A novel multisite silicon probe for laminar neural recordings

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PROBE DESIGN

The whole length of the silicon probe is 12 mm. The electrode shaft, that can be inserted into the brain tissue, is 7 mm long, 280 µm wide with a thickness of 80 µm. 24 square shaped and equally spaced platinum recording sites (with 30 µm x 30 µm contact size and 100 µm site spacing) were exposed at the end of the shaft (Fig.1.B,C, Fig.2.A, Fig.3.). The distance between the tip of the probe and the middle of the first Pt contact is 660 µm. Bonding pads were designed at the other end of the device in the form of 200 µm x 200 µm SiO_2-Pt microgrids.(Fig.1.B, Fig.3.) The recording sites were electrically connected to the bonding pads via 4 µm wide and 300 nm thick conductive paths made out of platinum.
A printed circuit board (PCB) specifically designed for this purpose with a 26 pole Preci-dip connector was used for packaging (Fig.1.A). First, the silicon chip was glued to the PCB with a two component epoxy resin and after that, the PCB and the probe were connected via ultrasonic wire bonding with 50 µm thick Al wires.(Fig.1.B.)
One of the main novel features of the probe that it can easily penetrate the dura and pia mater without bending, breaking or causing serious bleeding or brain damage due to its mechanical properties, rounded profile and yacht bow like, sharp tip geometry (Fig.2.B). This tip and shaft profile was implemented using a novel wet etching technique. The silicon probe can also be easily cleaned and reused several times.

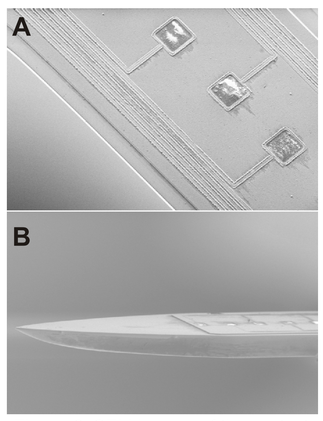

Figure 2. SEM images of the contacts (**A**) and the yacht bow like tip (**B**)

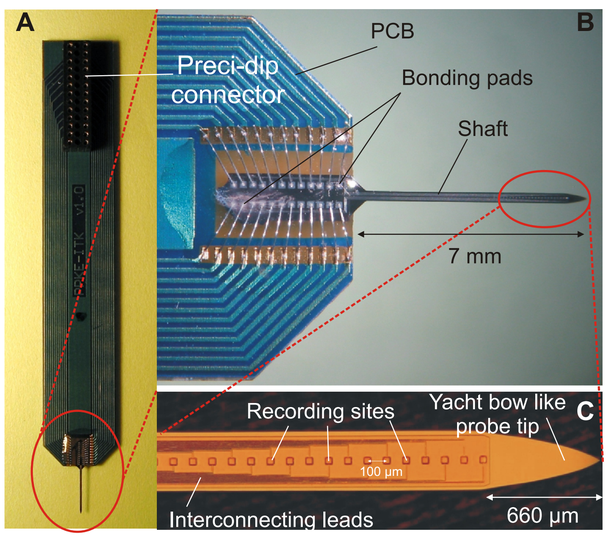

Figure 1. Outlook of the silicon probe **A** - The printed circuit board with the 26 pole connector **B** - The silicon chip with the Al bondings **C** - The tip and several Pt contacts of the electrode shaft

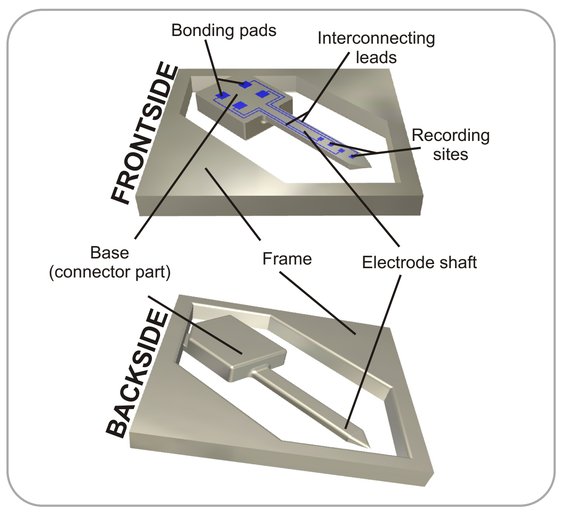

Figure 3. A simplified 3D model of the silicon probe with four recording sites and bonding pads.

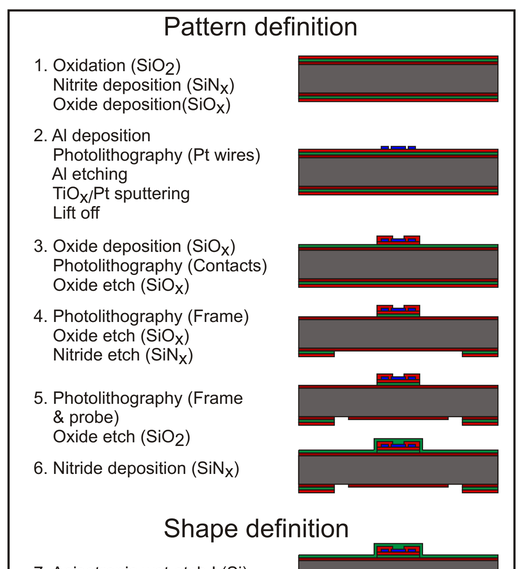

Figure 5. Main steps of the fabrication process

FABRICATION PROCESS

Standard 3-inch <100> oriented 200- µm-thick p-type silicon wafers polished on both sides were used (Fig.4.) for the probe fabrication process that proceeded in several technological steps (Fig.5.). The pattern definiton consisted of various thin-film depositions to form the bottom insulating layers, the Pt electrode contacts and wires, the passivation layer, the contact holes and the bonding pads (Fig.5. Steps 1-6.). The subsequent phases consisted of two anisotropic and one isotropic wet chemical etching step to define the shape of the probe in order to obtain the sharp tip with rounded edges (Fig.5. Steps 7-10.). The final phase was the removal of protecting and masking layers and packaging the probe (Fig.5. Step 11.). Four masks were used to define the laminar structure and the shape of the probe. The frontside masks were used to define the pattern of the probe, while the backside masks were used to define the probe shape and tip. The length, width and thickness of the electrode can be widely varied due to the silicon technology. A patent application describes the the fabrication process in detail.

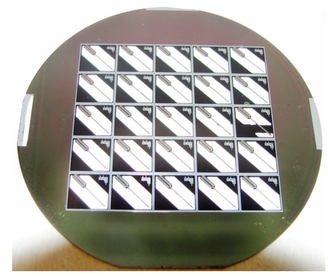

Figure 4. A 3-inch silicon wafer with 25 probes

IMPEDANCE REDUCTION

The impedance of the neural interface was measured with an electroanalytical activation and sensing instrument at 1 kHz in Ringer's lactate solution. The average site impedance and standard deviation (SD) was 1067.36±99.91 kΩ measured from overall of 72 contacts of 3 probes (Fig. 6.A). The electrode impedance can be reduced significantly by extending the surface of the platinum recording sites. This was managed with an electrochemical etching process, where +2V was applied through the electrode sites immersed in physiological saline solution for 10 seconds. Using this procedure, the average impedance of the probe dropped to 659.04±59.47 kΩ (Fig.6.B). With longer activation durations the impedances could be decreased further reaching an average of 200-300 kΩ (Fig.6.C).
Another method to lower the impedance of the probes is by depositing carbon nanotube (CNT) coatings onto the recording sites. CNTs have excellent electrical and physical properties, are chemically inert and biocompatible. They have extremely large surface areas, which decreases the impedance of the Pt-contacts to an average of 44±6.9 kΩ (Fig 6.D). Another advantages of CNT coatings are improved electrode sensitivity which occurs not at the expense of selectivity, high charge transfer characteristics which results in enhanced electrical stimulation of neurons and lower noise levels during recordings. The CNT coatings can withstand the mechanical and chemical effects of the implantation and ensure a long term recording stability.

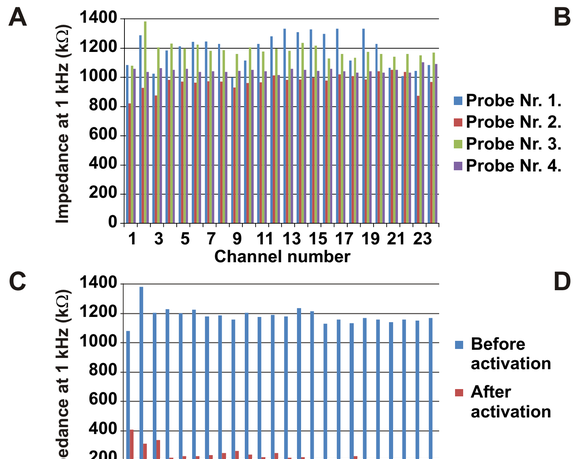

Figure 6. Impedance values of the probes measured at 1 kHz with an electroanalytical activation and sensing instrument in Ringer's lactate solution.
A - Original impedance values of four different electrodes.
B - Impedance reduction after a 10 sec long electrochemical activation by applying +2 V voltage through the platinum recording sites of one of the probes.
C - With a longer activation process (~30 sec) the impedances can be decreased further to reach an average of 200-300 kΩ.
D - Changes in the impedance values after several hours of in vivo experimentation. Every second site of the prove was coated with carbon nanotubes (even numbers).

ELECTROPHYSIOLOGICAL RECORDINGS

The probe provides high quality local field potential (LFP), multiple-unit (MUA) and single-unit (SUA) activity recordings (Fig.7.). The multielectrode attached to a micromanipulor was inserted into the trunk region of the primary somatosensory cortex of rats under ketamine/xylazine (KX) anesthesia. The electrical activity of the brain was recorded with a gain of 1000 on 24 channels. The sampling rate of the amplified wideband (0.1 Hz-7 kHz) signal was 20 kHz/channel and it was stored on a hard drive at 16 bit precision for further offline analysis.
The slow oscillation (SO) is a brain rhythm observed mainly in cortical and thalamic regions during natural slow-wave sleep or anesthesia, where the membrane potential of neurons alternates between a depolarized ("up-state") and a hyperpolarized ("down-state") state. The "up-state" is associated with increased synaptic activity, action potential generation of neurons and with surface positive and depth negative cortical LFP deflections. The "down-state" is characterized by neuronal silence probably occurring due to disfacilitation. The LFP of the cortical "down-state" is positive in the deep layers and has a negative polarity superficially (Fig.7.A,C).

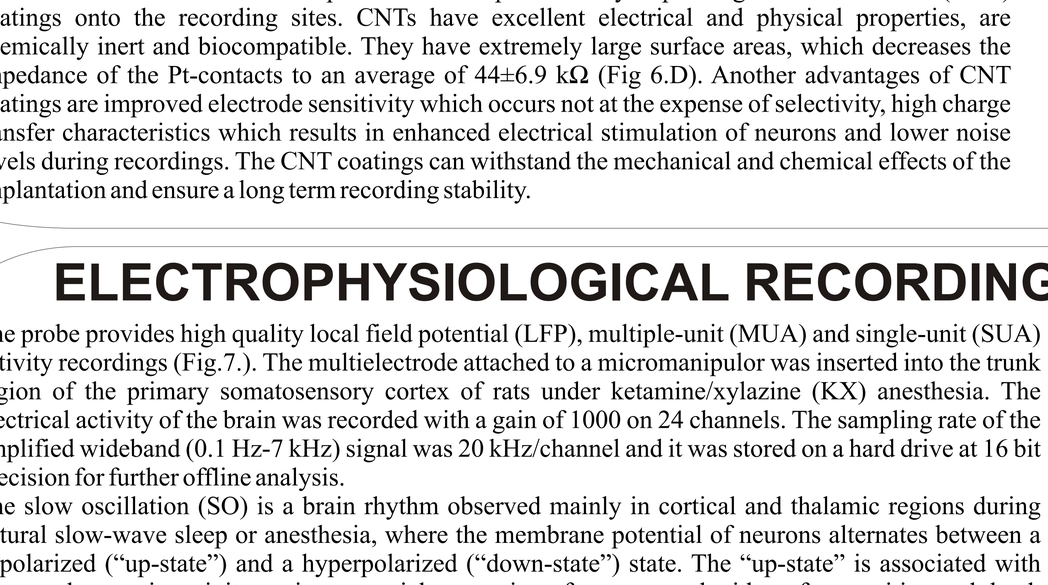

Figure 7. A - A seven second long segment of a 23 channel wideband (0.1 Hz- 7 kHz) local field potential (LFP) recording. The actual state of the slow-wave activity is shown under the bottom channel. **B** - The local field potential gradient (LFPg) derived from **A** (the LFPg is the first spatial derivative of the LFP) **C** - Multiple-unit activity (MUA) is calculated by filtering the recorded and rectifying the wideband signal between 500 and 5000 Hz. **D** - Current-source density (CSD) derived from **A** (the CSD is approximated with the negative second spatial derivative of the LFP). Positive deflections represent outward currents (source) while negative values indicate inward currents (sink).

The exact mechanism of generation of the SO is still unclear, but there are significant evidences of its cortical origin. Laminar electrophysiological analysis of the cortex during SO can yield important information about the undelying processes. For example current source density (CSD) shows the spatiotemporal changes of macroscopic transmembrane current sinks and sources in the extracellular medium. To calculate the CSD, multielectrodes with equidistant contact spacing implanted perpendicular in laminated brain structures are needed. Our probe fits these needs and because we can record from the whole cross-section of the rat cortex with 24 sites simultaneously, the CSD profile (Fig 8.A - LFP, LFP gradient (LFPg) and MUA profile are also shown) of the neocortex during the SO can be constructed. The obtained results are similar compared to electrophysiological profiles of the cortex recorded with laminar metal multielectrodes (Fig.8.B). The probe can detect also the activity of discharging neurons close to the contacts with good quality; therefore single unit analysis can be also performed on the data (Fig.9.).

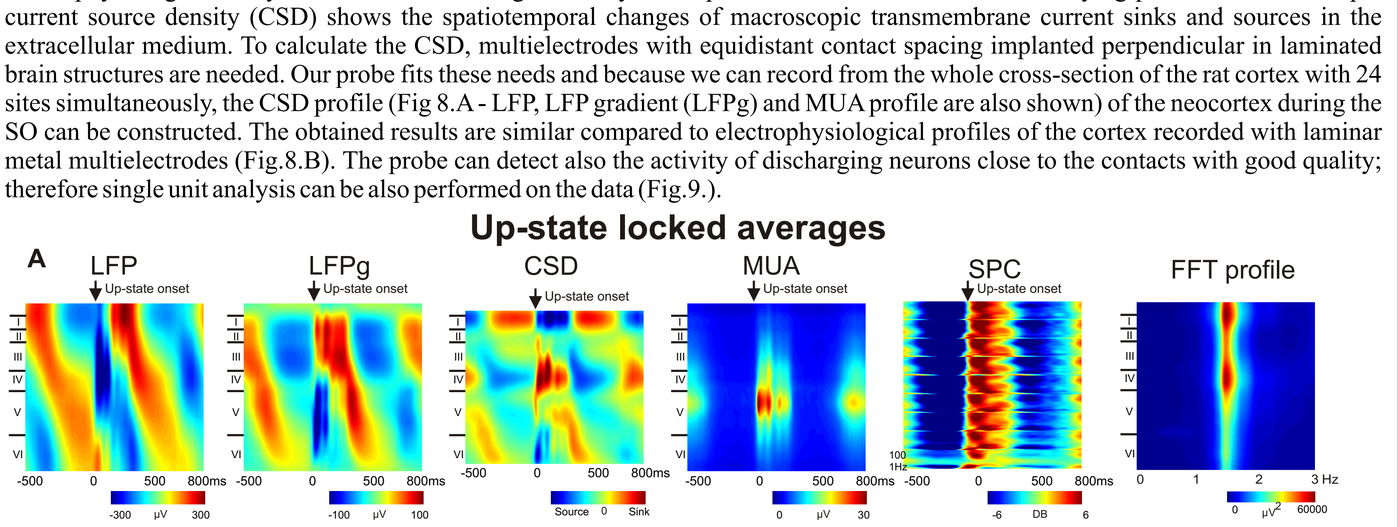

Figure 8. Laminar analysis of up-state locked averages **A-** Representative depth profile maps: up-state locked local field potential (LFP), local field potential gradient (LFPg), current-source density (CSD), multiple-unit activity (MUA) averages (x-axis: time, y-axis: cortical depth with corresponding laminarization, z-axis: colour coded amplitudes) and LFPg spectrograms (SPC) calculated from nine representative channels in layers I-VI. The last picture on the right is the depth distribution profile of the FFT power spectrum calculated from the LFP. It shows a peak around 1.5 Hz, which is a common frequency of the slow oscillation under ketamine-xylazine anesthesia.
B - The up-state locked averages constructed from recordings with a 24 contact metal multielectrode with 100 µm inter-electrode distance are similar compared to the recordings with the silicon probe. From left to right: depth profile maps of up-state locked LFP, LFPg, CSD and MUA averages.

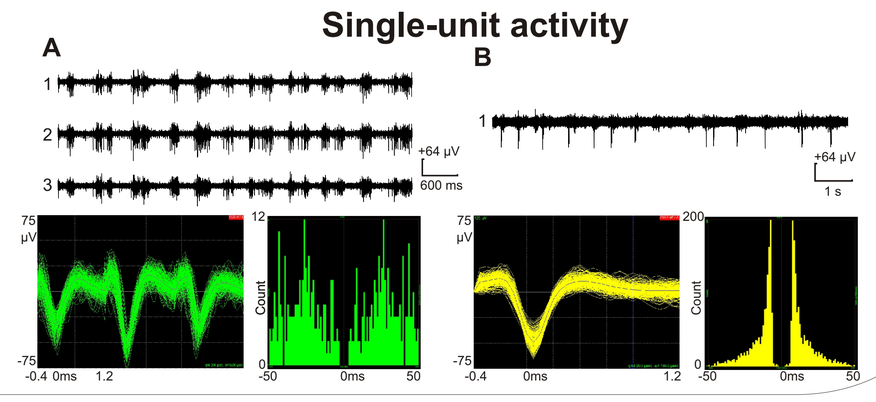

Figure 9. Single-unit activity recorded with the silicon probe.
A - 6 second long activity of several neurons on three adjacent recording sites.The action potentials of one of the recorded neurons is present on all three channels. Bottom pictures: sorted spikes of the before mentioned cell overlayed on each other (green) and the corresponding interspike interval (ISI) histogram.
B - A 10 second long recording showing the activity of a bursting neuron which was recorded from the bottom of the cortex. The left picture on the bottom shows 300 overlayed spikes (yellow) and the average action potential of the cell (gray line). On the ISI histogram (bottom right picture) a peak can be observed at around -10 and 10 ms. This peak is typical of bursting neurons.

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