Combined macro-/mesoporous microelectrode arrays for low-noise extracellular recording of neural networks

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Heim M, Rousseau L, Reculusa S, Urbanova V, Mazzocco C, Joucla S, Bouffier L, Vytras K, Bartlett P, Kuhn A, Yvert B. Combined macro-/mesoporous microelectrode arrays for low-noise extracellular recording of neural networks. J Neurophysiol 108: 1793–1803, 2012. First published June 27, 2012; doi:10.1152/jn.00711.2011.—Microelectrode arrays (MEAs) are appealing tools to probe large neural ensembles and build neural prostheses. Microelectronics microfabrication technologies now allow building high-density MEAs containing several hundreds of microelectrodes. However, several major problems become limiting factors when the size of the microelectrodes decreases. In particular, regarding recording of neural activity, the intrinsic noise level of a microelectrode dramatically increases when the size becomes small (typically below 20-μm diameter). Here, we propose to overcome this limitation using a template-based, single-scale meso- or two-scale macro-/mesoporous modification of the microelectrodes, combining the advantages of an overall small geometric surface and an active surface increased by several orders of magnitude. For this purpose, standard platinum MEAs were covered with a highly porous platinum overlayer obtained by lyotropic liquid crystal templating possibly in combination with a microsphere templating approach. These porous coatings were mechanically more robust than Pt-black coating and avoid potential toxicity issues. They had a highly increased active surface, resulting in a noise level ~3 times smaller than that of conventional flat electrodes. This approach can thus be used to build highly dense arrays of small-size microelectrodes for sensitive neural signal detection.

electrophysiology; nanotechnology; neural implant; prosthesis; multielectrode; neural probes

Yet, decreasing the size of microelectrodes is associated with an increase in their impedance and leads to several limiting factors. First, regarding recording of neural activity, the intrinsic noise level of microelectrodes dramatically increases when their size becomes small, namely for diameters typically <20 μm (Gesteland et al. 1959; Heuschkel et al. 2002), which is actually a general problem of microsensors (Imokawa et al. 2005). This phenomenon is due to both the intrinsic thermal noise of the electrode and their higher sensitivity to environmental perturbations at high impedance. Second, regarding electrical microstimulation, a small surface of the electrode-tissue interface strongly limits the level of currents that can be injected without degrading both the electrode and the tissue (Robblee and Rose 1990). A way to overcome these problems is to consider microelectrodes made of porous materials to increase the active surface of their electrode-medium interface while preserving a small geometric surface. Toward this goal, platinum black has been used as an overlayer of microelectrode surfaces, which indeed lowers their impedance and decreases the intrinsic noise level (Gesteland et al. 1959; Oka et al. 1999). However, platinum black remains very fragile and not mechanically robust on the long term. Although better durability has been obtained using electroplating of platinum black under sonication (Desai et al. 2010), this material is considered to be potentially toxic to cells for long-term implants (Malleo et al. 2008; Shoval et al. 2009; Su et al. 2010; Wang et al. 2006), but this strategy also suffers from limited mechanical stability and also raises concerns regarding long-term biocompatibility.

Here, we present a novel approach to achieve microelectrodes with a highly porous and organized platinum overlayer obtained by a combined lyotropic liquid crystal (mesoscale) and organized microspheres (macroscale) templating. We show that such a porous modification allows building low-noise microelectrodes for sensitive neural signal detection.

METHODS

Fabrication of MEAs with different electrode sizes. To highlight the influence of electrode diameter on intrinsic noise level and assess further the benefit of porous modifications, 56-channel MEAs with different electrode sizes (6, 8, 12, 16, 32, and 64 μm) have been
Innovative Methodology

LOW-NOISE MACRO-/MESOPOROUS MEAs

Fig. 1. Noise level and impedance increase as the size of microelectrodes decreases. A: microfabrication steps of the microelectrode arrays (MEAs). B: example of an MEA made on a glass substrate. C: close-up view of microelectrodes of different sizes. D: example of background noise recording with electrodes of different sizes. E: dependence of intrinsic electrode noise ($\sigma_e$) on electrode diameter (average ± SD over 236 electrodes). F: dependence of electrode impedance ($Z_0.3 \text{ V vs. Ag/AgCl and } F = 1 \text{ kHz}$) on electrode diameter ($n = 65$ electrodes). G: dependence of intrinsic electrode noise on electrode impedance ($n = 63$ electrodes).

developed (Fig. 1, A–C). In these arrays, electrodes are arranged in a $4 \times 15$ layout without corners, covering an area of $900 \times 12,600 \mu m^2$ adapted to the geometry of embryonic hindbrain-spinal cord preparations or a $1 \times 56$ linear arrangement with an interelectrode spacing of $150 \mu m$. The different electrode sizes were produced from a metal layer of unique size ($80 \mu m$) covered by a silicon nitride insulation layer having an opening of variable size. These arrays were compatible with Multi Channel Systems amplifier MEA1060 to be tested for neural recording. Figure 1A illustrates the fabrication process. First, an initial substrate consisting of either a glass or a silicon wafer covered by a 500-nm oxide layer is patterned with a metal layer defining the electrodes and their leads by a lift-off technique. For this purpose, the wafer is spin-coated with 2 $\mu m$ of nLOF 2020 Photoresist (Clariant), which is further removed at specific locations by photolithography to define the geometry of the metal layer. Next, 50 nm of titanium and 150 nm of platinum are deposited over the whole wafer. The wafer is then placed in an ultrasonic bath with a dedicated remover (AZ400) to remove the photoresist, leaving only the metal layer deposited directly on the initial substrate at the location of the electrodes and leads. In a second step, an insulation layer of silicon nitride is deposited by plasma-enhanced chemical vapor deposition over the whole wafer. This silicon nitride layer is then etched at the location of the electrodes. For this purpose, the wafer is protected by a nLOF Photoresist film ($2 \mu m$), which is etched over the electrodes to define the different
electrode sizes. Then, the silicon nitride is etched using a plasma of SF$_6$ gas at locations not protected by the photoresist (i.e., electrodes). nLOF is then removed. Finally, the wafer is cleaned and cut to separate the MEAs, and an annular glass ring is glued with polydimethylsiloxane to constitute a recording chamber around the electrodes. Figure 1B shows a MEA obtained on a glass substrate. Figure 1C shows an example of resulting electrodes of different sizes on a silicon substrate. All the tests and characterizations presented thereafter were performed on glass substrate MEAs.

Three different porous modifications of these flat microelectrodes were considered and evaluated: a mesoporous overlayer, a combined macro-/mesoporous overlayer, and a Pt-black overlayer.

Mesoporous modification of MEAs. Figure 2A outlines the key steps that were necessary to fabricate mesoporous microelectrodes on a MEA. A nanostructured metal film was obtained on the microelectrodes by electroplating a metal in the presence of lyotropic liquid crystalline phases, acting as a template (Attard et al. 1997). Here, platinum ions that were dissolved in the aqueous domains of the liquid

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**Fig. 2. Mesoporous modification of microelectrodes.** *A:* illustration of the steps involved in the fabrication of mesoporous microelectrodes using lyotropic liquid crystal templating. *B:* scheme illustrating the fabrication of macro-/mesoporous microelectrodes having a double-scale porosity using both colloidal spheres and surfactant molecules as macro- and mesotemplates, respectively. *C:* scanning electron micrographs (SEM) images showing 12-μm microelectrodes before the modification, after modification with a mesoporous (1 C/cm$^2$), a macro-/mesoporous overlayer, and a Pt-black deposit (both 5.5 C/cm$^2$). ø, Diameter.
crystalline phases were electrochemically reduced. This resulted in a platinum deposit around the surfactant molecules that were arranged in a rodlike configuration. Washing away the surfactant after the electroplating revealed an array of mesopores in the metal deposit.

The plating mixture consisted of a ternary system composed of 42% wt octaethylene glycol monododecyl ether (C\textsubscript{12}EO\textsubscript{8}; 98% purity; Sigma), 29% wt hexachloroplatinic acid hydrate (H\textsubscript{2}PtCl\textsubscript{6}·99.9% purity; Sigma), and 29% wt Milli-Q reagent water (resistivity \( \geq 18 \) M\text{Ω}-cm). The components were mixed in a glass vial vigorously for several minutes at room temperature until a gellike compound was obtained. The closed vial was then placed in a thermostated oven at \(-40\)°C for 30 min to allow the mixture to homogenize. Subsequent mixing and heating steps were repeated until a homogeneous solution was obtained.

Electroplating of platinum was performed using this mixture covering the MEA. An Ag/AgCl electrode and a platinum wire (1-mm diameter) were put into the plating mixture to serve as reference and counter electrode, respectively. The microelectrodes of the MEA were the working electrodes. To reduce the platinum ions present in the plating mixture, the potential was stepped from a value of +0.6 to \(-0.1 \) V until the desired amount of charge had passed. To study the influence of the film thickness on the physical properties of the electrode, different amounts of charge ranging from 1 to 8 C/cm\textsuperscript{2} were applied to single microelectrodes on the MEA. In some experiments, electrodeposition was carried out simultaneously on all microelectrodes with a fixed value of charge. After the electrodeposition step, the plating mixture was removed, and the microelectrodes were rinsed with copious amounts of water to wash away the surfactant. To make sure that no surfactant remains in the pores, a piranha solution was allowed to react with the microelectrodes for 10 min to oxidize the platinum and make it hydrophilic to ensure a good penetration of physiological liquid into the nanopores. This (hazardous) piranha solution, which was then washed with distilled water, was composed of 75% vol H\textsubscript{2}SO\textsubscript{4} (95–98% purity; Merck) and 25% vol hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}; 30% solution; FLUKA).

Macro-/mesoporous modification of MEAs. In a second step, we combined the mesoporous templating with a macroporous templating approach (Fig. 2B). First, an organized colloidal template consisting of five layers of silica beads (420-nm diameter) was deposited layer by layer on the MEA by using the Langmuir-Blodgett technique (Reculsa et al. 2011; Reculsa and Ravaine 2003). Subsequently, we partly filled the void space in this template by electrodepositing nanostructured platinum. Therefore, a plating solution consisting of 60 mM hexachloroplatinic acid hydrate, 0.1% wt SDS, and Milli-Q reagent water was prepared (Choi et al. 2003). Owing to the relatively low concentration of surfactant, the solution could easily penetrate into the whole void space of the colloidal template. A potential of \(-0.15 \) V vs. Ag/AgCl was applied to reduce the platinum ions using the previously described setup. In the present case, the formation of mesoporous platinum results from the deposition of platinum around the previously described setup. In the present experiments, the procedure starts (Yvert et al. 2011). In brief, embryonic mouse hindbrain-spinal cord preparations as previously described (Yvert et al. 2011). In brief, embryonic days 12.5–14.5
Fig. 3. Electrochemical characterization. Comparison of the cyclic voltammograms (CVs) of 12-μm microelectrodes obtained before (dashed line) and after their modification (plain line). Comparison of unmodified and mesoporous (A), macro-/mesoporous (B), and with Pt-black modified (C) electrode. A, inset: the CV of the nonporous microelectrode in detail. Note the increased reduction peak of platinum oxide after porous modification (hashed area of the solid CV).

(E12.5–E14.5) embryos were surgically removed from pregnant Oncins France 1 (OF1) mice (Charles River Laboratories, L’Arbresle, France), previously killed by cervical dislocation. Embryos were decapitated, their whole spinal cord and hindbrain were dissected in the physiological liquid gassed with carbogen (95% O2-5% CO2), meninges were removed, and the neural tube was opened along the rostrocaudal axis (open-book preparation) and then placed over the 4 × 15 MEA and superfused with physiological liquid, gassed with carbogen. A plastic net with small holes (70 × 70 μm²) was laid on the neural tissue to achieve a tight and uniform contact with the microelectrodes. Spontaneous rhythmic activity of this immature preparation was recorded for several hours at room temperature using the same apparatus as for the noise measurements. Spiking activity was extracted as follows: for each data sample, a moving average of the signal computed over a 10-ms window centered on this sample was first subtracted from the raw data [direct current (DC) removing], and then the obtained value was replaced by the average of the signal computed over a 1-ms time window centered on this sample (slight smoothing).

All experimental protocols conformed to recommendations of the European Community Council Directive of November 24, 1986 (86/609/EEC) and local French legislation for care and use of laboratory animals. B. Yvert received authorization no. 33 08 002 from Prefecture de la Gironde to carry out these experiments.

RESULTS

Noise level and impedance as a function of electrode diameter. We first assessed how the noise level and impedance of flat microelectrodes depended on their diameter. Figure 1D shows an example of noise recording for different electrode sizes. It can be seen that the smaller the electrode, the higher the noise, with peak-to-peak amplitudes of typical measurements in the range of 40–60 μV for electrode diameters <16 μm. As further quantified for all the electrodes of an array, the noise level (Fig. 1E) and the impedance (Fig. 1F) were inversely proportional to the electrode diameter. Consequently, and as shown in Fig. 1G, the electrode noise level increased with the electrode impedance, a result in accordance with previous theoretical predictions (Gesteland et al. 1959; Heuschkel et al. 2002).

Active surface of porous microelectrodes. Figure 2C shows examples of SEM images of 12-μm-diameter flat, mesoporous, macro-/mesoporous, and Pt-black microelectrodes. The mesoporous electrodes appear very smooth (nearly as flat electrodes) because the size of the mesopores defined by the lyotropic liquid crystal template used during the electrodeposition is very small (~2 nm) and cannot be resolved in SEM images. By contrast, macro-/mesoporous electrodes clearly display a nice organization of the macropores. The Pt-black microelectrode shows a typical mushroomlike surface.

To compare the active surface area of flat vs. modified microelectrodes, CV experiments were performed before and after electrodeposition. Figure 3A shows a typical CV for 12-μm-diameter platinum microelectrodes with and without a nanostructured surface. The peak for the platinum oxide reduction observed for the mesoporous electrode (hashed area) is >1 order of magnitude higher (×50) compared with the flat microelectrode. This increase is due to the well-ordered, highly accessible array of mesopores on the electrode surface. Figure 3B and C, further shows CVs for macro-/mesoporous and Pt-black microelectrodes, showing an increase in the electrode surface by several orders of magnitudes.

Impedance of porous microelectrodes. Impedance spectroscopy was performed (within the range 1–10,000 Hz) to determine the decrease of impedance obtained by mesoporous, macro-/mesoporous, or Pt-black modifications. Figure 4A illustrates the frequency-dependent impedance amplitude of 12-μm diameter mesoporous microelectrodes for different amounts of
charge used for their nanostructuration as well as of 12-μm-diameter macro-/mesoporous and Pt-black microelectrodes. In theory, the higher the charge, the thicker the nanostructured layer, and thus the lower the impedance. We found that nanostructuration reduced the impedance by up to 2 orders of magnitude, the highest decrease being obtained for the lower frequencies. Increasing the amount of charge used for the modification improved electrode impedance only moderately for mesoporous microelectrodes. The mesoporous film behaves like the idealized de Levie model for a porous electrode. Each pore acts like a transmission line so that at low frequency the charging/discharging of the pore wall capacitance progresses deeper into the pore than at high frequency. Hence increasing film thickness will have no effect beyond a certain value at a fixed frequency because the innermost part of the pore will no longer contribute (Elliott and Owen 2000).

We further evaluated the reproducibility of the noise improvement on an array composed of 56 12-μm microelectrodes modified with a charge of 4 C/cm². As shown in Fig. 5C, the noise improvement was homogenous across the array, decreasing from 8.4 ± 1.1 μV (mean ± SD) to 3.3 ± 1.2 μV. Figure 5D shows average noise improvements for the three different porous approaches: compared with flat microelectrodes, noise was reduced by a factor of 2.5, 3.4, and 5.8, with mesoporous, macro-/mesoporous, and Pt-black modifications, respectively.

**Neural recordings with mesoporous microelectrodes.** Figure 6A shows a whole embryonic mouse hindbrain-spinal cord prep-
aration opened on a mesoporous MEA. Figure 6B shows rhythmic activity (raw data) recorded in such a preparation using an MEA made of 12-μm-diameter mesoporous platinum microelectrodes. Activity was composed of rostrocaudal waves originating in the hindbrain and propagating caudally along the spinal cord (Fig. 6B) as described previously (Yvert et al. 2011). Figure 6C shows a close-up view of two successive episodes recorded on two electrodes of the array. Each episode is composed of a local field potential superimposed on a burst of spikes. Figure 6D shows the same data segment where only the spiking activity was extracted (see METHODS), which shows low-amplitude bursts of spikes. This embryonic preparation was very suitable to test the sensitivity of the recording because the spikes generated by this immature network are of low amplitude (typically 10–20 μV). Mesoporous modification of the electrodes allowed to detect clearly these low-amplitude bursts. However, in the presence of a tissue, the noise level of the mesoporous electrodes tended to increase with time when
recordings were performed during several hours. Figure 6 shows an example of noise increase over 1-h recording.

Neural recordings with macro-/mesoporous microelectrodes. To cope with this problem, we thus considered macro-/mesoporous microelectrodes. Also, to draw a direct comparison of the recording sensitivity between modified and nonmodified microelectrodes, we built 4×15 arrays with 2 columns of electrodes modified with the macro-/mesoporous approach and 2 columns not modified. As shown in Fig. 7A for an E14.5 preparation opened on such an array (left), activity could hardly be seen on the unmodified electrodes (middle), whereas spike bursts could be clearly detected on modified electrodes (right). This result shows that macro-/mesoporous microelectrodes are sensitive to low-amplitude neural activity. Moreover, unlike mesoporous electrodes, stable signals could be obtained over several hours with good sensitivity.

Finally, we compared this sensitivity with that of Pt-black microelectrodes. For this purpose, we considered an array with two columns of macro-/mesoporous electrodes and two columns of Pt-black electrodes. As shown in Fig. 7B for an E12.5 preparation opened on such array (left), spiking activity could be clearly detected on both types of electrodes, with a better signal-to-noise ratio with the Pt-black microelectrodes. For the spiking signals corresponding to the recording of Fig. 7, the full noise level (including electronic noise of the amplifiers) of the modified 12-μm-diameter electrodes was 1.93 ± 0.32 μV (mean ± SD) for the macro-/mesoporous microelectrodes and 1.34 ± 0.35 μV for the Pt-black microelectrodes.

Assessment of the mechanical stability of macro-/mesoporous vs. Pt-black microelectrodes. The mechanical robustness of mesoporous, macro-/mesoporous, and Pt-black microelectrodes was further assessed by recording the noise level of the microelectrodes before and after a standard cleaning procedure of the MEA. Cleaning was performed by filling the MEA chamber with a housekeeping dishwashing detergent (Paic citron) and brushing the array using a soft painting brush made of marten hair for ~1 min. This procedure was repeated 5 times, and noise was recorded after each cleaning step. As shown in Fig. 8A, we found that the noise of mesoporous microelectrodes was not affected by the different cleanings. Macro-/mesoporous microelectrodes had lower and more uniform intrinsic noise than the mesoporous microelectrodes (Fig. 8B). Following cleaning, a few macro-/mesoporous microelectrodes showed increased noise level, which we checked by SEM corresponded to a loss of their coating. However, most of the electrodes (20 out of 28) did not show any change in performance after repetitive cleaning (Fig. 8B), whereas all Pt-black microelectrodes were degraded (Fig. 8C).
DISCUSSION

Two templating approaches have been used to obtain organized porous films with well-ordered pore arrangements. Colloidal crystal templating has recently been used to obtain macroporous MEAs with macropores on the scale of hundreds of nanometers (Urbanova et al. 2011). This strategy, however, only led to a moderate noise reduction. In the present work, well-ordered mesopores (typically 2 nm) were generated in the platinum deposit, and a much higher porosity was obtained, resulting in very high active surface areas. A comparable nanoporous strategy has recently been considered to improve the charge injection limit of stimulation microelectrodes (Park et al. 2010) but was not developed or evaluated for neural recording. Here, we show how to build mesoporous and combined macro-/mesoporous microelectrodes to achieve low-noise neural recordings. Mesoporous microelectrodes showed lower noise level than flat electrodes, but better achievement was obtained with combined macro-/mesoporous microelec-

Fig. 7. Low-noise recording of neural activity using macro-/mesoporous MEAs. A: example of wave recording in a E14.5 preparation on a hybrid MEA composed of half flat (2 columns) and half macro-/mesoporous microelectrodes. Signals processed to extract spiking activity are shown on 8 channels of each type, with a close-up view of 1 of each (frame). Activity hardly can be seen on unmodified electrodes, whereas bursts clearly appear on macro-/mesoporous microelectrodes. B: recording of an E12.5 preparation on the same array after the flat electrodes have been coated with Pt-black. Bursting activity can be clearly seen on both types of electrodes.
trodos. Thus the combination of macro- and mesoporous approaches led to better results than either one used in isolation. Pt-black electrodes gave even lower noise levels than either organized porous approach. However, regarding mechanical stability, mesoporous and macro-/mesoporous coatings were more stable than Pt-black overlay, which did not resist standard cleaning procedure (Fig. 8).

The most significant improvement of microelectrode performance was obtained for diameters of 12 µm, for which noise was reduced by roughly a factor of 3. As shown in Fig. 5B, the noise level of smaller microelectrodes could also be significantly reduced, although the improvement factor was slightly smaller. This may be understood in the light of a previous work detailing the influence of several parameters on electrodeposited mesoporous platinum films (Elliott et al. 1999). This study indeed showed that, when the size of the electrode is <200 µm, its roughness factor, which represents a measure for the gain of active surface area for a mesoporous compared with a smooth film, decreases for decreasing electrode size. This finding was attributed to lower efficiencies in the electrodeposition process when micro- instead of macroelectrodes were used. The fact that radial diffusion occurs more efficiently on microelectrodes can cause interaction reaction products to diffuse away from the electrode surface before being reduced to solid platinum. The faradaic efficiency of the Pt deposition in this case varies with the size of the electrode and time and eventually because of the reduction of dissolved oxygen (Gollas et al. 2000). In the same regard, we observed during the electrodeposition experiments that a fixed amount of charge density was injected faster into smaller microelectrodes, indicating that diffusion characteristics changed as a function of the microelectrode diameter.

The good reproducibility of the results obtained in different electrodeposition experiments provides evidence for the high degree of ordering of the lyotropic liquid crystal template, which is maintained in the final pore structure of the mesoporous film. This mesoporous strategy thus offers an interesting alternative to other porous modifications (such as platinum black or carbon nanotubes) that have so far shown limited mechanical stability. Moreover, mesoporous and macro-/mesoporous overlayers also offer perspectives for functional grafting of the microelectrodes for very local endogenous compound (e.g., neurotransmitters) detection. Interestingly enough for end users such as neuroscientists, mesoporous modification of the microelectrodes can be achieved using rather simple laboratory equipment (namely a voltage supply) from a chemical mixture quite easily prepared in the laboratory from commercially available chemicals. Yet, combined macro-/mesoporous modification requires a specific Langmuir-Blodgett apparatus to deposit organized layers of silica beads even though other assembly techniques relying on standard laboratory equipment are also described in the literature. An important step in the fabrication of meso- and macro-/mesoporous MEAs is the need to render the platinum hydrophilic. For this purpose, we used a piranha solution that oxidizes the platinum. MEAs should then be stored in distilled water to avoid drying.

It should be noted that the mesoporous and the combined macro-/mesoporous approaches could equally be applied to other types of MEAs, the only constraint for the macro-/mesoporous approach being that the surface of the array needs to be compatible with the Langmuir-Blodgett procedure (as in Fig. 2B, left). In particular, macro-/mesoporous plating could be ideal for applications where platinum black electrodes, which have a lower intrinsic noise, cannot be used due to their lower mechanical stability compared with the better robustness of this new coating. One of such applications could be the coating of in vivo acute or chronically implanted penetrating silicon probes.

Finally, future developments may seek to combine mesoporous and macro-/mesoporous templating with conductive polymers showing promising performances for neural interfacing (Kang et al. 2011; Venkatraman et al. 2011). Besides the improvement with respect to the signal-to-noise ratio, demonstrated in this study, future work will also evaluate the performance of such nanostructured electrode arrays in the frame of neural stimulation. Indeed, low-impedance materials should improve in two ways the performance of MEAs regarding electrical microstimulation. First, using the porous approach proposed in this study, the amount of charge that can be injected without damaging the electrode material should be higher without changing the geometric electrode surface. Moreover, focalization of stimulation can be achieved using a ground surface surrounding the electrodes of an array, with the highest performance when the impedance of the material of this surface is lowest (Joucla et al. 2012; Joucla and Yvert 2009).

In conclusion, a new method has been developed that can be used to build highly dense arrays of small-size microelectrodes with enhanced mechanical robustness for sensitive neural signal detection.


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