Supporting information

SWCNTs/PEDOT:PSS modified microelectrode arrays for dual-mode detection of electrophysiological signals and dopamine concentration in the striatum under isoflurane anesthesia

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MATERIAL AND METHODS

Reagents and apparatus

Isoflurane ($C_3H_2ClF_5O$) was purchased from RWD Life Science Co., Ltd (China). Poly (sodium-4-styrenesultanate) (PSS) was obtained from HEROCHEM (China). 3,4-ethoxylene dioxy thiophene (EDOT) was purchased from Aladdin (China). Carboxylic ultrapure single-wall carbon nanotube (SWCNTs, OD: 1–2 nm, length: 5–30 μm) aqueous dispersion (0.15 wt%) were obtained from XFNANO (China). Multi-walled carbon nanotubes (MWCNT, OD: 30–50 nm, length: 10–20 μm) were obtained from XFNANO (China). Dopamine (DA) hydrochloride was obtained from Acros Organics (USA). All other chemicals were analytic grade and used as received unless stated otherwise.

Sprague–Dawley (SD) rats were anesthetized with the anesthesia machine (R580S, RWD life science, China). The microelectrode array (MEA) was positioned and implanted into the brain by a stereotaxic frame (model 51650, Stoelting, USA) and a micropositioner (model 2662, David KOPF instrument, USA). The homemade dual-mode recording system was used to record electrophysiological signals and DA concentration. Electrochemical deposition and electrochemical impedance spectroscopy (EIS) were tested by a Gamry electrochemical workstation (Gamry Reference 600, Gamry Instruments, USA).

Dual-mode recording MEA Fabrication

The details of the MEA fabrication process were shown in the following steps: (1) Standard photolithography was performed to define the conductor structure using AZ1500. (2) The sputter of Ti/Pt (30 nm/250 nm) conductive layer was followed by a lift-off process. (3) A SiO_2/Si_3N_4 (300 nm/500 nm) insulating layer was deposited by plasma-enhanced chemical vapor deposition (PECVD). (4) A second photolithography was performed to expose the microelectrodes, reference electrodes and bonding pads. (5) The insulating layer was selectively removed with CHF_3 reactive ion etching (RIE) to expose the microelectrodes, reference electrodes and bonding pads. (6) A third photolithography was performed to define the shape of the probes. (7) The top silicon layer was selectively etched down by inductively coupled plasma deep reactive ion etching (ICP-DRIE). (8) The wafer was covered with the melting asphaltum to protect the top side of the silicon. (9) The back side of the silicon was wet etched in a KOH solution (50%, 80 °C) until it stopped automatically, and then the buried oxide layer was removed in the solution of HF buffer. (10) The probes were released from the wafer and then assembled on the interfacing printed circuit boards (PCB) through pressure welding.

Dual-mode recording equipment Design

As previously described[1, 2], the homemade dual-mode recording equipment was designed for synchronous detection of neural electrophysiological activities and neurotransmitter signals. Briefly, it comprised a dual-function head-stage and a low noise detection instrument. The dual-function head-stage was designed for simultaneous preprocessing of 16-channel electrophysiological signals and 1-channel electrochemical signal. Its light weight and small size made it possible to locate on the head of the SD rat to ensure high signal-to-noise ratio (SNR). The low noise detection instrument mainly consisted of electrophysiological amplifier, electrochemical module, data acquisition (DAQ) module and Signal Acquisition Software (SAS). The dual-mode signals from the dual-function head-stage were further processed by the electrophysiological amplifier and electrochemical module, and then were simultaneously input to the DAQ module in real-time. The DAQ module was interfaced with the SAS implemented on a personal computer (PC) via a universal serial bus (USB 2.0) port. The SAS could perform data processing, online spike detection, online spike firing rate calculation, electrochemical working potential setting, data storage and result display in real-time. It also offered electrochemical analysis methods such as chronoamperometry and cyclic voltammetry.

Protocol for in vivo dual-mode recording under isoflurane anesthesia

The whole experiment comprised an anesthesia machine, a mask, a modified MEA and a homemade dual-mode recording equipment, as shown in Figure S1. The anesthesia machine ventilated the rat through the mask and mediated the degrees of anesthesia by changing the concentration of isoflurane. The dual-mode signals in rat striatum were recorded by the MEA and transmitted to the homemade dual-mode recording equipment for noise removal and offline analysis.

SD rats were provided by the Experimental Animal Center of Peking University. All animal experiments were performed with permission from the Ethical Committee of Peking University. Male SD rats (190 g

~ 200 g, n = 5) were selected for this experiment. All animal experiments were performed in a professional biological laboratory, with the room temperature in the range of 22-25 ℃, the relative humidity in the range of 30-50 %, natural light-dark cycle, uninterrupted food and water supply.

Briefly, the MEA was implanted into the rat striatum, and then the concentration of isoflurane was changed to induce different degrees of anesthesia. Rats were anesthetized by 2.0% isoflurane, and then properly placed in a stereotaxic frame in a flat skull position, where bregma and lambda were lying in the same horizontal coronal plane[3]. Three craniotomies were carried out as show in Figure S2a: a recording position H1 (AP: 1.2 mm, ML: 2.4 mm), an electrochemical reference position H2 (AP: -1.5 mm, ML: -2 mm) and a random ground position H3 at cerebellum. The MEA was implanted into the striatum (DV: 4.85 mm) using a micropositioner to simultaneously detect the electrophysiological signals (action potentials and local field potentials (LFPs)) and DA concentration, as show in Figure S2b,c,d. Each SWCNTs/PEDOT:PSS modified microelectrode was selected to either detect electrophysiological signals or detect DA concentration. For the detection of DA concentration, an Ag|AgCl electrode was placed in the position H2, and Chronoamperometry of electrochemical method (160mV vs Ag|AgCl) was applied to the microelectrode. A special-purpose stainless steel bone screw with cooper wire was twisted into the position H3 to connect the skull to the ground. The entire experiment was conducted in a well-shielded cage to exclude external electromagnetic interference. After 15 minutes of the MEA implantation, the concentration of isoflurane varied to regulate the depth of anesthesia in three anesthesia cycles according to a sequence (1.5% → 1.0% → 0.5%). In the anesthesia-induced death experiments, the concentration of isoflurane changed from 0.5% to 3% to produce overdose anesthesia.

The MEA was connected to the dual-function head-stage of the homemade dual-modal recording equipment. In the experiments, the sampling rate of the system was 30 kHz/channel. The LFPs and action potentials (spikes) were separated from the raw neural electrophysiological signals by software filters with the bandwidth of 0 - 200 Hz and 0.25 - 5 kHz, respectively. Then, they were classified and processed through the commercial software “Offline Sorter x64 V3” and “NeuroExplorer 4”. The DA concentration was recorded with chronoamperometry. The sampling rate of the concentration of DA signal was downsampled to 10 Hz with oversampling techniques. The concentration of DA signal was analyzed and processed using software “Matlab 2016b”.

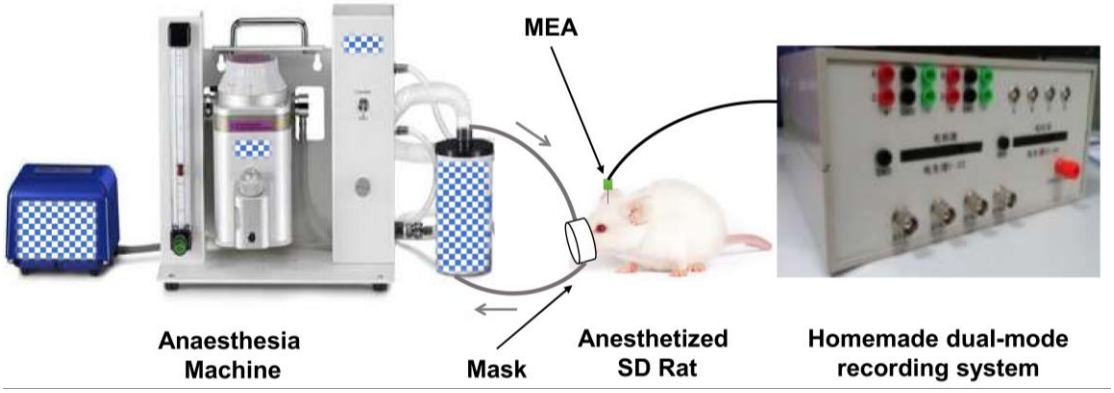

Figure S1. The whole experiment is composed of an anesthesia machine, a mask, a modified MEA and a homemade dual-mode recording equipment.

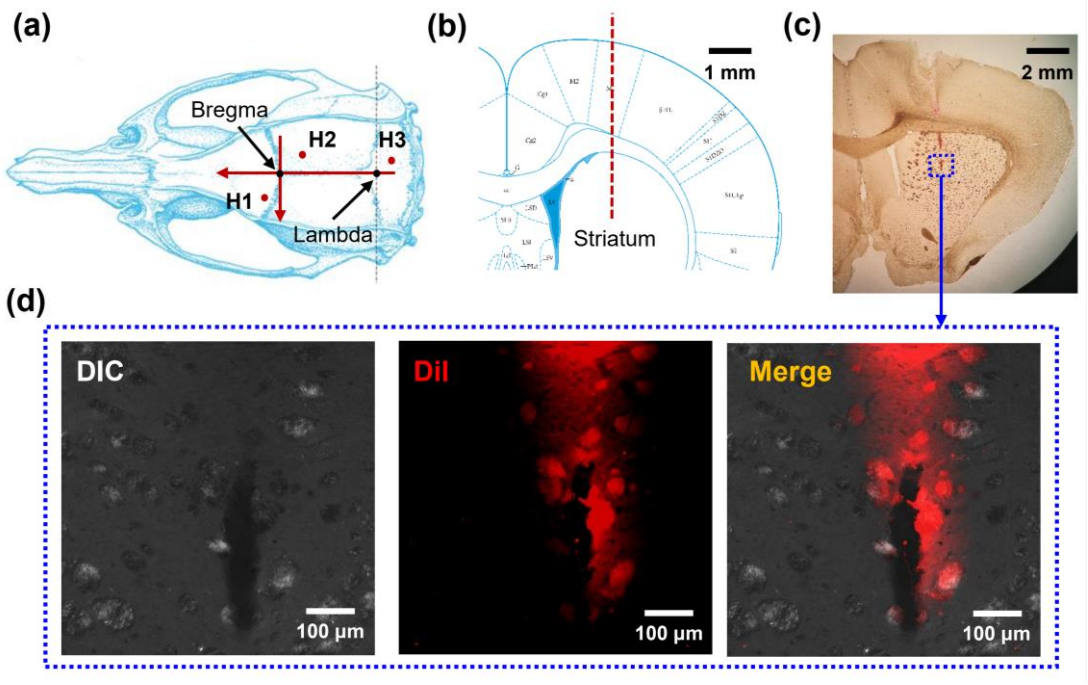

Figure S2. The implantation position of the MEA. (a) Craniotomy position diagram[3]. A recording position H1 (AP: 1.2 mm, ML: 2.4 mm), an electrochemical reference position H2 (AP: -1.5 mm, ML: -2 mm) and a random ground position H3. (b) The picture shows the desired implantation position in the striatum according to rat brain atlas[4] (DV: 4.85 mm). (c) Implantation pathways marked with red fluorochrome in the brain slices of the experimental rat. (d) The DiI (red) trace indicated that the MEA was implanted in the striatum. DIC, differential interference contrast imaging.

RESULTS AND DISCUSSION

Comparison of active surface area of SWCNTs/PEDOT:PSS- and MWCNTs/PEDOT:PSS- fmodified microelectrodes

The differences in active surface area of these two types of MEAs were calculated. The active surface area which can estimate the numbers of active sites of the MEA is responded by the double layer capacitance (C_{dl}). Therefore, according to the reported methods[5, 6], measurements of the C_{dl} from cyclic voltammetry (CV) experiments in a solution containing only supporting electrolyte, where no faradaic current was present, was used for estimations of the active surface area of the MEA.

As shown in Figure S3a,b, the C_{dl} from cyclic voltammetry experiments was applied to evaluate the active surface area of the SWCNTs/PEDOT:PSS and MWCNTs/PEDOT:PSS modified microelectrodes, where the cyclic voltammetry curves was between 0 and 0.4 V at a different scan rate from 20 mV/s to 100 mV/s. The C_{dl} was determined by measuring the capacitive current associated with double-layer charging from the scan-rate dependence of cyclic voltammogram. The Δj could be acquired by cyclic voltammetry measurement under the potential at 0.2 V (j_a - j_c), where j_a was anode current at 0.2 V, and j_c was cathode current at 0.2 V. The C_{dl} was estimated by plotting the Δj against the scan rates (Figure S3c). The results that the C_{dl} of SWCNTs/PEDOT:PSS modified microelectrodes is 0.852 nF, and the C_{dl} of MWCNTs/PEDOT:PSS modified microelectrodes is 0.296, which means that SWCNTs/PEDOT:PSS modified microelectrodes have large active surface areas and more active sites than MWCNTs/PEDOT:PSS modified microelectrodes.

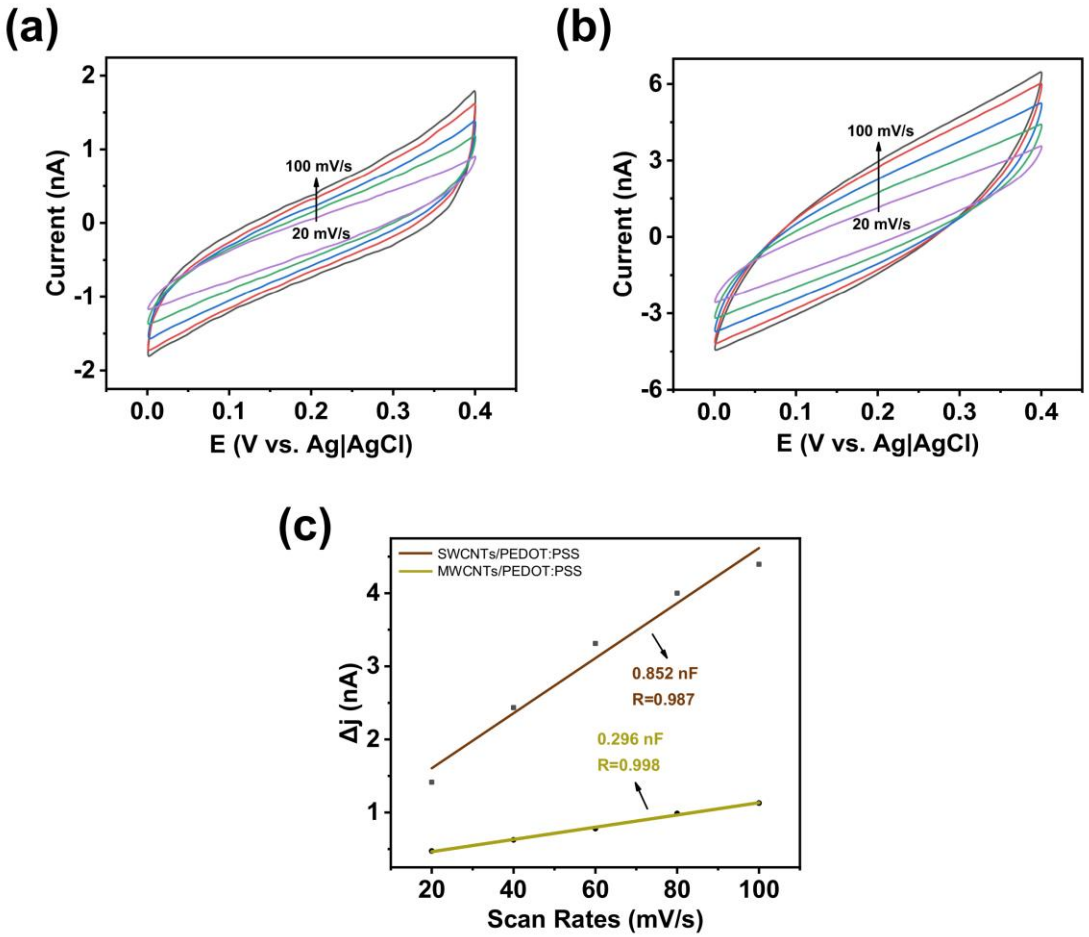

Figure S3. Measurements of the C_{dl} from cyclic voltammetry experiments. (a) The cyclic voltammetry curves of the MWCNTs/PEDOT:PSS modified microelectrodes at different scan rates. (b) The cyclic voltammetry curves of the SWCNTs/PEDOT:PSS modified microelectrodes at different scan rates. (c) The double layer capacitance of the MWCNTs/PEDOT:PSS and SWCNTs/PEDOT:PSS modified microelectrodes.

The stability of SWCNTs/PEDOT:PSS nanocomposites on microelectrodes

To evaluate the stability of SWCNTs/PEDOT:PSS nanocomposites on microelectrodes, we monitored the morphology change of SWCNTs/PEDOT:PSS modified MEA before and after implantation. As shown in Figure S4a, the morphology of two representative microelectrodes do not change significantly before and after implantation, even after washing with water. Further, we detected the impedance and the phase of the SWCNTs/PEDOT:PSS modified microelectrodes before and after implantation. As shown in Figure S4b, at 1 kHz frequency (central frequency of the neural activity), the impedances of the modified microelectrodes are 15.6 ± 1.67 kΩ before implantation and 15.8 ± 2.51 kΩ after implantation ($n = 5$, $p > 0.05$), and the phases of the modified microelectrodes are -25.8 ± 3.39° before implantation and -25.1 ± 3.83° after implantation ($n = 5$, $p > 0.05$), which mean that the electrical performance of the microelectrodes do not change significantly before and after implantation. The above results prove that the SWCNTs/PEDOT:PSS nanocomposites on microelectrodes are stable before and after implantation, and the nanocomposites will not detach from the MEA.

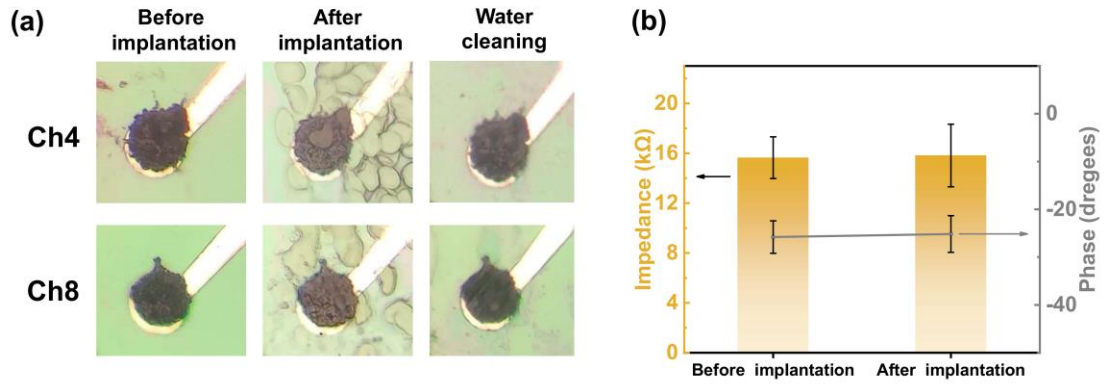

Figure S4. The stability of SWCNTs/PEDOT:PSS nanocomposites on microelectrodes before and after implantation. (a) The morphology change of SWCNTs/PEDOT:PSS modified microelectrodes before and after implantation. (b) At 1 kHz frequency, the impedances and the phases of the SWCNTs/PEDOT:PSS modified microelectrodes before and after implantation (n=5, $p > 0.05$).

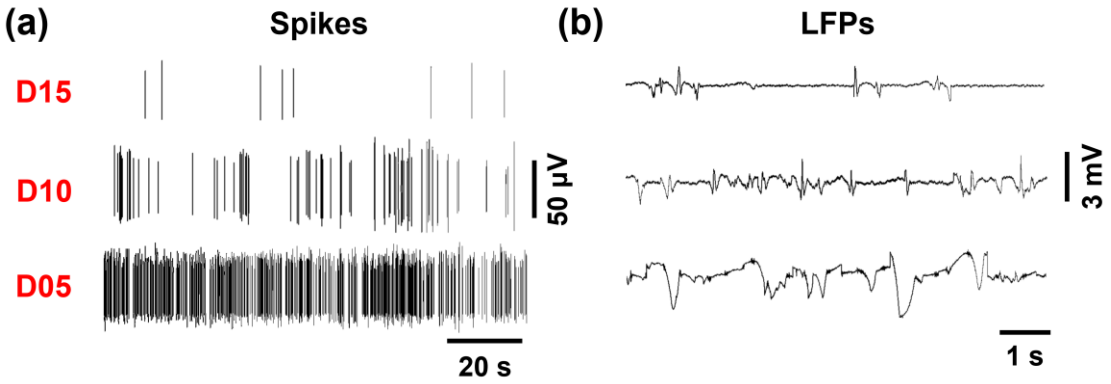

Figure S5. Comparison of typical electrophysiology patterns in the three stages of D15, D10 and D05. (a) Typical firing pattern of action potentials. (b) Typical pattern of LFPs.

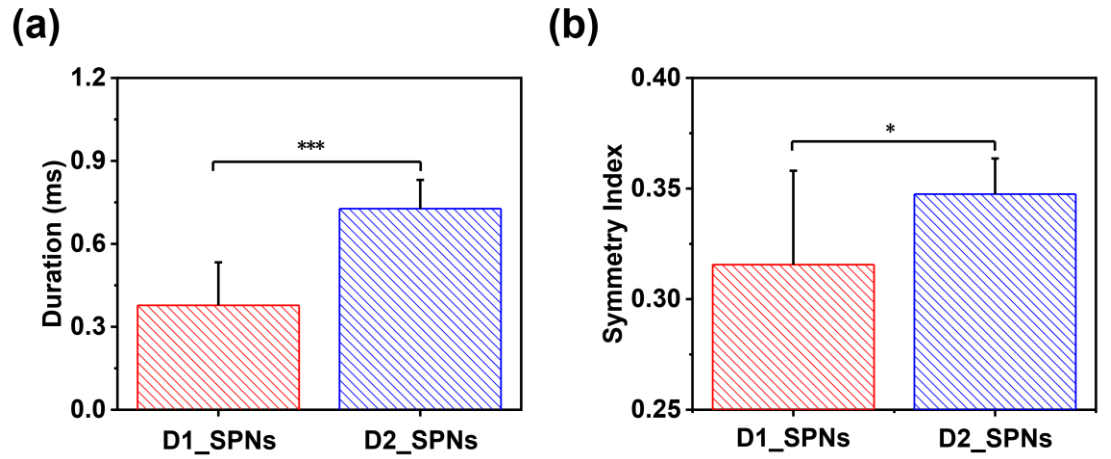

Figure S6. Comparison of the characteristics between D1_SPNs and D2_SPNs. (a) Duration is defined as the time from peak to trough of action potential. (b) Symmetry index is defined as the difference between the first

and second positive peaks of the action potential, which is sensitive to both duration of action potential and to the speed of action potential repolarization. (* P < 0.05; **P < 0.01; ***P < 0.001)

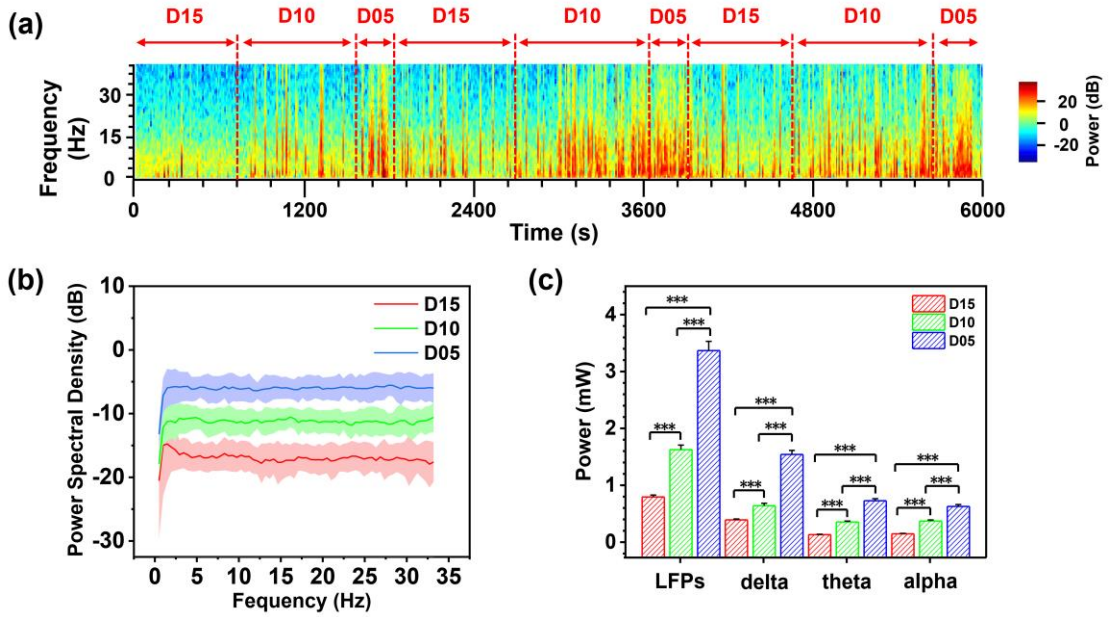

Figure S7. Characteristics of the LFPs at different degrees of isoflurane anesthesia. (a) The power density spectrogram of LFPs in the frequency band 0-33 Hz during the whole anesthetic procedure. (b) The PSD of spikes in the frequency band of 0-33 Hz. The shadow is the error bar calculated from the PSD of the D2_SPN spikes recorded by 8 channels. (c) Statistical analysis of the average power of delta rhythm, theta rhythm and alpha rhythm at the different degrees of isoflurane anesthesia. (n = 8; ***P < 0.001).

Table.S1 The compare of CNTs/PEDOT:PSS modified MEAs.

Nanocomposites	Electrochemical deposition	Target Detection	Electrode diameter	Impedance	Oxidation potential	Sensitivity	Method	Matrix	Reference
PEDOT/CNT/MEA	I-T	DA	37 μm	3.8 ± 0.4 kΩ	180 mV	14.7 pA/μM	SWM	Striatum	[7]
PEDOT/CNT-$(COOH)_x$/PSS/Au-MEA	V-T	ER	18 μm	90.3 ± 8.1 kΩ	---	---	---	Cortex	[8]
PEDOT/ox-SWCNH/PSS/ Au-MEA	CV	ER and ES	60 μm	---	---	---	---	in Vivo and in Vitro	[9]
CNT/PEDOT/PSS/MEA	I-T	ER and ES	---	0.0937 Ω/cm2	---	---	---	Cortex	[10]
CNT/PEDOT/PSS/MEA	V-T	ER and ES	---	---	---	---	---	in Vivo and in Vitro	[11]
MWCNTs/PEDOT/ PSS	CV	---	20 μm	32.47 ± 4.22 kΩ	250 mV	19.6 pA/μM	---	---	This work

SWCNTs/PEDOT/PSS	CV	ER and DA	20 μm	16.20 ± 1.68 kΩ	160 mV	42 pA/μM	I-T	Striatum	This work

* I-T—Chronoamperometry; V-T—Galvanostatic mode; CV—Cyclic Voltammetry; ER—Electrophysiological Recording; ES—Electrical Stimulation; DA—Dopamine; SWM—Square Wave Voltammetry

The prediction method for fatal overdose isoflurane anesthesia

As shown in Table S2, isoflurane anesthesia-induced death procedure is divided into four stages: Shallow Anesthesia – Overdose Anesthesia - Deep Anesthesia (Ⅰ, Ⅱ, Ⅲ) - Brain Death. We can determine the anesthesia stage by quantifying three indicators (Spike Firing Rate, LFP Power, DA Concentration). We use the stage of shallow anesthesia as a baseline (100 %). When the spike firing rate drops to 3 - 20 %, the LFPs power drops to 15 - 25 %, and the DA concentration drops to 75 - 100 %, the rat is overdose anesthetized. When the spike firing rate drops to 0 - 3 %, the LFPs power drops to 5 - 15 %, the rat is deeply anesthetized. Furthermore, the stage of deep anesthesia can be divided into stages Ⅰ, Ⅱ and Ⅲ by DA concentration. In detail, when the DA concentration drops to 20 - 75 %, the rat enters stage of deep anesthesia stage I, and the brain of rat begins to be injured. When the DA concentration is < 20 %, the rat enters stage of deep anesthesia stage Ⅱ, and the brain of rat is in dangerous. When the DA concentration rises to 20 – 145 %, the rat enters stage of deep anesthesia stage Ⅲ, and the brain of rat begins to die. When the spike firing disappeared, the LFPs power drops below 5 %, and the DA concentration is 120 - 145 %, the rat is dead. Quantitative dual-mode signals can provide a prediction method for fatal overdose isoflurane anesthesia. However, a lot of further works are still needed to establish this model, and we are working hard on this exploration.

Table.S2 Dynamic process of quantitative dual-mode signal of isoflurane anesthesia-induced death procedure.

Stage	Shallow Anesthesia	Overdose Anesthesia	Deep Anesthesia Ⅰ	Deep Anesthesia Ⅱ	Deep Anesthesia Ⅲ	Brain Death
Spike Firing Rate	100 %	3 - 20 %		0 – 3 %		0 %
LFP Power	100 %	15 – 25 %		5 – 15 %		< 5 %
DA Concentration	100 %	75 – 100 %	20 – 75 %	< 20 %	20 – 145 %	120 – 145 %

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