

# **Supplementary Materials and Methods**

## **Monkey surgery**

Monkeys were first injected with Zoletil-50 (4 mg/kg) for inducing anesthesia. The head was then fixed on the operating table and the body covered with a heating blanket. The anesthesia was maintained during surgery with isoflurane (~1.75%, volume fraction) mixed in oxygen gas (O<sub>2</sub>) by a breathing machine (RWD R620-S1, China). Basic physiological vital signs, including heartbeat, oxyhemoglobin saturation, carbon dioxide density, and rectal temperature were monitored concurrently (MINDARY iPM12, China).

## **Generation of Monkey Model of AME**

We employed three adult male rhesus monkeys for this study. Every monkey was individually housed in cages with free access to water. Bi-weekly weight assessments were conducted, and the monkeys received a balanced diet comprising food, fruits, and vegetables to maintain optimal body weights, ensuring prevention of obesity. Regular daily checks and periodic veterinary evaluations confirmed their sustained health. All experimental procedures received approval from the Animal Committee of the Institute of Neuroscience and Center for Excellence in Brain Science and Intelligence Technology, Chinese Academy of Sciences. Animal care protocols adhered to the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals.

To simulate the self-administration patterns observed in amphetamine users, we designed the following experimental paradigm, which was consistent with our previous reports<sup>1</sup>. Each monkey underwent initial habituation to wearing a nylon collar and sitting in a standard monkey training chair for several weeks in their regular housing environment. Following adaptive behavior training, surgical implantation of intravascular catheters for methamphetamine administration was performed under general anesthesia. Basic physiological vital signs, including heartbeat, oxyhemoglobin saturation, carbon dioxide levels, and rectal temperature, were continuously monitored (MINDARY iPM12, China). A silicone rubber catheter (POLYSITE 3007ISP, France)

was implanted in the internal jugular or femoral vein, exiting in the mid-scapular region. The monkeys were allowed a 2-week recovery period post-surgery before commencing subsequent experiments.

During the experimental treatment, monkeys were transferred to a ventilated and soundattenuating chamber equipped with an operation panel (AniLab Software & Instruments) in front of the training chair. The panel featured a hand-pressing lever and two signaling lights of different colors. The white and green lights signaled trial onset and reward delivery via lever pressing, respectively. All data on light signals, lever pressing, as well as Meth dose and duration were collected using commercial software (AniLab Software & Instruments).

For Meth treatment conduction, during the initial 2–3 rounds, monkeys self-administered Meth for 7 days (2 hours per day), with a total Meth dosage of approximately 1 mg/kg per day—lower than the dosage commonly taken by meth abusers (~2–10 mg/kg per day). In the last round (3rd or 4th round), Meth self-administration was combined with experimenter-initiated supplementary injections to increase the total daily dose to 1.5 mg/kg.

### **Monkey electroencephalogram (EEG) data recording**

Surgical implantation of electroencephalogram (EEG) electrodes for epidural EEG recording was performed under general anesthesia, as previously described. Two EEG electrodes (bipolar electrodes) were implanted on the surface of the dura mater via metal nails screwed through the skull over the left temporal region and frontal lobe, roughly corresponding to the T5 and Fz locations for human EEG recording, respectively. An electromyography (EMG) electrode was also implanted in the monkey's trapezius muscle. The wireless implant (L-03, Data Sciences International, DSI) was subcutaneously implanted on the monkey's back. Monkeys had a 2-week recovery period post-surgery before the initiation of subsequent experiments. All animals received intramuscular injections of sufentanil (2.5 µg/kg) twice a day for three days or

longer if signs of discomfort were exhibited. During the baseline period and the AME period, one set of data was respectively excluded during the quality control process.

### **Electroencephalogram (EEG) data preprocessing**

The initial sampling rate was set to 1000. EEG data were analyzed using EEGLAB and Fieldtrip toolboxes in MATLAB software (2019a, MathWorks Inc.) with customized codes. The Harvard Automated Processing Pipeline for EEG was employed to reduce noise and remove artifacts from EEG signals. The EEG signals were initially filtered at 1–250 Hz, along with a notch filter at 50 Hz to eliminate line noise. Channels with high impedance or displacement during recording were rejected, and eye- and muscle generated EEG artifacts were removed using wavelet-enhanced principal component analysis. Further artifact removal was conducted with the Multiple Artifact Rejection Algorithm for components labeled with >50% chance of being artifacts. Bad channels were interpolated using spherical splines. The cleaned EEG signals were re-referenced by the average signal from all recording channels. Noise and artifacts in epidural EEG signals from monkeys were manually removed based on visual inspection of raw signals and spectrograms.

### **Sleep stage classification**

The sleep stages of monkeys were classified by custom-developed software codes based on AASM manual for sleep scores of adult humans, further confirmed by visual evaluation<sup>2</sup>. In short, 5-sec epochs with high delta power, low beta power, low EMG activity, and absence of locomotion were classified as NREM sleep; epochs with high theta power, low beta power, low EMG activity and absence of locomotion were classified as REM sleep; and the rest epochs were classified as the awake state.

### **Neural oscillations identification**

In the offline analysis, the algorithm developed by Maingret and colleagues was employed to detect slow oscillations, delta oscillations, and spindle oscillations<sup>3</sup>. The

LFP average across all recording channels, underwent a band-pass Butterworth filter (2nd order, zero-phase shifted, with a cutoff from 0.1 Hz to 4Hz). Subsequently, zero crossings from positive to negative during non-rapid eye movement sleep were identified, along with preceding peaks, following troughs, and surrounding zero crossings from negative to positive.

Positive and negative thresholds were respectively defined for the up-states and downstates of SO/delta. Each identified wave was classified as an SO if the trough was lower than the negative threshold (indicating up-state), the preceding peak was higher than the positive threshold (indicating down-state), and the duration between the peak and the trough was within the range of 150 ms to 500 ms. Conversely, oscillation activities were identified as delta waves if the trough was lower than the negative threshold (indicating up-states) and the up-state was preceded by a maximum voltage lower than the positive threshold within 500 ms.

For the identification of spindles, the LFP in each channel were z-scored, averaged, and subjected to band-pass filtering within the spindle frequency range (8–14 Hz) using a 6th order, zero-phase shifted Butterworth filter with a cutoff from 8 Hz to 14 Hz. Subsequently, the smoothed envelope of this processed signal, obtained by computing the magnitude of the Hilbert transforms convolved with a Gaussian window. Two thresholds for spindle detection were determined based on the mean and standard deviation of the spindle band envelope during NREM sleep. The upper and lower thresholds were respectively set at mean +2.5 std and mean -1.5 std. Spindles were identified if the spindle power exceeded the upper threshold for at least one sample and exceeded the lower threshold for at least 500 ms. The start and stop of each spindle were determined based on these values, and the duration of each spindle was calculated accordingly.

### **Spindle Nesting Analyses**

The nesting analysis basically followed the algorithm developed by Kim and colleagues<sup>4</sup>. The term of “nesting” indicates temporal coupling of spindles relative to

SO and delta waves. For the nesting of spindles to SO (SO-Nesting), each spindle was calculated its latency to the closest SO. The time difference between the peak of the spindle and the up-state of the closest SO was measured for each detected spindle ( $\Delta t_{\text{SO-Spindle}}$ ). The nesting time window was set at  $-500$  ms to  $+1000$  ms from time point of spindle peak. If  $\Delta t_{\text{SO-Spindle}}$  was in the nesting time window, that spindle event was considered a SO-nested spindle. The nesting of spindles to delta was identified similarly to SO-Nesting, measuring time differences between the peak of the spindle and the time of the delta up-state ( $\Delta t_{\text{delta-Spindle}}$ ). To quantitatively assess changes in the temporal relationship of nesting, the nesting index of spindles to SO (SO-Nesting index) was measured. The time lag of the spindle from the closest SO ( $\Delta t_{\text{SO-Spindle}}$ ) was determined for each spindle event, and the rate of spindles with  $\Delta t_{\text{SO-Spindle}}$  within the nesting time window was measured, i.e., SO-Nesting index. The “nesting index” of spindles to delta (delta-Nesting index) was measured similarly to the SO-Nesting index, using time lags between the peaks of spindles and the up-states of the linked delta ( $\Delta t_{\text{delta-Spindle}}$ ).

Finally, the competition index was calculated as the ratio of spindle participant in delta nesting to spindle participant in SO nesting, where spindle participant was computed as the ratio of nested spindle incidence to the total spindle incidence.

## References

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