by standard neural recording systems. Unlike passive electrode arrays that detect voltage fluctuations, IGTs act as active transducers, generating a current output proportional to the neural signal. This fundamental distinction, voltage recording vs. current recording, necessitates a specialized readout circuit to effectively convert the IGT's current output into a measurable voltage.

To address this requirement, we implemented a common-source amplifier configuration. This setup allows IGT channel current variations (corresponding to neural signal changes at its gate) to be converted into voltage fluctuations at the output. The conversion is achieved by placing a load resistor across the IGT's drain and providing a fixed bias, ensuring that current variations through the transistor are linearly mapped onto voltage drops across the resistor. This configuration not only facilitates the essential current-to-voltage conversion but also amplifies the neural signal for precise measurements.

We developed an adaptor (custom PCB) solution as interim measure until a customized CMOS chip becomes available. This approach ensures continuity in testing and validation of the IGT concept, guaranteeing that the readout scheme of IGTs has already been rigorously tested and optimized. As a result, future integration and performance assessment will be significantly facilitated.

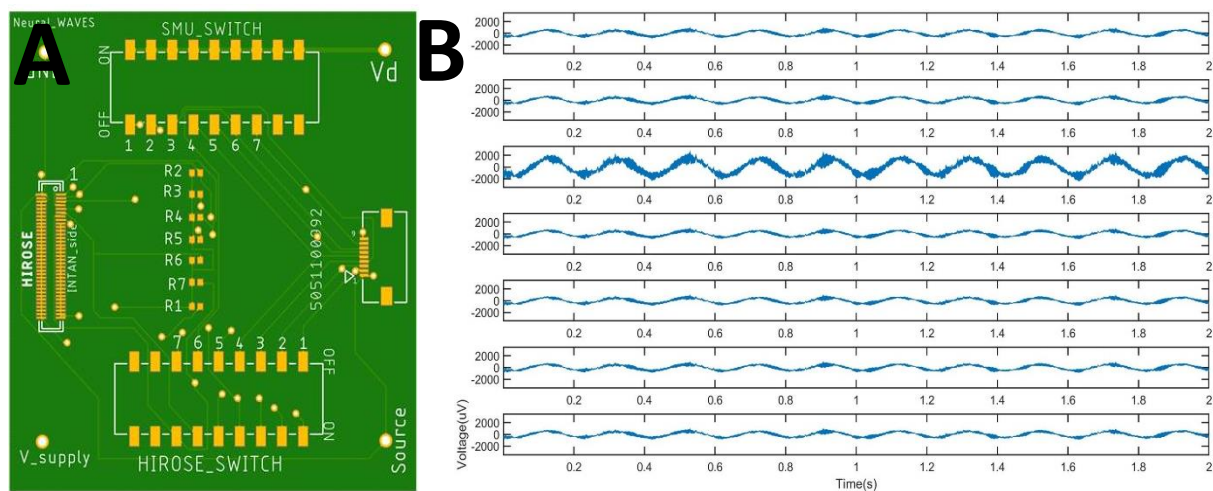


Figure 1: (A) Layout for the 1st generation IGT interface board. (B) Measured amplification of the common-source amplifier for one input signal (5 mV).

To facilitate bench-top testing of the IGTs, we designed and fabricated a custom PCB that can connect to multiple IGTs interface them with both standard neural recording equipment (in common source amplifier mode with Open Ephys) and source measurements units (SMU). Figure 1A illustrates the design of the 1st generation PCB, which includes passive components and connectors to bias the transistors, condition their outputs, and allow flexible readout options. Key components on the board are resistors, configurable switches, a zero insertion force (ZIF) connector to plug in and test various IGT probes, and a Hirose connector to link the board to the multichannel acquisition system. Each IGT transistor line has an adjustable resistor to optimize biasing for the common source amplifier. We incorporated switches that serve two main purposes: **(1) IGT activation control** – these switches allow for individual activation of an IGT channel, and **(2) measurement routing** – they allow each transistor to be alternatively connected to an external SMU for direct electrical characterization. This switchable design provides great flexibility to either record neural-like signals through the on-board amplifier or to directly measure transistor characteristics.

During the measurements, we encountered crosstalk issues, where the output signal from one transistor was detected in adjacent channels (Figure 1B). This interference likely occurred due to the signal propagating through the shared bias network, as all the resistors were connected to the same bias line. This not only caused crosstalk but also a V_D drop at all resistors hence lowering the signal amplification at

the main channel. To resolve this issue, we made several improvements to the current design which include: adding decoupling capacitors, broadening the supply line, and expanding the ground plane. The capacitors filter out high-frequency noise from the power supply line, reducing signal transfer between transistor lines. These changes successfully reduced crosstalk, and signal noise and ensured that each transistor could operate independently with clean signal readings.

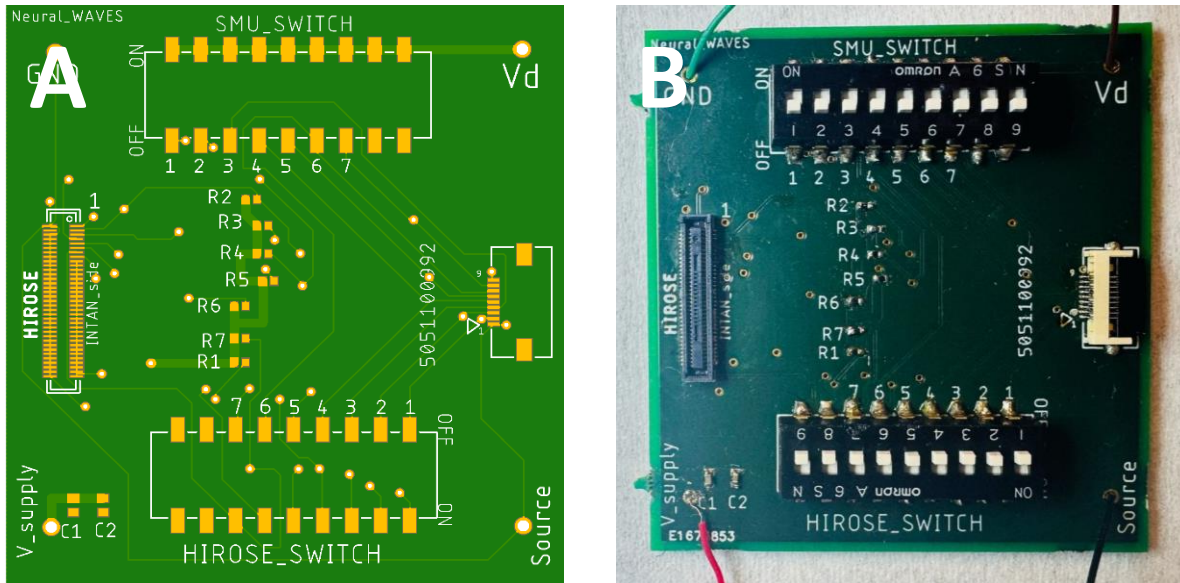


Figure 2: (A) Layout for the 2nd generation IGT interface board. (B) IGT interface board consisting of load resistors, capacitors, switches, HIROSE EIB, and back-end connector.

After the fabrication of 2nd generation PCB, we tested its performance (Task 3.2.1 related work) to ensure it works as expected. We characterized the board for proper voltage supply distribution, verified the functionality of each switch setting, and confirmed that the Hirose connector pinout correctly maps each transistor’s output to the acquisition system channels. Preliminary bench tests demonstrated that the PCB can successfully provide the required bias to each transistor and read out the small signal variations from the IGT through the common-source amplifier topology.

We used the PCB interface to measure signal amplification and channel isolation (crosstalk) in the system. A small sinusoidal voltage signal was injected into the gate of each IGT to simulate a neural signal input, and we measured the output voltage across various load resistances. We tested input amplitudes of 2 mVpp and 5 mVpp. Figure 3A shows that applying a 2 mVpp gate signal resulted in a linear amplification factor of five. A similar trend is observed in Figure 3B when a 5mVpp gate voltage is applied. The common-source amplifier configuration successfully amplified small input signals. The results demonstrate a consistent gain, confirming that the amplification is linearly related to the resistor value.

Crosstalk analysis was conducted to ensure that activity in one IGT channel did not induce any interference in adjacent channels on the board (Figure 3C). Two or more IGTs were activated simultaneously, each receiving a different frequency input. As an example, Channel 2 (500 Ohm) received an input of 10Hz while Channel 7 (5 kOhm) had an input of 20Hz. The resulting measurements showed amplified voltage with

minimal interference. The inactive transistor channels remained at baseline noise levels regardless of the frequency or amplitude driving the active channels.

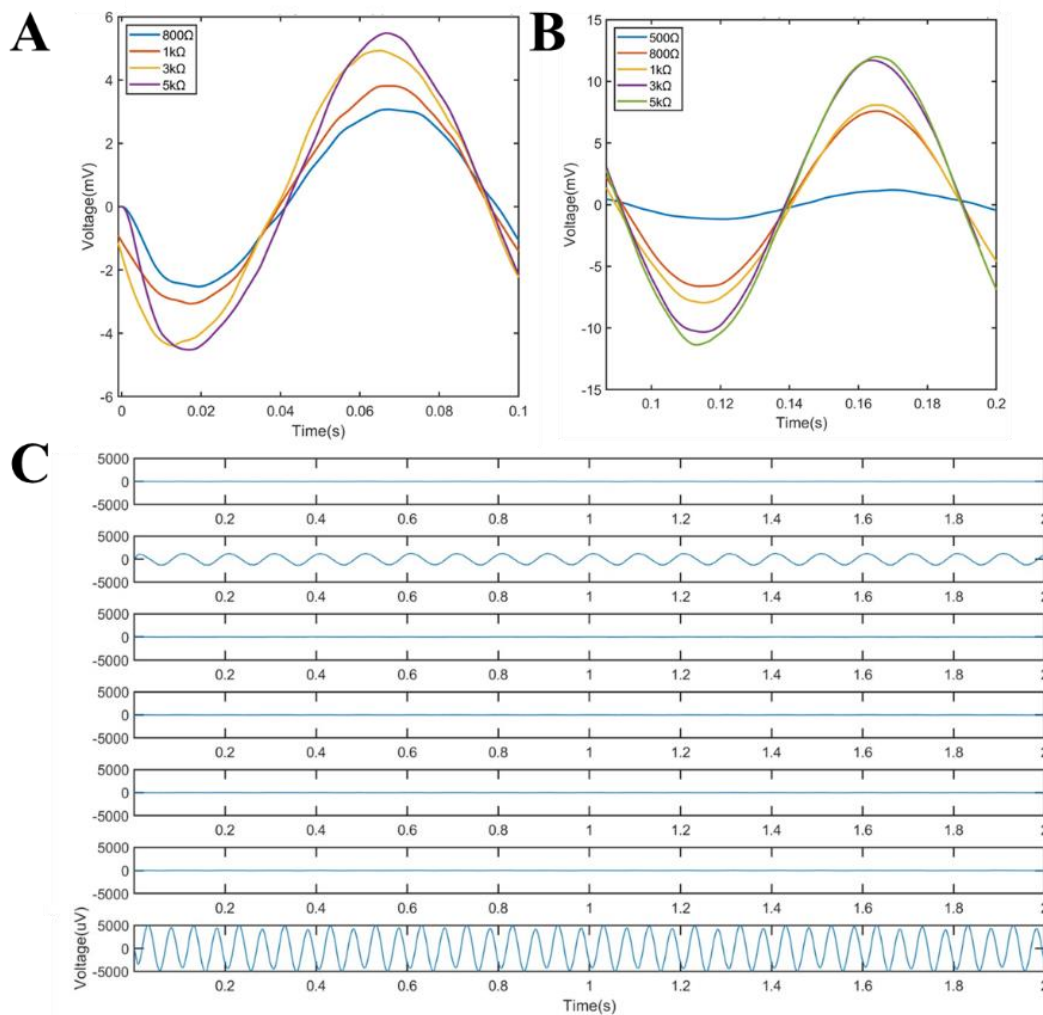


Figure 3: Characterization of the IGT interface PCB. (A) and (B) Measured amplification of the common-source amplifier for two input signal levels (2 mV and 5 mV) across increasing load resistor values, respectively. The output voltage shows a linear increase with resistor size, confirming linear gain tuning. (C). Overall, the PCB maintains signal integrity and isolation across channels.

Based on these results, the next step is to miniaturize this PCB so that it can eventually function as an adaptor that can be fitted together with the Open Ephys headstage on an animal cap for chronic recordings. Furthermore, this gives us confidence that once the custom chip is available, it can be integrated via a similar frontend configuration to reliably capture neural signals with on-chip amplification.

1.2 Chronic EEG signal acquisition from a depressed-like phenotype rodent

Because of the aforementioned, we proceed with to the goal of acquiring high-quality EEG signal from chronic implantations performed on rats by utilizing high-density passive microelectrode arrays with very

low impedance, instead of IGT arrays. A 64-channel passive micro-electrode array (MEA) – fabricated on a flexible polymer substrate – was implanted in a rodent cortex for long-term recording of local field potentials (LFPs) (Figure 4).

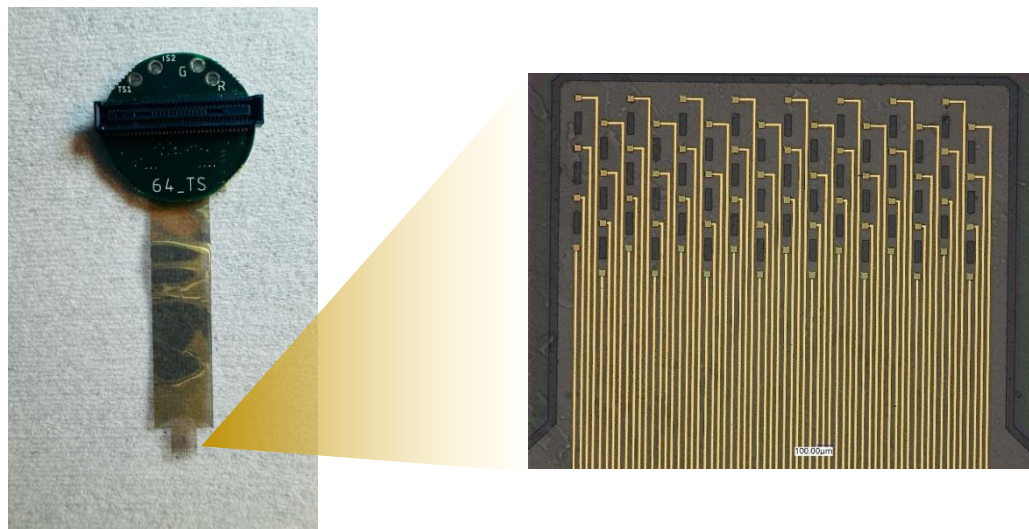


Figure 4: The 64-channel passive flexible electrode probe used for chronic recordings. Inset: Microscope image showing the flexible array after the peel-off process of the PaC sacrificial layer.

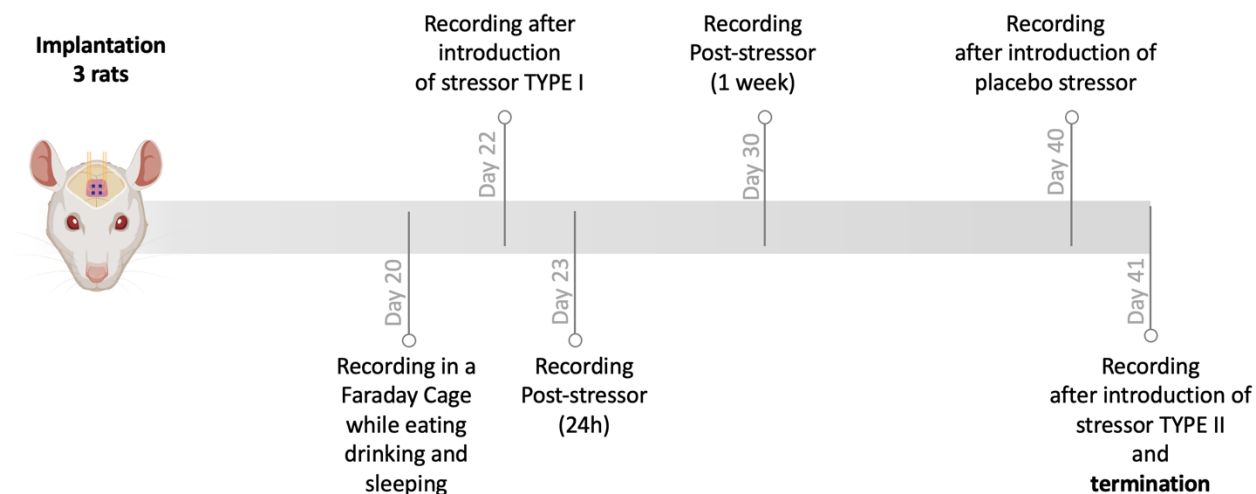


Figure 5: Chronic implantation protocol of recording EEG signals using passive electrode arrays for > 1 month.

Figure 5 illustrates the chronic implantation protocol, highlighting key recording events and two distinct stressor protocols. Stressor Type I involves space constriction, while Stressor Type II consists of exposure to predator odor.

To secure the acquisition board to the animal's head, a **3D-printed headpiece** was designed and fabricated (Figure 6A). This headpiece provided a stable mounting platform for the recording system, ensuring that the electrodes remained in place while minimizing movement artifacts (Figure 6B). The design allows for

easy attachment and detachment, facilitating chronic recordings. The animal was allowed to recover and was monitored to ensure it could move freely with the implant (Figure 6C and 6D).

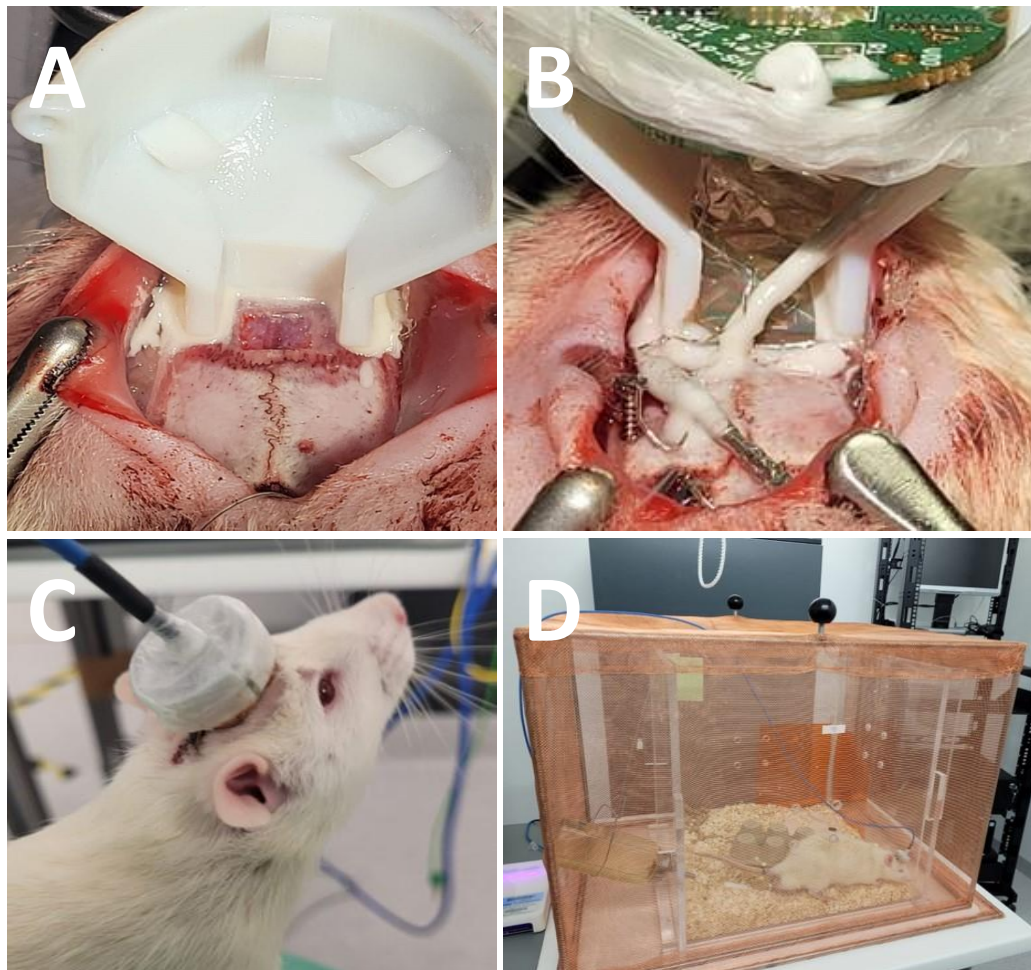


Figure 6: Post-surgery implantation of the 64-channel passive electrode array in the rodent. (A) A 3D-printed headpiece was cemented to the head for the accommodation of the acquisition board. (B) The electrode array (not visible, placed on the cortex) is connected to the headstage fixed on the skull with dental cement. The small form-factor of the headstage minimizes discomfort and allows the subject to carry it easily. (C) and (D) Post surgery images showing that the animal free to move normally with the headstage.

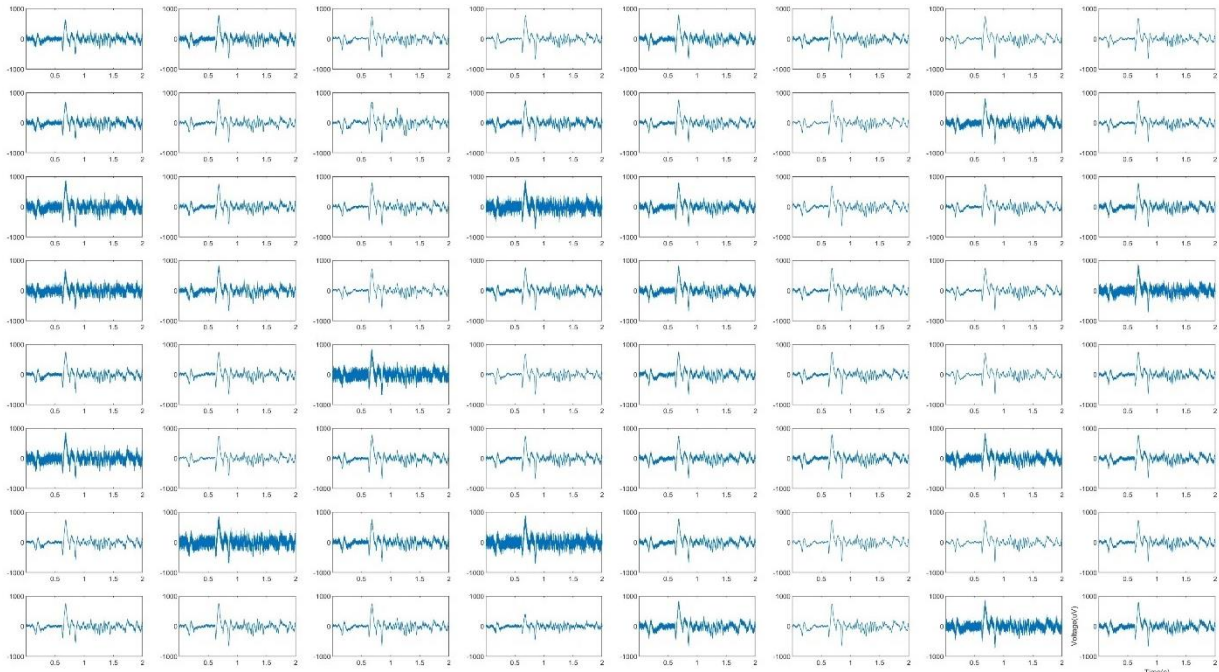


Figure 7: Sample LFP recordings from the chronic 64-channel passive electrode array after implantation (DAY 1). Traces from four example channels over a 2-second window are shown, illustrating typical neural LFP activity.

During chronic recording sessions, we successfully captured local field potential (LFP) signals from the 64-channel array. Figure 7 shows an example of LFPs recorded from the implantable array in Day 1. The signals are in the order of hundreds of microvolts in amplitude and exhibit characteristic fluctuations corresponding to the animal's brain activity. Notably, the LFP traces contain oscillatory patterns that are indicative of the brain's neural network dynamics. All 64 channels were functional, and signal quality remained consistent over 4 weeks, indicating good implant stability.

To analyze the frequency content of the recorded neural signals, we performed a spectral analysis of the LFP data. In particular, we were interested in identifying known brain oscillations such as **beta (15–30 Hz)** and **low-gamma oscillations (30–50 Hz)** in our recordings. Power spectral density calculations were computed for each channel and each recording session

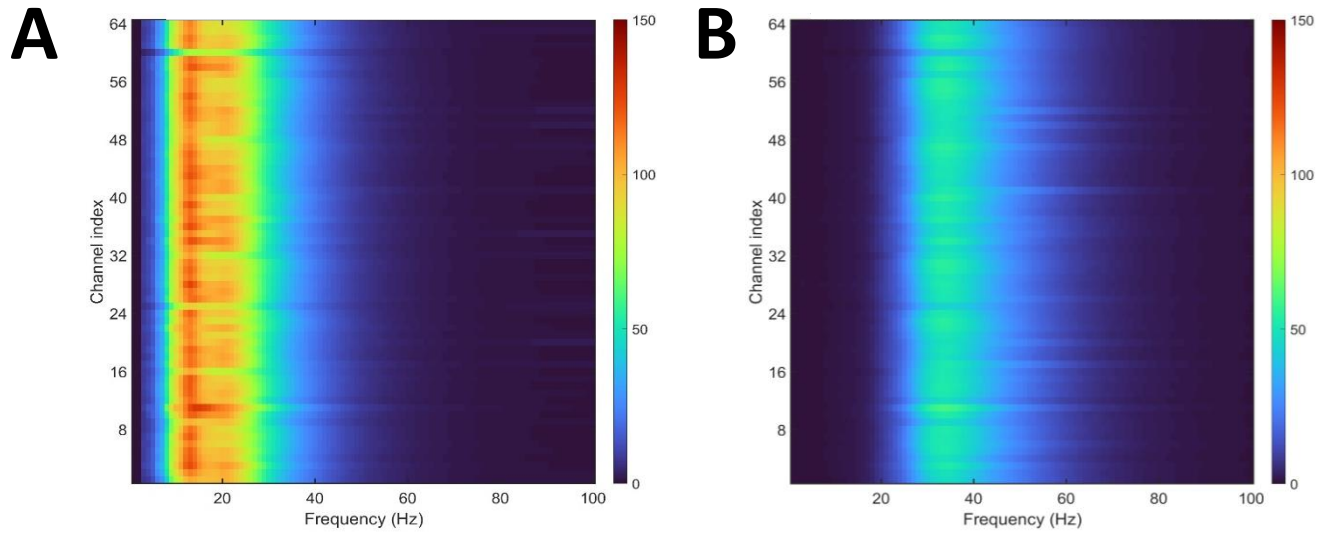


Figure 8: Time-frequency spectrogram of the LFP activity from the passive electrode array (one channel, 2-second segment) after implantation (DAY 1). Warmer colors indicate higher power at a given frequency over time. The spectrogram shows persistent (A) beta and (B) low-gamma oscillations.

Additionally, time-frequency analysis (spectrograms) was used to examine how these oscillations persist or vary over time. The spectrogram of the LFP data (Figure 8) illustrates that beta and low-gamma activity were continuously present during quiet waking periods. Importantly, no significant interference or noise

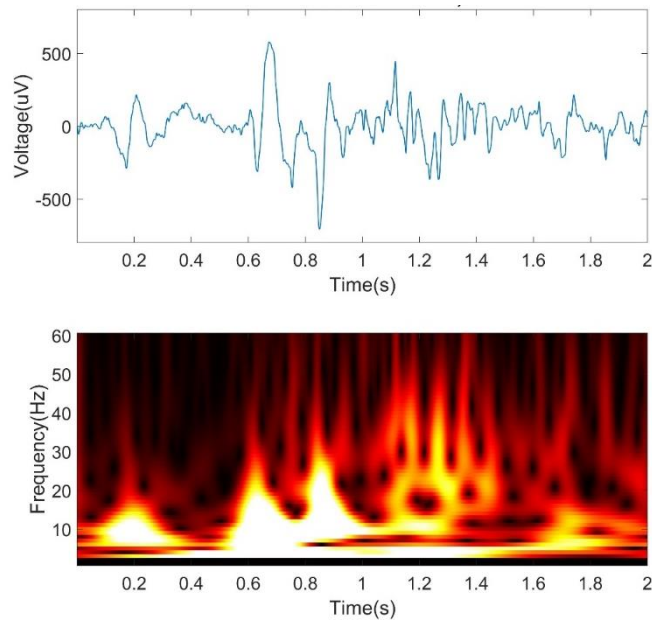


Figure 9: Power spectral density of an LFP signal from the chronic recording after implantation (DAY 1) (single channel, 2 sec of data).

artifacts at these frequencies were observed, which means the recorded oscillations are genuinely neural in origin.

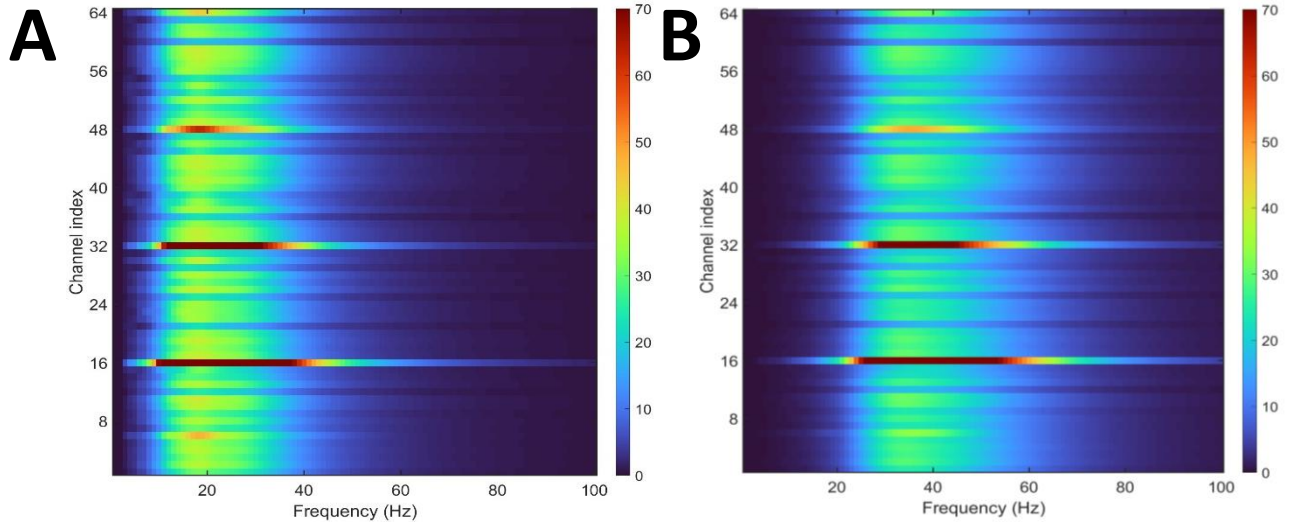


Figure 10: Time-frequency spectrogram of the LFP activity from the passive electrode array (one channel, 2-second segment) during DAY 41. Warmer colors indicate higher power at a given frequency over time. The spectrogram shows persistent (A) beta and (B) low-gamma oscillations.

Figure 10A and B demonstrate beta and low gamma oscillations obtained during the 41st day. The single-channel LFP data and the corresponding spectrogram are depicted in Figure 11.

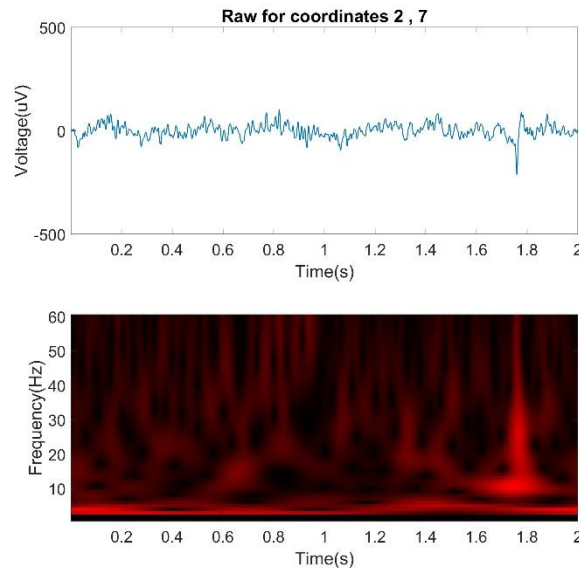


Figure 11: Power spectral density of an LFP signal from the chronic recording during DAY 41 (single channel, 2 sec of data).

Conclusions

In summary, the chronic implantation of passive 64-channel arrays in 3 rats was successful and yielded high-quality neural recordings over an extended period (41 days). We detected key neural oscillations (beta and gamma bands) in the rodent cortex, demonstrating the capability of our system to stably capture relevant biomarkers. These results serve as an important database for developing biomarker detection algorithms and mark the completion of Deliverable 2.3.

Moreover, the development and validation of the second-generation PCB confirmed its ability to provide stable biasing and effective amplification of neural signals using the IGT common-source amplifier topology. We successfully mitigated crosstalk and achieved reliable signal transmission from every transistor line. Moving forward, we are focused on miniaturizing the PCB to function as an adaptor for the Open Ephys headstage while also contributing to the design of a custom CMOS chip that can directly interface with IGT transistor probes.