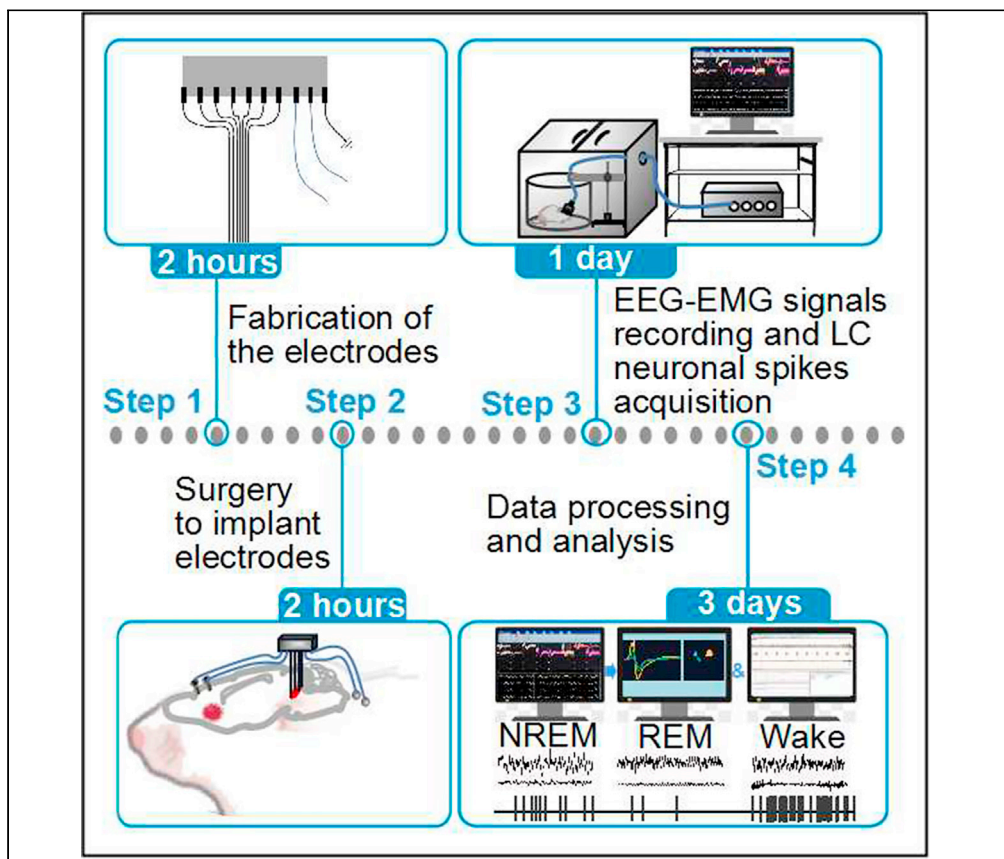**Protocol**

Protocol for recording the discharge of locus coeruleus neurons in free-moving mice during different sleep-wake stages

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Highlights

The technology of EEG-EMG recording combined with *in vivo* multichannel recording

Recording neuronal activity during different sleep-wake stages in free-moving mice

The locus coeruleus (LC) neurons are more active during wakefulness

Cortical electroencephalography (EEG) is generally used to detect the different sleep-wake states of animals. EEG combined with *in vivo* multichannel recording provides a powerful tool for decoding the neural network of sleep-wake regulation. Here, we detail a protocol using cortical EEG combined with *in vivo* multichannel recording to examine the activity of locus coeruleus (LC) neurons in free-moving mice at different sleep-wake stages. The procedures for electrode fabrication, the surgery to implant electrodes, and post-recording data analysis are also included.

Protocol

Protocol for recording the discharge of locus coeruleus neurons in free-moving mice during different sleep-wake stages

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SUMMARY

Cortical electroencephalography (EEG) is generally used to detect the different sleep-wake states of animals. EEG combined with *in vivo* multichannel recording provides a powerful tool for decoding the neural network of sleep-wake regulation. Here, we detail a protocol using cortical EEG combined with *in vivo* multichannel recording to examine the activity of locus coeruleus (LC) neurons in free-moving mice at different sleep-wake stages. The procedures for electrode fabrication, the surgery to implant electrodes, and post-recording data analysis are also included.
For complete details on the use and execution of this protocol, please refer to Liang et al. (2021).

BEFORE YOU BEGIN

Animal

C57BL/6N mice were purchased from Shanghai SLAC Laboratory Animal Company. The mice were housed at a controlled temperature ($22 \pm 2°C$) and humidity (60%–80%) under a 12 h light-dark cycle (lights on at 7:00 and off at 19:00) and provided free access to food and water. Adult male mice (6–8 weeks old) were used in the experiments. All experiments were performed in accordance with the guidelines for animals used in biological studies of Tongji University and were approved by the Animal Study Committee at Tongji University School of Medicine, Shanghai, China.

Fabrication of the electrodes

⊙ Timing: 2 h

Note: To monitor the sleep-wake state of animals and the discharge of LC (locus coeruleus) neurons simultaneously, we make multichannel electrodes by ourselves.

Micro-wire array electrodes play an important role in multi-site, multiple single-unit *in vivo* recording experiments (Nicolelis et al., 2003). Although they can be purchased from commercial vendors and are reliable for many experiments, it is worthwhile to consider home-made electrodes for economic reasons, as well as for the flexibility of experimental design (Tsai and Yen, 2003).
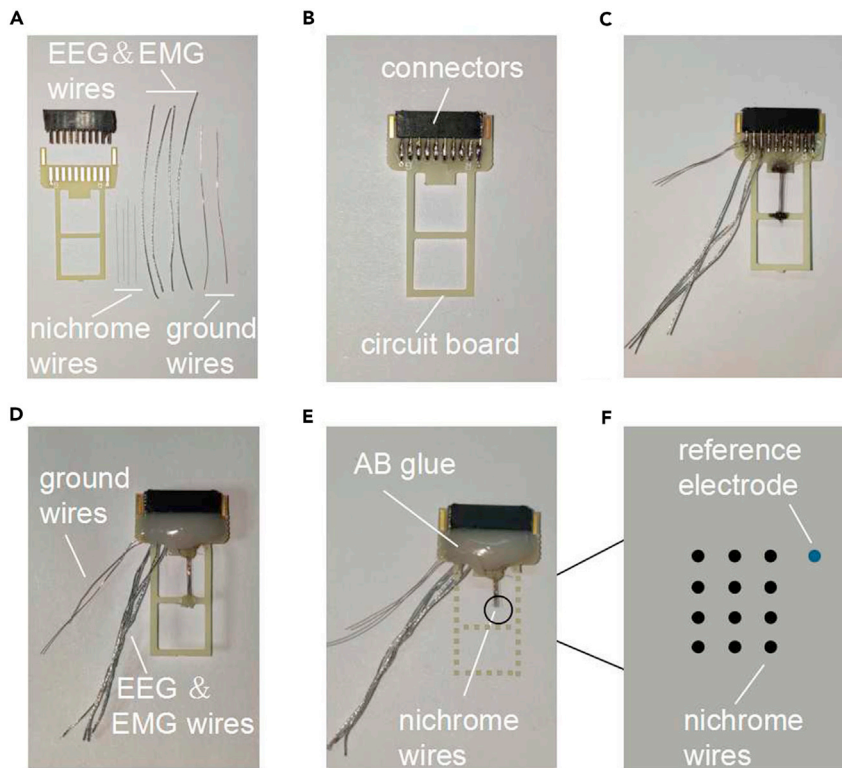
Figure 1. Homemade multichannel electrodes

(A) Accessories for making electrodes.

(B) A 20-pin connector was soldered to a 20-pin circuit board.

(C) Arranged insulated nichrome wires, two insulated EEG wires, two insulated EMG wires and two ground wires were soldered to the 20-pin circuit board.

(D) The welds were sealed with AB glue.

(E) The nichrome wires were cut to the appropriate length, and the anchoring part (the yellow dotted line in the picture) of the circuit board was removed.

(F) A schematic diagram of the arrangement and distribution of electrode wires. Thirteen individually insulated nichrome wire arrays were arranged in a 3 + 3 + 3 + 3 + 1 pattern (approximately 200 μm between lines), one of which was a separate arrangement of the reference electrode.

Note: In this study, we obtained all recordings through chronically implanted micro-wire array electrodes.

1. Make these 16-channel home-made arrays as follows:
   a. Arrange 13 individually insulated nichrome wires (35 μm internal diameter, 300–900 Kohm impedance, Stablohm 675) in arrays in a 3 + 3 + 3 + 3+ 1 pattern (Li et al., 2016).
   b. Connect a circuit board to the nichrome wires.
   c. Connect a miniature 20-pin connector to another side of the circuit board.

Note: In the arrays, the distance between nichrome wires in a row vary from 100 to 200 μm, and the distance between the rows vary from 200 to 300 μm.

   d. Coat the circuit board with epoxy for insulation.
   e. Solder two insulated EEG wires and EMG (Electromyography) wires to four pins of the 20-pin circuit board.
   f. Solder two ground wires to both sides of the 20-pin circuit board respectively.
   g. Seal the welds with AB glue (Figure 1).

Note: Several key steps in the fabrication of a good electrode include: (1) arranging nichrome wires into the desired configuration, (2) maintaining the corresponding sequence of the nichrome wires.

Trimming the electrodes

⏱ Timing: 5 min

Before surgical implantation of electrodes, cut the insulated nichrome wires to the appropriate length according to the depth of the target nucleus, and remove the anchoring part of the circuit board (Figure 1F).

2. Cut the electrode wire to a length of approximately 6 mm for LCneurons recording.
3. Remove the anchoring part of the circuit board.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Neuro-Dil	Biotium	Cat#: 60016
paraformaldehyde (PFA)	Sigma-Aldrich	Cat#: P6148
sucrose	Sigma-Aldrich	Cat#: V900116-500 G
isoflurane	RWD Life Science	Cat#: R510-22
$Na_2HPO_4 \cdot 12H_2O$	Sinopharm	Cat#: 10020318-500 g
$NaH_2PO_4 \cdot 2H_2O$	Sigma-Aldrich	Cat#: 71500-1 KG
NaCl	Sigma-Aldrich	Cat#: S5886-5 KG
sodium pentobarbital	Biohao	Cat#: P6031
corn oil	Absin	Cat#: abs42015838
Experimental models: Organisms/strains		
Mouse: C57BL/6N	SLAC Laboratory	N/A
Software and algorithms		
SleepSign 3.0	KISSEI COMTEC	http://www.sleepsign.com
Neuroexploer 5.0	Plexon	https://www.neuroexplorer.com/
Offline Sorter	Plexon	https://plexon.com/products/offline-sorter/
Prism 7.00	GraphPad Software	https://www.graphpad.com/scientifific-software/prism/
Adobe Illustrator	Adobe	RRID:SCR_010279
Adobe Photoshop	Adobe	RRID:SCR_014199
Other		
freezing microtome	Leica Biosystems	CM1950
Fluorescence microscope	Carl Zeiss	Zeiss Imager M2
In vivo multi-channel neural signal acquisition system	Bio-Signal Technologies	Zeus
headstage of 32 channels	Bio-Signal Technologies	Cat#: 2002
eletrode adaptor, O32-S16	Bio-Signal Technologies	Cat#: 2202
insulated nichrome wires	Bio-Signal Technologies	Cat#: CFW2036043
20-pin circuit board	Bio-Signal Technologies	N/A
20-pin connectors	Bio-Signal Technologies	N/A
ground wires	Bio-Signal Technologies	N/A
active-photoelectric integrated commutator	Doric Lenses	AHRJ-OE_PC_AD_12_HDMI_B2
insulated sliver wires for EEG and EMG recording	Cooner Wire	Cat#: AS633
stereotaxic frame	RWD Life Science	Cat#: 68018

MATERIALS AND EQUIPMENT

PBS		
Reagent	Final concentration (mM)	Amount (g) for 1000 mL
$Na_2HPO_4 \cdot 12H_2O$	8.6	3.08
$NaH_2PO_4 \cdot 2H_2O$	1.8	0.28
NaCl	145.4	8.5
Store at room temperature for 15 days		

4% PFA solution		
Reagent	Final concentration (mM)	Amount (g) for 1000 mL
$Na_2HPO_4 \cdot 12H_2O$	8.6	3.08
$NaH_2PO_4 \cdot 2H_2O$	1.8	0.28
NaCl	145.4	8.5
PFA	1333	40
Store at 4°C for 14 days		

30% Sucrose solution		
Reagent	Final concentration (mM)	Amount (g) for 1000 mL
$Na_2HPO_4 \cdot 12H_2O$	8.6	3.08
$NaH_2PO_4 \cdot 2H_2O$	1.8	0.28
NaCl	145.4	8.5
Sucrose	87.6	300
Store at 4°C for 7 days		

STEP-BY-STEP METHOD DETAILS
The surgery to implant electrodes

⊙ Timing: 2 h

For *in vivo* multichannel electrophysiological recordings of LC neuronal activity during the spontaneous sleep-wake cycle, electrode bundles are implanted into the target nucleus through strict and meticulous operation, and the collector of cortical EEG-EMG signal is implanted (Figure 2) (Ren et al., 2018).

1. Sterilize the surgical scissors, hemostatic forceps, stainless skull screws and other surgical instruments by autoclaving before the operation. Spray alcohol on the operating tables, stereotaxic frame, and electrode bundles for disinfection. Use the iodophor to disinfect the scalp of mice with hair removed. Wear gloves and masks during the operation, and strictly implement aseptic operation to alleviate the pain of mice as far as possible.
2. Anesthetize an adult mouse with 1% sodium pentobarbital (50 mg/kg, i.p.) and place it on a stereotaxic frame. Remove the hair on the head of the mouse, and fix the head of the mouse by an adaptor of a stereotaxic frame (Figure 2A).
3. Cut the mouse scalp to expose the skull, insert two stainless skull screws into the frontal region, and place two others in the lateral parietal region.
4. Adjust the skull plane to ensure that the bregma and lambda are at a horizontal level on the stereotaxic apparatus.

Figure 2. The surgical procedure for implanting electrodes

(A) Adult mice were anaesthetized, the head was fixed in a stereotaxic frame, and the hair on the head of the mouse was removed.

(B) Two skull screws were inserted in the frontal skull for EEG recording, and two others were placed in the skull of the lateral parietal region for grounding. The skull was leveled using a stereotaxic frame, and the location of the target nucleus was marked. Scaling bar: 5 mm.

(C) The electrode bundle of the homemade multichannel electrode was slowly inserted into a hole at the targeted location on the skull.

(D) The electrode bundle was secured by dental cement.

(E) Two EEG wires were soldered to two skull screws in the frontal region for EEG recording, and two ground wires were soldered to two other screws for grounding.

(F) A large spherical solder was soldered at the terminals of the EMG wires.

(G) Two EMG wires were inserted into the neck muscles, and the skin was sutured.

(H) Dental cement was used to secure the exposed skull and electrode.

(I) The mouse was recovered after surgery.

5. Calculate and mark the location of the target nucleus (LC: AP = −6.00 mm; ML = ± 0.95 mm) on the skull according to the mouse atlas by Paxinos and Watson (Figure 2B).

6. Move an electrode over the target brain region, and drill a hole at the desired location on the skull. To verify the location of the implant at the end of the experiment, the electrode tips are dipped in fluorescent Neuro-Dil dye (dissolve at 1–2 mM in vegetable oil by heating to 55°C) prior to surgery insertion, which can then reveal the electrode track under a fluorescent microscope.

7. Remove the dura of the hole carefully, and insert the electrode bundle slowly into the brain tissue (Figure 2C) (Ma et al., 2013).

8. Secure the electrode bundle on the skull using 3M Vetbond tissue glue and dental cement sequentially (Figure 2D).
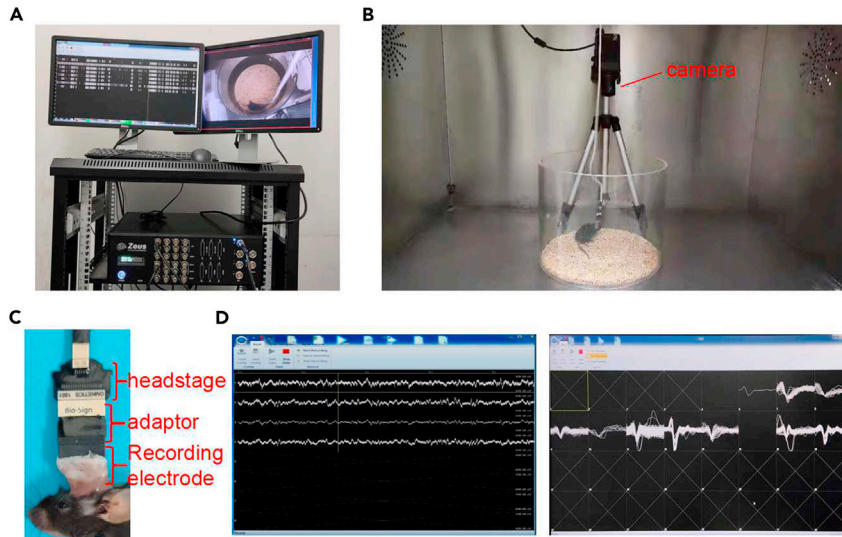
Figure 3. EEG-EMG signal recording and LC neuronal spike acquisition
(A) Recording system (Zeus, Bio-Signal Technologies, McKinney, TX, USA).
(B) The freely moving mouse in the shielding box with the head connected to the recording system.
(C) The connection of the recording apparatus included the headstage, adaptor and electrode.
(D) (Left) LFP and (right) spike signals were recorded.

9. Solder two insulated silver EEG wires to two screws in the frontal skull, and solder two ground wires to two other screws in the lateral parietal skull respectively (Figure 2E).
10. Insert two EMG electrodes into the neck muscles, and suture the neck skin (Figures 2F and G).
11. Fix the electrode finally to the skull with more dental cement (Figure 2H).
12. After the dental cement is solidified, remove the mouse from the stereotaxic frame and place on an electric blanket to keep warm and accelerate recovery from anesthesia (Figure 2I).

   ⚠ CRITICAL: Soldering of insulating silver wire and stainless-steel screw as quickly as possible to prevent brain damage caused by high temperature. Skull screws for EEG recording should not contact the scalp to avoid recording the heartbeat of mice. Skull screws should not be inserted too deep to avoid damage to brain tissue, and dental cement should be covered around the skull screws to provide better sealing.

EEG-EMG signal recording and LC neuronal spike acquisition

⏱ Timing: 1 day

One week after the electrode is implanted, mice have no hemiplegia, no inflammation and edema in the dental cement cover. And mice appear healthy with normal activity and body weight regain to more than or equal to 90% of their presurgery level. At this point, we can record the discharges of LC neurons in different sleep stages (including wakefulness, NREM and REM sleep) (Figure 3).

13. LC neuronal spike acquisition
    a. Record neuronal signals in a quiet room with a shielded box starting on the 7th day after implantation of the electrodes into the mice.
    b. Connect mice with a commutator at least 1 day before the recording for habituation. To avoid electrode shedding, anesthetize the mice lightly with isoflurane before connecting the electrodes to an adaptor of the recording system.
    c. Filter the spike and LFP (local field potential) signals online at 300–7,000 Hz and 0.7–200 Hz, respectively.
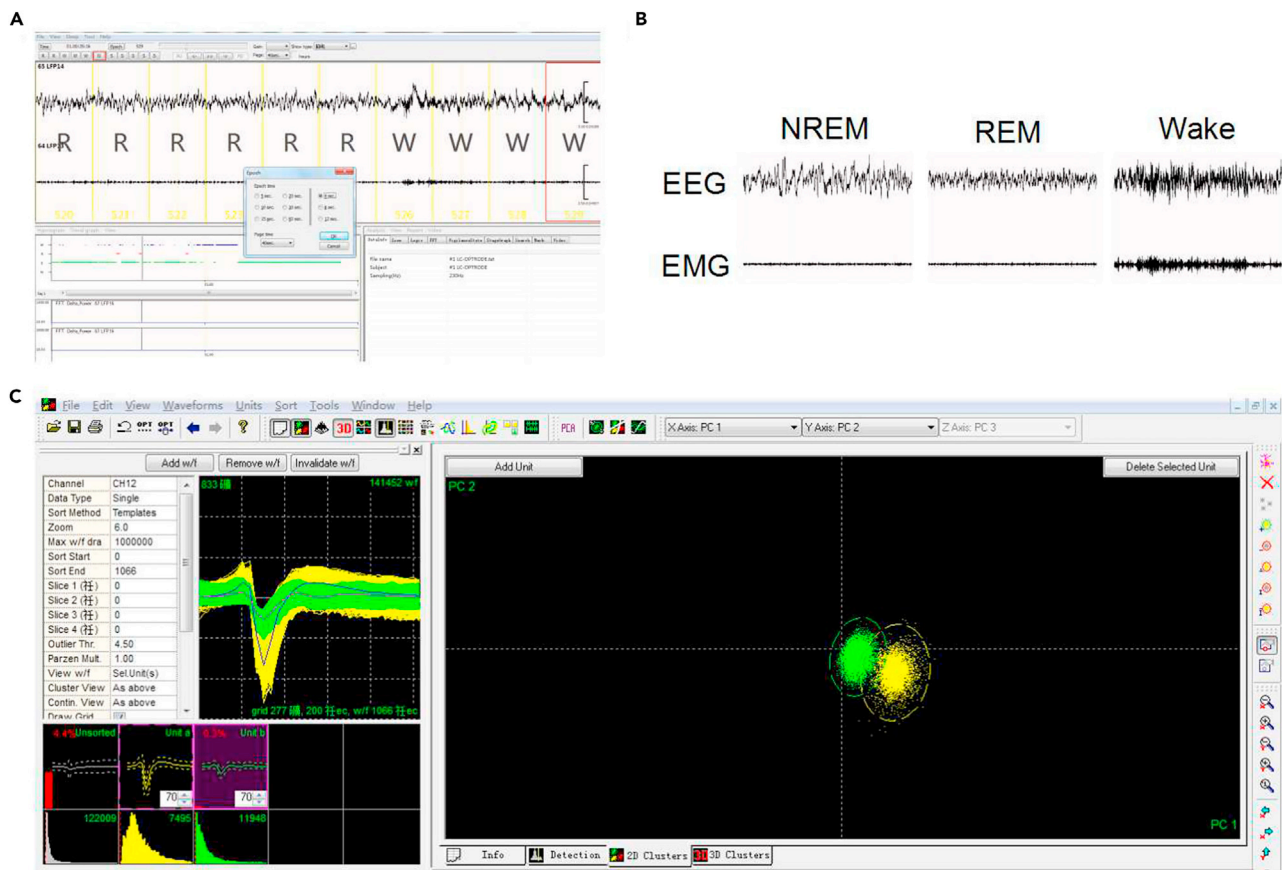
A

B

C

Figure 4. LFP and spike signals data processing
(A) Definition of LFP signal as wakefulness, NREM sleep, or REM sleep in 4-s epochs using SleepSign.
(B) EEG and EMG recording traces during wakefulness, NREM sleep, and REM sleep.
(C) Action potential waveforms of two sorted LC neurons from a single electrode channel and clusters formed by the distinct action potential waveforms plotted in principal component space are shown in Offline Sorter.

14. Record LFP and spike signals simultaneously. The LFP signals of channel 2 and 4 represent EEG signals, channel 1 and 3 represent the EMG signals, and the spike signals of channels 5 to 16 collect the discharges of LC neurons.

15. Record LFP and spike signals continuously for approximately 1 h during zeitgeber time(ZT) 2 to ZT 14, and the mice experience stages of wakefulness, NREM sleep and REM sleep.

⚠ CRITICAL: The recording time should not exceed 1 h, otherwise the recording file will be very large, approximately 8 GB. The big data file is not conducive to data processing, and different sleep states should be recorded in one file to facilitate later data processing.

Data processing and analysis

⏱ Timing: 3 days

Save spike waveforms, time stamps and LFP signals to Plexon data (*.plx) files. Analyze LFP signals by SleepSign software (Kissei Comtec, Japan). Sort the spikes by the valley seek method with Offline Sorter software (Plexon, USA) (Fukunaga and Mantock, 1983). The sorted units are analyzed with NeuroExplorer 5.0 to obtain a graph, and a chart of the firing rate of the neurons and timestamps is exported for further analysis in GraphPad Prism (Figure 4).

LFP signal processing

⏱ Timing: 6 h

16. Exporting Plexon data (*.plx) files to spreadsheet txt (*.txt) files by Spike2 software.
17. Open spreadsheet txt (*.txt) files in SleepSign software, and digitize the LFP signal data at a sampling rate of 230 Hz using SleepSign.
18. Polygraphic recordings are automatically scored offline as wakefulness, NREM sleep, or REM sleep in 4 s epochs using SleepSign according to standard criteria. We define wakefulness as desynchronized, low-amplitude EEG rhythms and elevated EMG activity with phasic bursts; NREM sleep is defined as synchronized, high-amplitude, low-frequency (0.5–4 Hz) EEG activity in the absence of motor activity compared with wakefulness; REM sleep is defined as containing a pronounced theta (4–10 Hz) rhythm and muscle atonia (Luo et al., 2018). Visually examine and manually correct defined sleep-wake stages if necessary.
19. Select the time range in which the mice are in wakefulness, NREM or REM sleep (the duration of episode: wakefulness and NREM sleep $\geqq$ 6 min; REM sleep $\geqq$ 30 s).

Spike signal sorting

⏱ Timing: 2 days

20. Open recorded neural activity in a .plx file by Plexon Offline Sorter.
21. Selecting and opening the channel that records the neuronal spike signal.
22. Remove the obviously distinct, mussy waves manually.
23. Automatic clustering
    a. To obtain the best principal component calculation effect, it is necessary to align the action potential waveform (align waveforms). When operating, choose to align the waveform at the maximum or minimum value of the action potential waveform (Min et al., 2014).
    b. Recalculate the principal components (RPC).
    c. Then, the waveforms are clustered automatically by the valley seeking method, and units are added.
24. Remove the remaining mussy waves manually in a three-dimensional feature space by the template method (Figure 5).
25. Save the sorted file as -01.plx (*.plx) files.

Integration of LC neuronal activity in different sleep stages

⏱ Timing: 18 h

26. Opening a -01.plx (*.plx) file by NeuroExplorer software.
27. Select the analysis type of "Firing Rates Analysis" and fill in the time ranges of wakefulness, NREM and REM sleep.
28. Obtain the firing rates of different neuronal units in different brain states, and then summarize the data by Prism.

Detection of electrode implantation position

⏱ Timing: 2 days

For facilitating the identification of electrode array position, the electrode tips are dipped in fluorescent Neuro-Dil dye which then can reveal the electrode track.
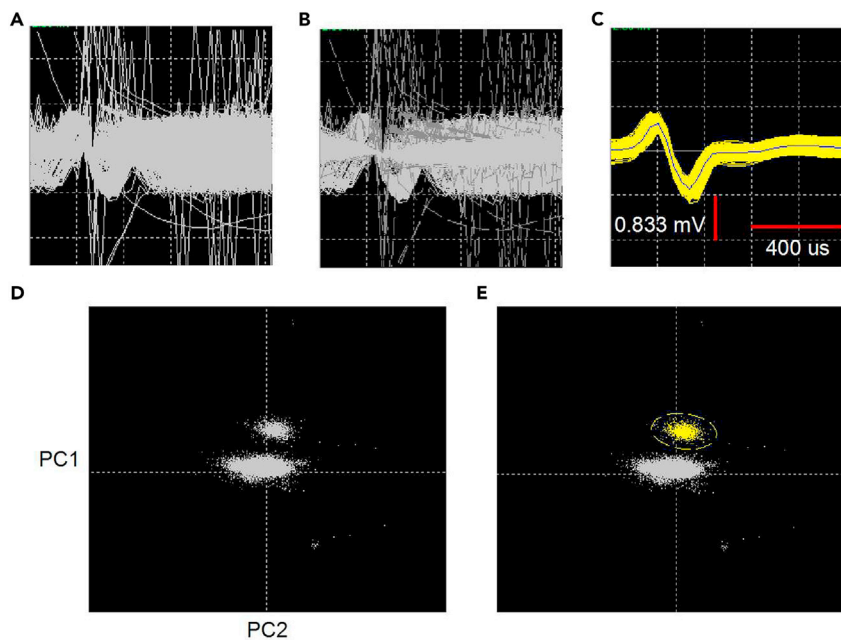
Figure 5. Action potential waveforms and clusters of LC unit spikes in different stages of sorting

(A) Action potential waveforms of LC unit in the initial file.

(B) Action potential waveforms of LC unit after remove the obviously distinct, mussy waves manually.

(C) Action potential waveforms of LC unit after the completion of sorting.

(D) Clusters formed by the distinct action potential waveforms plotted in different principal component spaces in the initial file.

(E) Clusters formed by the distinct action potential waveforms plotted in different principal component spaces after the completion of sorting.

29. Following completion of the behavioral experiments, anesthetize mice with 1% sodium pentobarbital (50 mg/kg, i.p.) and transcardially perfuse with $\sim$ 25 mL of 37°C phosphate-buffered saline (PBS) followed by $\sim$ 25 mL of ice-cold 4% paraformaldehyde (PFA) in PBS.

30. Remove the electrode carefully, then remove the brains carefully from the skull, postfixed for 12 h at 4°C in 4% PFA, and then dehydrate with 30% sucrose at 4°C overnight ($\geqq$ 16 h).

31. Cut the brain sections serially in the coronal plane at a thickness of 30 μm with a freezing microtome (CM1950, Leica, Wetzlar, Germany).

32. Then select the sections containing the brain region of LC and excite the fluorescent Neuro-Dil dye by 556 nm laser under the fluorescence microscope.

EXPECTED OUTCOMES

A multichannel electrode recording technique is used to detect the discharges of neurons in the LC and combined with EEG-EMG recording to determine the activity of LC neurons in different brain states. Firstly, we evaluate recording quality and success probability of LC unit recording by multi-channel electrode. The result show that 52.1% of the channels can be successfully recorded to the LC unit, and 8.3% of the successful channels are single electrode recording two LC units at the same time (Figure 6). We also count the amplitude and waveform width of LC unit spike (Figure 7). Then, we detect the electrode implantation site in the LC, and mice with inaccurate locations are discarded (Figure 8A). The results show that LC neurons exhibit a higher firing rate during wakefulness than during NREM and REM sleep. However, we do not find differences in the mean firing rates of LC neurons between NREM and REM sleep (Figures 8B and 8C) and analyze the possible reasons (Liang et al., 2021).
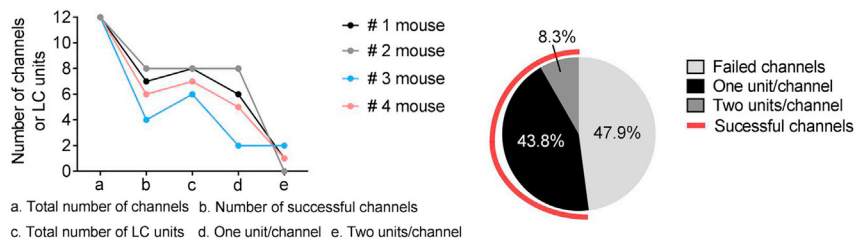
Figure 6. Evaluation of recording quality and success probability of LC discharge recording by multichannel electrode

Left: Statistics of the number of various channels and LC units per mouse (n = 4 mice). Right: The proportion of successful channels and failed channels in all available channels of 4 mice.

LIMITATIONS

First, our homemade multichannel electrodes have only 12 channels to record neuronal spikes, and the number of neurons recorded per mouse is limited, which may increase the time cost and the number of animals to complete the experiment. In addition, compared with microdrive array electrodes, our electrodes are unable to be adjusted, so we cannot record the discharges of neurons in different layers of the nucleus in one experiment. Finally, for very small nucleus recording, our electrode bundle may be too large to exclude the possibility of recording neurons outside; hence, this electrode bundle may not as favorable as silicon electrodes.

TROUBLESHOOTING

Problem 1: Noise in the neural recordings

Neural recordings may display high amounts of electrical noise and a low signal-to-noise ratio (step 13).

Potential solution

There are several methods for reducing electrical noise and increasing the signal-to-noise ratio. First, we ensure that the neural recording system is grounded. Normally, the ground wire of the recording system is connected with the shielding box, and the shielding box is grounded. Then, the recording system is separated from other electrical devices to avoid 50 Hz power-frequency interference. In addition, during the surgery, we ensure that the electrode is well grounded on the skull screw, mainly by checking whether the soldering is firm and whether the ground wire is conductive. Finally, lowering the electrode very slowly during surgery is also important for obtaining high-quality recordings.

Problem 2: Noise in the LFP recordings

LFP signals are not a simple EEG-EMG signal and cannot reflect the sleep-wake state (step 14).

Potential solution

First, the skull screw and silver wire of EEG recordings do not touch the scalp of mice during the surgery, and the skull screw is fixed as far as possible from the blood vessels to prevent the recording of
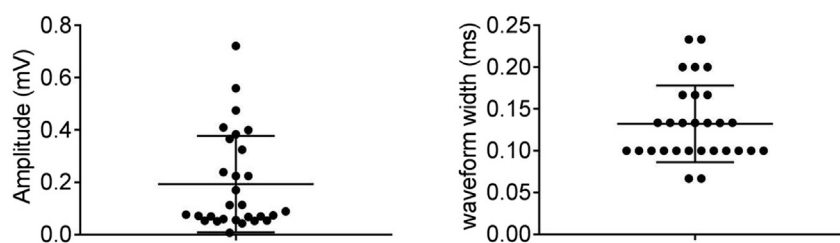

Figure 7. The amplitude and waveform width of LC unit spike

Left: The mean (± S.D.) amplitude of LC unit spike was 0.1938 ± 0.1848 mV (cells = 29 from 4 mice). Right: The mean (± S.D.) waveform width of LC unit spike was 0.1322 ± 0.04858 ms (cells = 29 from 4 mice).
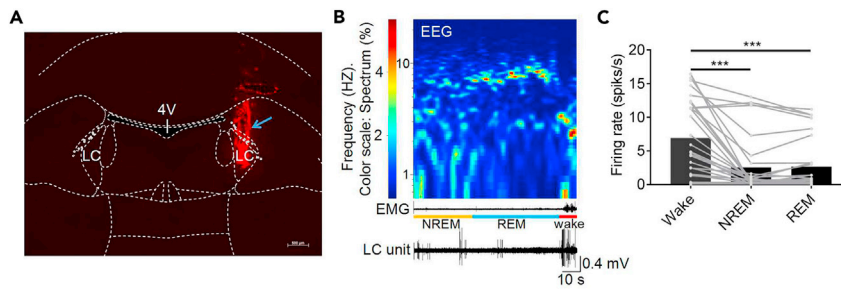
Figure 8. LC neuronal activity during different sleep stages

(A) A brain slice from a mouse with electrodes implanted in the LC. Blue arrow indicates the electrode track. Scaling bar: 500 μm.

(B) Representative EEG power spectrum, EMG trace and firing rates of an example LC neuron during different brain states (color coded).

(C) Average firing rate of 29 LC neurons in each state. Each line shows firing rates of one unit. (***p < 0.001, cells = 29 from 4 mice). Data are represented as the mean ± SEM. The statistical significance of the data was calculated with one-way ANOVA.

vascular pulsation or heartbeat. Then, insulated ground, EEG and EMG wires should be used, and separate the EEG wires from other wires and skull screws to avoid short circuits. When the recording of EMG signals is not obvious or fails, make sure that the EMG wires are indeed placed into the neck muscle of mice during the surgery, and fix the EMG wire with tissue glue and suture, which can prevent any potential movement of the wires in the neck muscle while the mouse moves. Moreover, a large spherical solder can be soldered at the terminals of the EMG wire to increase the contact area with the neck muscles and improve the recording of EMG (Figure 2F).

Problem 3: Difficulties in sorting neural activity

It is difficult to remove noise and sort different neuronal spikes during sorting neural activity to obtain a high-quality spike signal (step 23, 24).

Potential solution

When the recorded file is large and there may be considerable noise, we can use low-cut filtering primarily to remove signals of lower than 250 Hz or 300 Hz. Offline Sorter provides an automated principal component analysis that clusters the waveforms. It is easier to cluster when the noise and signals are separate; however, it usually takes a longer time to cluster and manually sorter when the noise is fully integrated into the signals.

Neuronal clusters should be well isolated and easy to differentiate when we use different 2D cluster views; however, if some parts are mixed together and cannot be separated automatically in one 2D cluster view, we should try to use different 2D cluster views or 3D cluster views (Figure 9).

To obtain high-quality data, theoretically, all time intervals of discharge (interspike interval, ISI) of a single neuron should not be shorter than the refractory period (1–2 ms) of the neuron (Figure 10). However, in practice, a pollution rate of 0.5%–1% is generally allowed; that is, the proportion of ISI with a duration less than the refractory period should not exceed 1% of all ISI (Lewicki, 1998; Takekawa et al., 2010). Note that when removing noise, do not remove it in stages, but select it together throughout the recording period, so that even if the noise removal is not very clean, it is evenly distributed throughout the recording period and will not be biased.

Problem 4: No spike of neurons can be recorded.
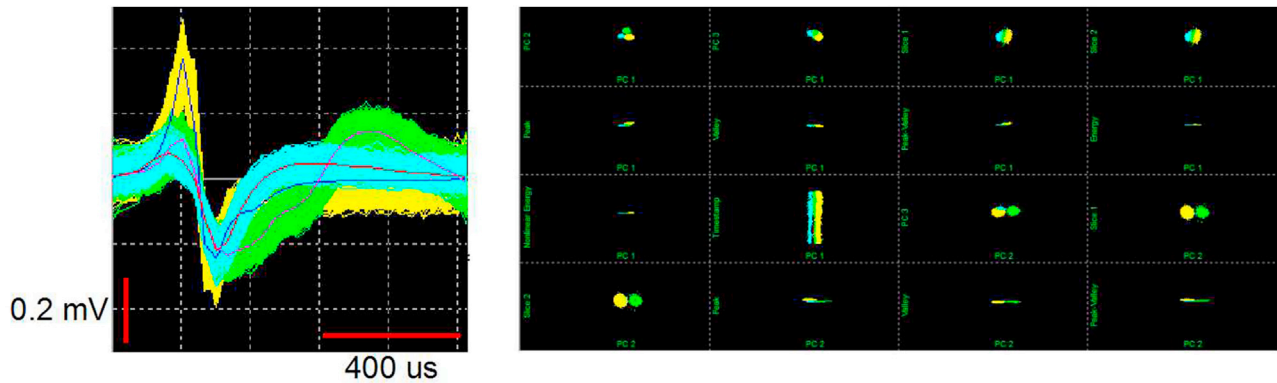
There are three conditions,

Figure 9. Distribution of multiple unit signals in different 2D cluster views
Left: Action potential waveforms of three sorted LC neurons from a single electrode channel. Right: Clusters formed by the distinct action potential waveforms plotted in different principal component spaces.

Case 1, the spikes are obscured in the excessive noise (step 13, 14). Case 2, problems with the animal, such as poor electrode quality, the electrode is not firmly implanted, and so on (Fabrication of the electrodes, step 1 and the surgery of implanting electrodes step 8). Case 3, problems with the recording system, such as poor contact of recording system, and so on (step 13).

Potential solution
The solution to noise in case 1 can be found in the section of troubleshooting "problem 1". For case 2 & 3, an emulator can be used instead of the mouse to troubleshoot the problem. If the data
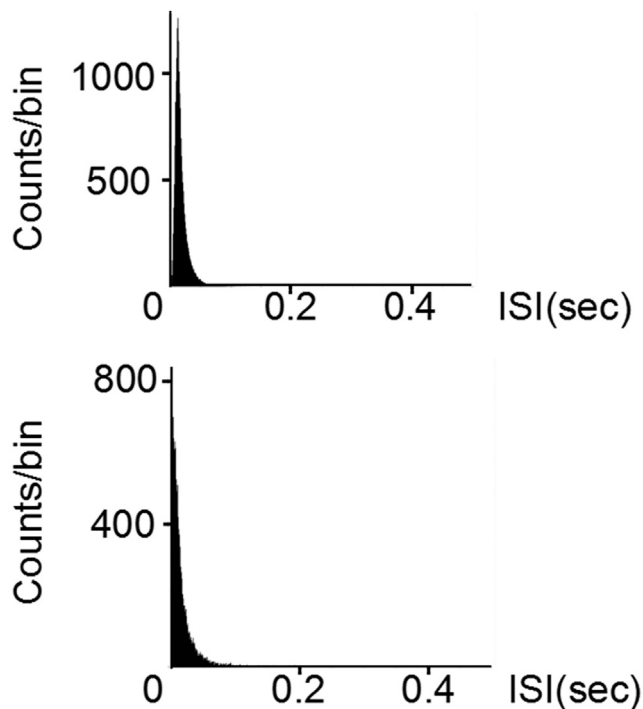

Figure 10. Interspike interval (ISI) histograms of LC neuron
Top: ISI histograms of LC neurons with successful noise removal. Bottom: ISI histograms of LC neurons with incomplete noise removal.

acquisition system is connected to the emulator and the simulation waveform can be recorded, that means there are problems of animal side, and replace other mice to record it.

On the contrary, if the acquisition device is connected to the emulator, the simulator waveform cannot be recorded, that means there are problems of system side, and carefully check the circuit connection, such as headstage and other interfaces. And whether the record computer is set up correctly.

Problem 5: Surgical risk
Due to the complexity of implanting recording electrodes, improper operation is likely to cause poor recovery or even death of mice, resulting in unnecessary sacrifice of mice and waste of materials and time (step 2, 3, 7, 9, 12).

Potential solution
Select adult mice in good health. Use the safer isoflurane anesthetic system as far as possible, monitor the body temperature of mice in real time, and use electric blanket throughout the whole process if necessary. If conditions permit, aseptic operation should be ensured during surgery, including skin disinfection of mice and autoclaving of surgical instruments. Prepare gelatin sponges for possible bleeding during surgery. The implanted screws should not be too deep (approximately 1 mm) to prevent damage to cortical tissue and cause hemiplegia in mice. Implanting electrode slowly (100 um/s) to reduce brain tissue damage. Practice welding technology in advance to avoid high temperature damage to mice. After surgery, appropriate antibiotics are injected to prevent infection. After recovering from consciousness on the electric blanket, the animals were raised in a single cage.

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ling Zhang (lzhang0808@tongji.edu.cn)

Materials availability
This study did not generate new unique reagents.

Data and code availability
This study did not generate/analyze [datasets/code].

AUTHOR CONTRIBUTIONS
L.Z. and Y.L. conceptualized the method. W.S. designed and made the multichannel electrodes. Y.L. and D.D.H. completed surgery and recording. Y.L., W.S., and A.F.X. drafted the manuscript. All authors read and approved the manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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