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Research Article

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Competitive roles of SO/delta-nesting-mediated sleep disruption under acute methamphetamine exposure in monkeys

Xin LV*, Jie LIU*, Shuo MA*, Yuhan WANG, Yixin PAN, Xian QIU, Bomin SUN✉, Shikun ZHAN✉

Department of Functional Neurosurgery, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Abstract: Abuse of amphetamine-based stimulants is a primary public health concern. Recent studies have underscored a troubling escalation in the inappropriate use of prescription amphetamine-based stimulants. However, the neurophysiological mechanisms underlying the impact of acute methamphetamine exposure (AME) on sleep homeostasis remain to be explored. This study employed non-human primates and electroencephalogram (EEG) sleep staging to evaluate the influence of AME on neural oscillations. The primary focus was on alterations in spindles, delta oscillations, and slow oscillations (SO), and their interactions as conduits through which AME influences sleep stability. AME predominantly diminishes sleep-spindle waves in the non-rapid-eye-movement 2 (NREM2) stage, and impacts slow oscillations and delta waves differentially. Furthermore, the competitive relationships between SO/delta waves nesting with sleep spindles were selectively strengthened by methamphetamine. Complexity analysis also revealed that the SO-nested spindles had lost their function of maintaining sleep depth and sleep stability. In summary, this finding could be one of the intrinsic electrophysiological mechanisms by which AME leads to disruption of sleep homeostasis.

Key words: Amphetamine; Sleep stage; Slow oscillation; Delta oscillation; Addiction; EEG

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1 Introduction

Despite the efficacy of amphetamine-based stimulants in treating conditions such as narcolepsy and attention-deficit/hyperactivity disorder, amphetamine abuse has emerged as a prominent substance use disorder, affecting over 35 million people globally and leading to significant mental disorders, violence, and disability(Fazel et al., 2009; Whiteford et al., 2013; Sun et al., 2014; Baingana et al., 2015; Mcelroy, 2017; Bassetti et al., 2019; Posner et al., 2020). Individuals affected by the disorder may engage in "self-medication" with methamphetamine and related substances to cope with stress or negative emotions, or to enhance performance in daily life(Winslow et al., 2007; Van Emmerik-Van Oortmerssen et al., 2012). Statistical data indicate a rapid rise in the misuse of prescription amphetamine-based stimulants in recent years, resulting in more cases of acute adaptation disorders associated with excessive use(Cruickshank and Dyer, 2009; Vivolo-Kantor et al., 2020; Berro et al., 2022).

Sleep disorders represent one of the most significant health issues resulting from acute methamphetamine exposure(Herbeck et al., 2015; Vrajová et al., 2021). Research on individuals who abuse methamphetamine has revealed that amphetamine-related effects on sleep manifest primarily as compromised sleep depth, shortened

✉ Shikun ZHAN, shikun_zhan@hotmail.com
Bomin SUN, sbm11224@rjh.com.cn
* These authors contributed equally to this work
Shikun ZHAN, https://orcid.org/0000-0001-8769-8760

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sleep duration, and decreased sleep quality(Rommel et al., 2015). Specific manifestations include prolonged sleep latency, reduced time in rapid eye movement (REM) sleep, shortened NREM2 sleep, and more REM sleep episodes(Herrmann et al., 2017).

Due to their similarity to humans in terms of brain development, non-human primates serve as excellent pathological models for studying sleep disturbances induced by methamphetamine(Nunes et al., 2020; Vrajová, et al., 2021). Based on human subjects, past research relied mainly on rough sleep data obtained through wristband measurements of limb activity. In recent years, more precise sleep staging based on EEG has gradually been applied to this problem(Berro, et al., 2022). Non-human primate sleep structure in closely resembles that of humans, defined (from shallow to profound sleep) as the REM stage, NREM1 stage, NREM2 stage, and NREM3 stage(Ishikawa et al., 2017).

Rich sleep spindles, delta oscillations, and slow oscillations characterize NREM sleep(Schönauer and Pöhlchen, 2018; Kjaerby et al., 2022; Ruch et al., 2022). They play a crucial role as cortico-thalamic neurogenic oscillations mediating sleep homeostasis and sleep-related physiological functions(Bandarabadi et al., 2020). Sleep spindles not only directly contribute to the stability and quality of deep sleep but also engage in temporal coupling with delta/slow oscillations, mediating the reactivation of memory information during sleep and thus promoting emotional-cognitive enhancement processes(Maingret et al., 2016; Mendes et al., 2021; Lv et al., 2022; Kim et al., 2023).The phenomena of mutual exclusion and competition between delta-spindle and SO-spindle temporal coupling occur during sleep, facilitating memory consolidation and forgetting(Kim et al., 2019). However, it remains unclear whether these mechanisms are involved in another core function of sleep spindles, namely maintaining sleep stability.

Prior research has clarified the significant disruptions of sleep duration and sleep architecture induced by AME(Berro, et al., 2022). Based on this foundation, the present study primarily focuses on how neural oscillations and their interactions directly impact the destabilization of sleep homeostasis during AME. By recruiting oscillation coupling patterns that influence brain-wide sleep depth, we explored the underlying mechanisms of disrupted neural oscillatory interactions(Maingret, et al., 2016).

In this study, we assessed the impact of self-administered methamphetamine on EEG parameters in monkeys, using a wireless recording system that allowed continuous recording of EEG and electromyogram (EMG). By evaluating the influence of methamphetamine on sleep staging, we identified characteristic changes in sleep spindles and differentiated functionally distinct delta oscillations and slow oscillations within similar frequency ranges. We examined the competitive effects of delta-spindle and SO-spindle temporal coupling under the influence of methamphetamine, and observed a significant reduction in the suppressive effect of delta-spindle nesting on SO-spindle nesting. Furthermore, to assess the direct relevance of this process to sleep stability, we extracted sample entropy around delta-nested spindles and SO-nested spindles to characterize local sleep depth, revealing differences in baseline functionality between the two spindle types; SO-nested spindles were weakened under the influence of methamphetamine.

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2 Results

2.1 Acute methamphetamine exposure (AME) experimental design and procedure

To investigate the effects of acute methamphetamine exposure on monkey sleep structure, we implanted electrocorticography (ECOG) electrodes into the cortex of three macaques and recorded their limb movements with an accelerometer. Electrode contact points were reconstructed using a 3D-slicer and CT data to ensure consistency of location across individuals (Fig 1a, Fig.S1a). Baseline data were continuously recorded for 5 days (approximately 120 hours), after which each monkey received intravenous injections of methamphetamine at 1.5 mg/kg daily for 7 days (approximately 180 hours) (Fig 1b). Based on circadian rhythms, each monkey was observed for at least one sleep period lasting more than 5 hours daily. We extracted these continuous sleep periods and classified sleep stages based on synchronized EEG data, activity data, and a specific sleep-stage

identification method, categorizing sleep into wakefulness, REM sleep, non-REM 1, non-REM 2, and non-REM 3 (Fig. 1c).

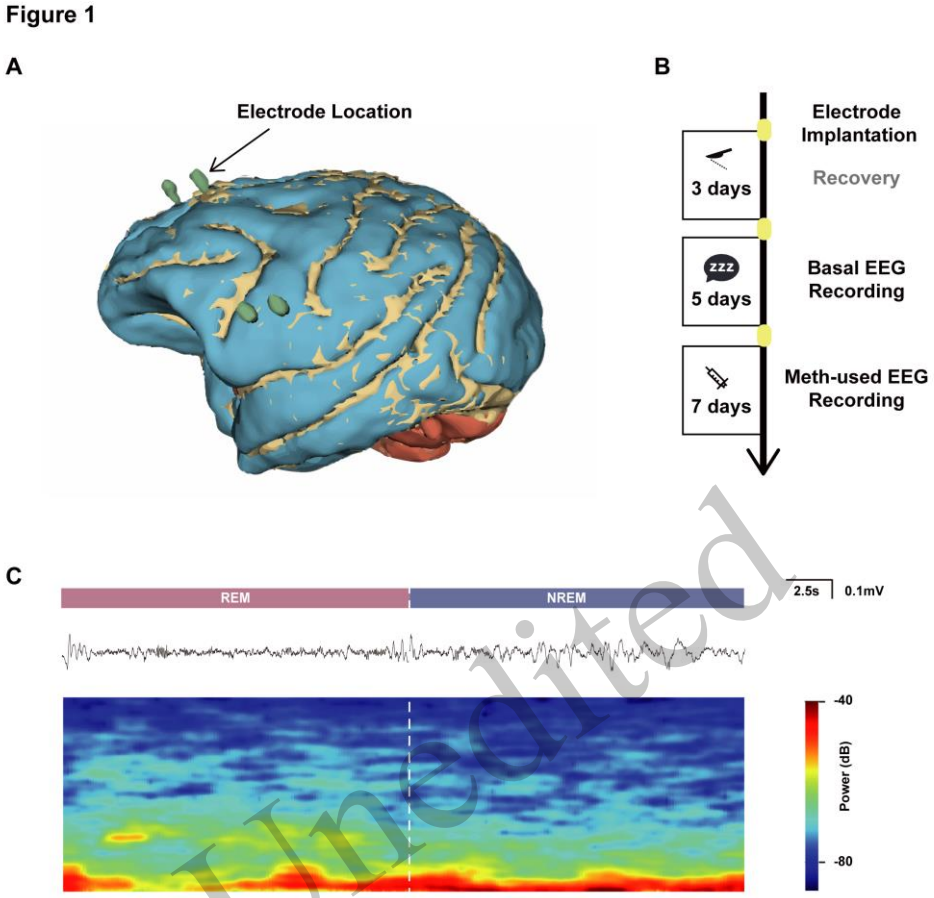

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Figure 1 Acute methamphetamine exposure experimental design and procedure
(A) Schematic of surgical electrode implantation and electrode-position reconstruction, showing the precise arrangement of electrodes in specific locations.
(B) Experimental procedure schematic illustrating the entire experimental process.
(C) Sleep stage diagram depicting sleep stages of REM and NREM with raw wave representation and its heatmap features.

2.2 AME impairs functional sleep structure

Significant alterations in the typical sleep structure were induced by AME, resulting in a noteworthy decrease in both REM and NREM sleep (Fig. 2a). This indicated that AME directly led to a reduction in total sleep time, a decrease in sleep quality, and disruption of monkey sleep homeostasis, which is consistent with early studies(Daley et al., 2006; Herrmann, et al., 2017; Berro, et al., 2022). To further investigate whether AME hindered the progression of sleep towards deeper stages, we calculated probability matrices for transitions between different sleep stages and visualized the comparisons in the form of chord diagrams (Fig. 2b). The results indicated that AME did not impede transitions between any of the sleep stages.

Based on these results, to identify the specific sleep stages disrupted by AME and investigate the steady state, we measured the duration of each sleep stage before the transition to the next stage. Longer duration indicated greater stability in maintaining a sleep stage. We found a significant increase in REM sleep duration after AME, while NREM2 sleep duration significantly decreased; there were no significant changes observed in other stages (Fig. 2c). These results indicated that NREM, particularly the NREM2 stage, may represent the sleep stages most susceptible to the influence of AME. It was necessary to conduct further analysis on the

factors during this period that influence sleep stability.

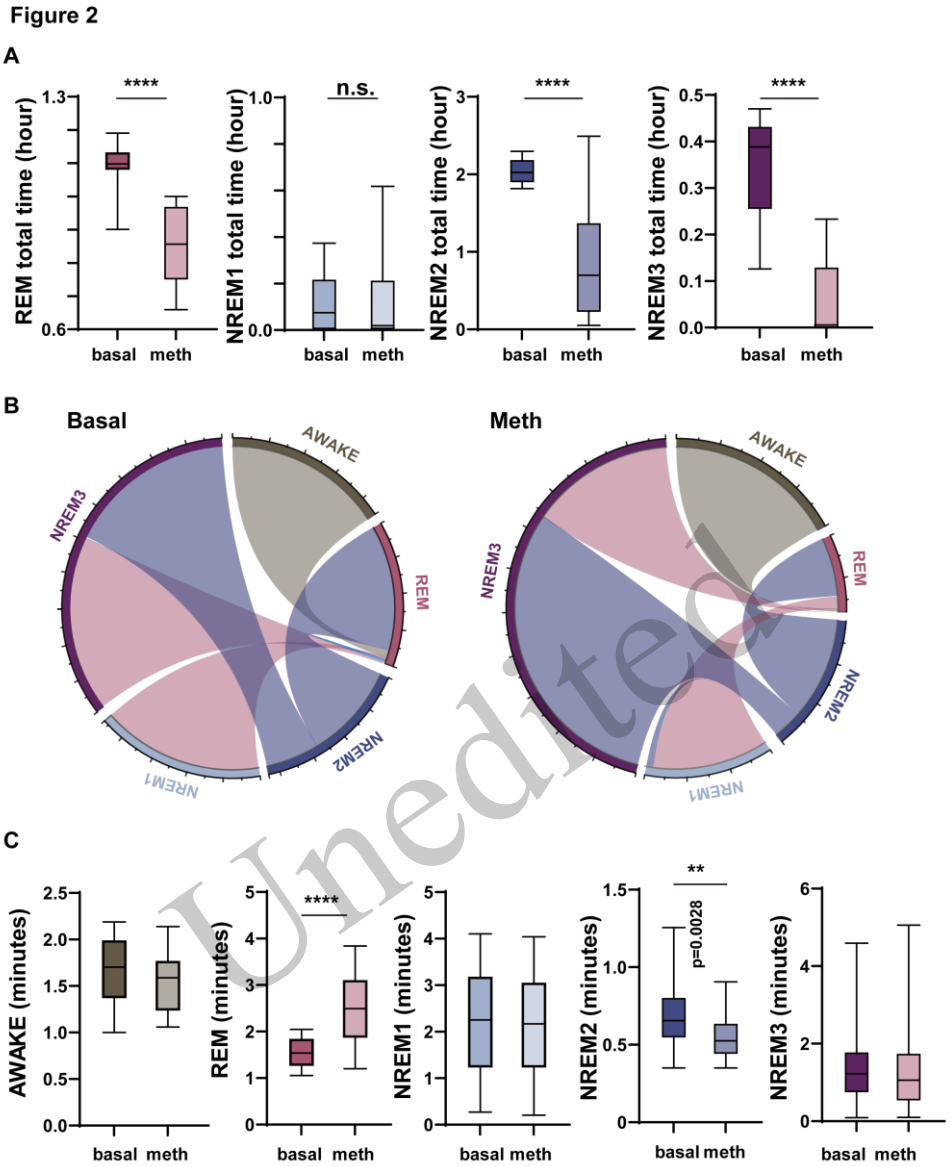

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Figure 2 Acute methamphetamine exposure impairs functional sleep structure.

(A) The total time of each sleep stage (REM, NREM1, NREM2, NREM3) changes after AME. AME significantly prolongs the total duration of REM sleep and shortens the total time of NREM2 and NREM3 sleep (for REM, n=14 in basal versus n=20 in meth, Mann-Whitney test of non-parameters test, p= 0.0002; for NREM1, n=14 in basal versus n=20 in meth, Mann-Whitney test of non-parameters test, p=0.7178; for NREM2, n=14 in basal versus n=20 in meth, Mann-Whitney test of non-parameters test, p=0.0001; for NREM3, n=14 in basal versus n=20 in meth, Mann-Whitney test of non-parameters test, p<0.0001) .

(B) Chord diagram illustrating the probability of transitions between sleep stages. The left panel represents the baseline state, while the right panel depicts the state after AME. AME does not fundamentally block progression from REM sleep to NREM sleep.

(C) Average duration of segments of each sleep stage (REM, NREM1, NREM2, NREM3). In addition to the significantly prolonged duration of individual REM sleep stages, impairment of NREM sleep stability mainly occurs during the NREM2 stage (for AWAKE, n=51 in basal versus n=34 in meth, Mann-Whitney test of non-parameters test, p=0.0720; for REM, n=202 in basal versus n=381 in meth, Mann-Whitney test of non-parameters test, p<0.0001; for NREM1, n= 238 in basal versus n= 310 in meth, Mann-Whitney test of non-parameters test, p=0.4821; for NREM2, n= 72 in basal versus n= 22 in meth, Mann-Whitney test of non-parameters test, p=0.0012; for NREM3, n= 156 in basal versus n=98 in meth, Mann-Whitney test of non-parameters test, p=0.2720).

* indicates p < 0.05, ** indicates p < 0.01, *** indicates p < 0.001, **** indicates p < 0.0001.

2.3 AME attenuates sleep-spindle waves in the NREM2 stage

Sleep-spindle waves are an intrinsic neural oscillatory mechanism involved in maintaining sleep homeostasis, and exhibit widespread activity throughout the cortex. We chose these electroencephalogram features for study because of their role in maintaining sleep stability. We extracted sleep-spindle waves and analyzed changes in their physiological characteristics after AME (Fig. 3a). The results revealed a significant attenuation of sleep-spindle waves after AME, with lower incidence (Fig. 3b), flattening of peak-trough amplitudes (Fig.S1b-c), reduction in average power (Fig. 3c), shortened duration (Fig.S1d), and uneven distribution (Fig. 3d, Fig.S1e). Among these features, the incidence of sleep-spindle waves is the most crucial factor in maintaining sleep homeostasis. We assessed the changes in the incidence of sleep-spindle waves during different sleep stages before and after AME. The results showed a significant decrease in the incidence of sleep-spindle waves during NREM2 sleep stages (Fig. 3e), with no significant change observed in NREM3 sleep (Fig. 3f). Additionally, no sleep-spindle waves were observed during NREM1 sleep. Thus it seems that disruption of sleep-spindle waves is one of the intrinsic mechanisms of sleep homeostasis disruption induced by AME.

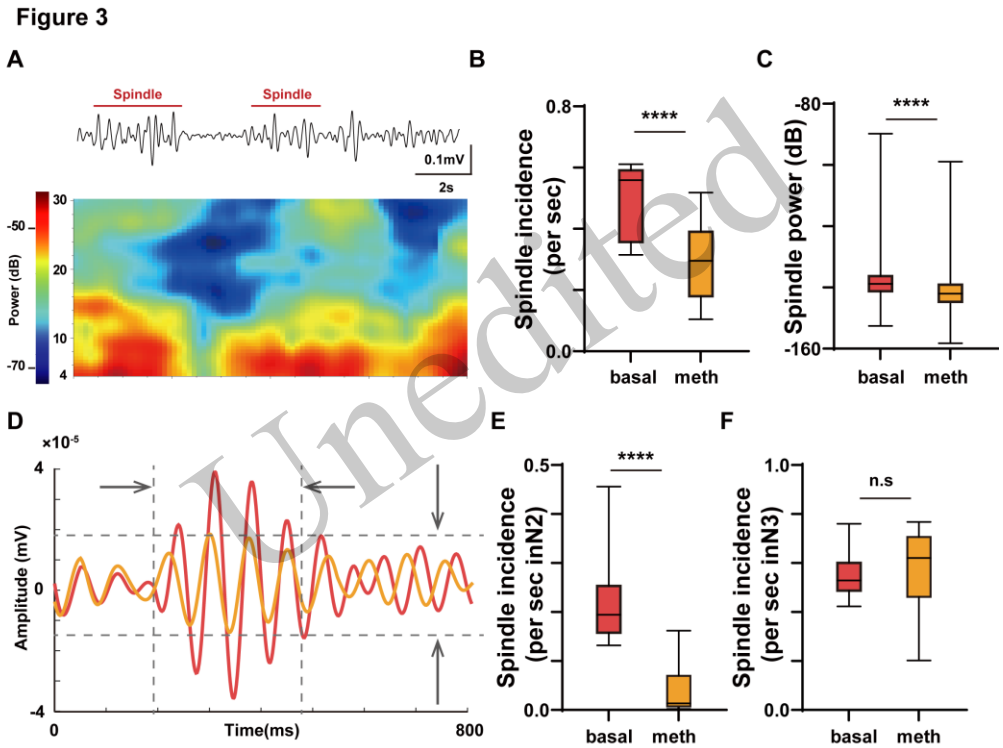

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Figure 3 Acute methamphetamine exposure attenuates sleep-spindle waves in the NREM2 stage.

(A) Schematic representation of spindle waves identified in the raw waveform after 4-12Hz filtering during NREM sleep.

(B) Spindle-wave incidence (count per second) during basal sleep and AME sleep period (n=14 in basal versus n=20 in meth, Mann-Whitney test of non-parameters test, p<0.0001).

(C) Average power of spindle waves during basal sleep period and AME sleep period (n=30145 spindles in basal versus n=28904 spindles in meth, Mann-Whitney test of non-parameters test, p<0.0001).

(D) Average spindle waveform during basal sleep period and AME sleep period. AME induced a flatter waveform with lower amplitude and shorter duration, which leads to functional loss in sleep stability.

(E) Spindle-wave incidence in the NREM2 stage (count per second) during basal sleep period and AME sleep period (n=14 in basal versus n=20 in meth, Mann-Whitney test of non-parameters test, p<0.0001).

(F) Spindle-wave incidence in the NREM3 stage (count per second) during basal sleep period and AME sleep period (n=14 in basal versus n=20 in meth, Mann-Whitney test of non-parameters test, p= 0.3451).

* indicated p < 0.05, ** indicated p < 0.01, *** indicated p < 0.001, **** indicated p < 0.0001.

2.4 AME differentially altered features of delta waves and slow oscillations

Slow oscillation/delta waves are also considered essential oscillatory elements for maintaining sleep homeostasis, and have slight physiological differences despite their overlapping frequency ranges. The former primarily induces deep sleep, while the latter participates in mediating cognitive functions. Using the method reported by Kim et al., we extracted SOs and delta oscillations during the entire sleep period and classified them based on peak amplitude, trough amplitude, and power(Kim et al., 2019). Similar to previous findings, SOs and delta oscillations were mainly distinguished by their peak amplitude, with similar trough amplitude distributions and significant overlap in average power distribution (Fig. 4a). SOs exhibited a significantly higher peak amplitude (Fig. 4b-f).

We compared the relevant features of slow oscillation/delta waves after AME. The results demonstrated that AME differentially affected delta oscillations and SOs. Firstly, AME significantly reduced the incidence of delta oscillations to nearly half the original value (Fig. 5a) and slightly lowered their average power (Fig. 5b). With regard to the waveform features, AME increased the amplitude of delta oscillations (Fig.S2a-b) but prolonged their latency, resulting in more uneven distribution (Fig.S2c). AME did not alter the incidence of SOs (Fig. 5c), but reduced average power (Fig. 5d) and amplitude without a significant impact on latency (Fig.S2d-f). AME also induced differences across various sleep stages. In the NREM2 stage, AME only resulted in a decrease in delta occurrence without affecting SOs (Fig.5e-f). In the NREM3 stage, it led to the disappearance of SOs and delta oscillations in some individuals, resulting in a significant overall decrease (Fig. 5g-h). These results indicated differential effects of AME on SO and delta waves, laying the groundwork for further investigation of the intrinsic mechanisms of AME-induced sleep homeostasis disruption.

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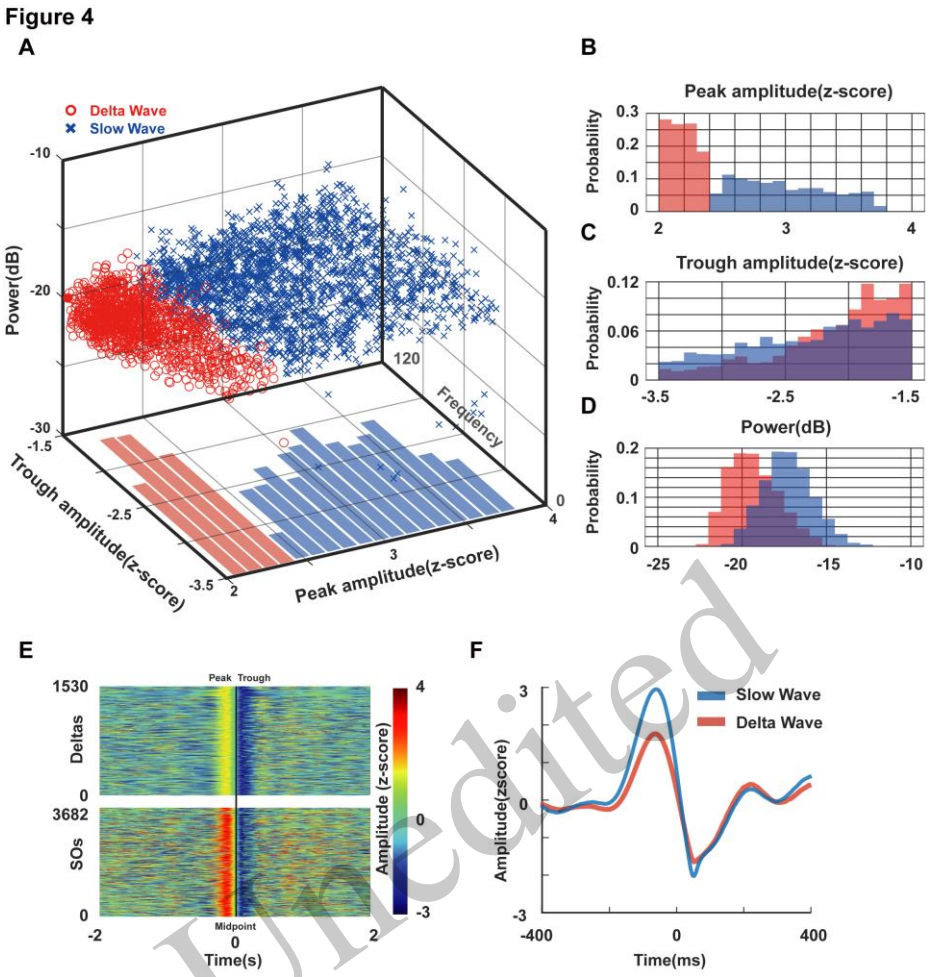

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Figure 4 Dissociation of delta waves and slow oscillations

(A) Delta waves and slow oscillations from single monkeys in the basal sleep period stage (n=5). The scatter plot is based on z-scored peaks of up states, z-scored troughs of up states, and average power.

(B) Distribution of z-scored peaks of up states. The peak distribution of the delta wave is completely separate from the slow oscillation.

(C) Distribution of z-scored troughs in the up states of delta waves and slow oscillations.

(D) Distribution of average power of delta waves and slow oscillations. Slow oscillations exhibit slightly higher power than delta waves.

(E) Heat-map matrix which vertically stacks all original waveform heat maps of delta and slow oscillations. On the left side of the midpoint is the peak amplitude, and on the right side are the trough amplitudes.

(F) Average waveform of slow oscillations (n=1530 from 5 trials) and delta waves (n=3682 from 5 trials).

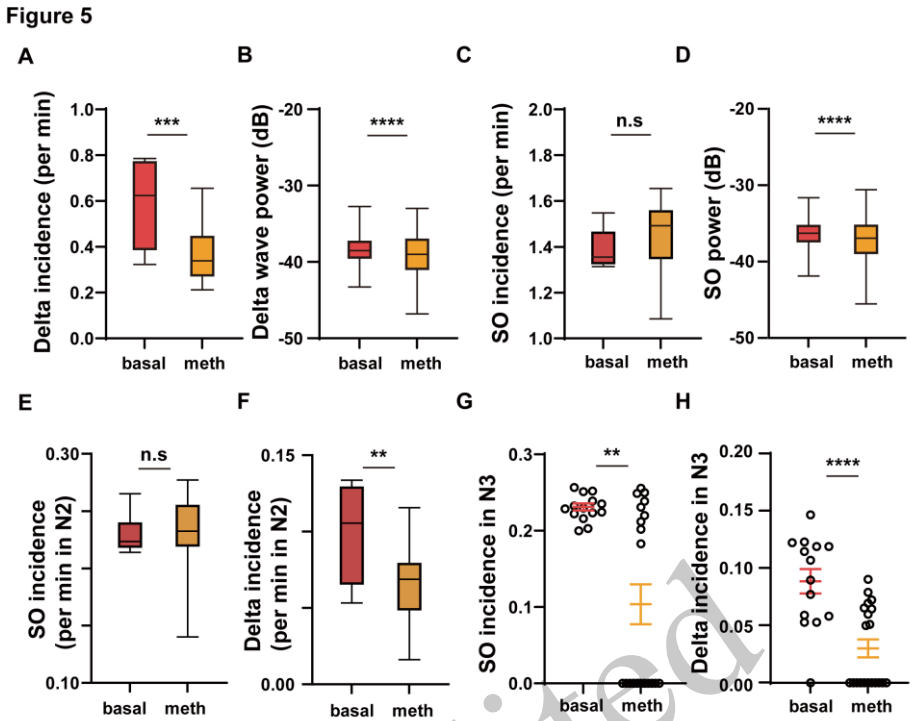

Figure 5 AME differentially alters features of delta waves and slow oscillations.

(A) Comparison of the incidence of slow oscillations (per minute) during NREM in basal and AME periods (n=14 in basal versus n=20 in meth, unpaired t-test, p=0.0002).

(B) Comparison of the average power of slow oscillations (per minute) during NREM in baseline and AME periods (n=93204 SOs in basal versus n=134091 SOs in meth, Mann-Whitney test of non-parameters test, p<0.0001).

(C) Comparison of the incidence of delta waves (per minute) during NREM in baseline and AME periods (n=14 in basal versus n=20 in meth, unpaired t-test, p=0.1496).

(D) Comparison of the average power of delta waves (per minute) during NREM in baseline and AME periods (n=39300 deltas in basal versus n=50043 deltas in meth, Mann-Whitney test of non-parameters test, p<0.0001).

(E) Comparison of the incidence of slow oscillations (per minute) during NREM 2 sleep (n=14 in basal versus n=20 in meth, unpaired t-test, p=0.9343).

(F) Comparison of the incidence of delta waves (per minute) during NREM 2 sleep (n=14 in basal versus n=20 in meth, unpaired t-test, p=0.0015).

(G) Comparison of the incidence of slow oscillations (per minute) during NREM 3 sleep (n=14 in basal versus n=20 in meth, Mann-Whitney test of non-parameters test, p=0.0033).

(H) Comparison of the incidence of delta waves (per minute) during NREM 3 sleep (n=14 in basal versus n=20 in meth, Mann-Whitney test of non-parameters test, p=0.0004).

* indicated p < 0.05, ** indicated p < 0.01, *** indicated p < 0.001, **** indicated p < 0.0001.

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2.5 Competitive nesting of SO/delta and sleep-spindle waves mediates AME-induced sleep disruption

Competitive nesting between SO/delta waves and sleep-spindle waves has been shown to play a role in cognitive processes. Increased nesting of sleep-spindle waves with delta waves leads to decreased nesting with SO waves, promoting memory consolidation(Kim, et al., 2019). Building upon this conclusion and the afore-mentioned results, we explored the role of this competitive mechanism in AME-induced disruption of monkey sleep homeostasis.

First, we identified nesting of delta waves with sleep-spindle waves and nesting of SO waves with sleep-spindle waves and quantified them separately (Fig. 6a). We found a clear segregation effect, with AME leading to a significant decrease in the incidence of delta-spindle nesting and a significant increase in the incidence of SO-spindle nesting (Fig. 6b-c). Subsequently, changes in the distribution of spindle probabilities around the nesting time window were consistent between delta and SO waves: both decreased after AME (Fig.

6d-e). This indicates a weakening of the recruitment of spindle waves by both delta and SO waves after AME. To further quantitatively describe recruitment of spindles by SO/delta waves, we obtained the corresponding nesting index by calculating the firing rate of spindles within nesting time windows (Fig. 6f-g). Finally, to determine the competitive relationship between SO and delta waves while eliminating the influence of baseline firing rates, we calculated the ratio of the proportion of delta-nested spindles to SO-nested spindles as the delta/SO competing index. It showed a significant decrease in the delta/SO competition index after AME, with an almost twofold difference in average value (Fig. 6h). This implies that the enhancement of coupling between SO waves and spindles is established on the basis of their competitive interaction with delta-spindle coupling.

The enhanced competitive coupling between SO waves and spindles does not directly lead to conclusions regarding its impact on sleep stability. Therefore, we hypothesize that there might be some physiological abnormalities associated with SO-nested spindles. To validate this hypothesis regarding the role of AME in disrupting sleep stability, we separately extracted SO-nested spindles, delta-nested spindles, and non-nested spindles (Fig. 7a). The overall sample entropy of EEG around the three types of spindle waves was calculated, and lower sample entropy represented a lower overall wakefulness level of the brain, directly related to sleep depth (Fig. 7a). For SO-nested spindles, although AME increased their occurrence and competitive role, the process of reduced entropy mediated by them under baseline conditions (purple line at top of Fig. 7b) showed significant attenuation after AME (purple line at bottom of Fig. 7b). It was evident that there was a significant increase in complexity around SO-nested spindle waves, indicating a gradual loss of physiological function in some spindles after AME. These spindles became coupled with SO waves, contributing to the overall disruption of sleep homeostasis. In Fig. 7b, it can be observed that the complexity around non-nested spindles remained relatively stable both before and after AME. Meanwhile, the trend of sample entropy changes around delta-nested spindles exhibited an opposite pattern to that of SO-nested spindles overall. This indicates physiological differences between the two, with the former potentially associated with cognitive consolidation, and the latter more involved in sleep homeostasis, leading to the disruption of sleep structure after AME.

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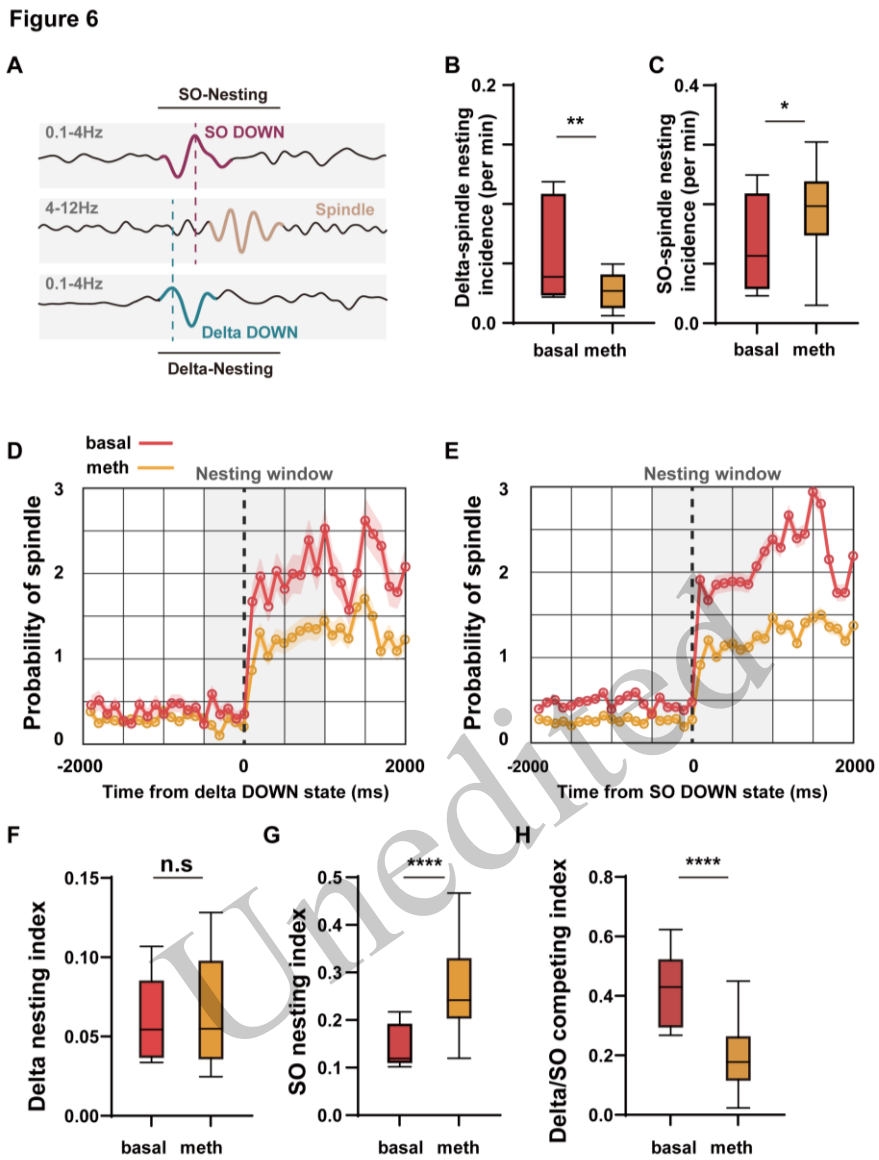

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Figure 6 AME strengthens competitive spindle nesting of slow oscillations over that of delta waves

(A) Schematic diagram illustrating the independent nesting of slow oscillations and delta waves with spindles. The waveforms represent the slow oscillation, spindle wave, and delta wave from the filtered signal. The dashed line indicates the starting point of the down state, which is also the beginning time-point of nesting.

(B) Delta-spindle nesting incidence (per minute) changes from basal to AME periods (n=14 in basal versus n=20 in meth, unpaired t-test, p=0.0051).

(C) Slow oscillation-spindle nesting incidence (per minute) changes from basal to AME periods (n=14 in basal versus n=20 in meth, unpaired t-test, p=0.0238).

(D) Peri-delta-nesting distribution of spindle wave in basal (red line) and AME periods (yellow line). Lines and dots represent the means, and shading represents the standard error of the mean (SEM).

(E) Peri-SO-nesting distribution of spindle waves in basal (red line) and AME periods (yellow line). Lines and dots represent the means, and shading represents the standard error of the mean (SEM).

(F) Delta-nesting index changes from basal to AME periods (n=14 in basal versus n=20 in meth, unpaired t-test, p=0.8559).

(G) SO-nesting index changes from basal to AME periods (n=14 in basal versus n=20 in meth, unpaired t-test, p<0.0001).

(H) SO/delta competing index changes from basal to AME periods, calculated based on the ratio of spindles involved in delta nesting to the ratio of spindles involved in SO nesting, reflecting the competitive recruitment effects of the two types of nesting (n=14 in basal versus n=20 in meth, unpaired t-test, p<0.0001).

* indicated p < 0.05, ** indicated p < 0.01, *** indicated p < 0.001, **** indicated p < 0.0001.

Figure 7

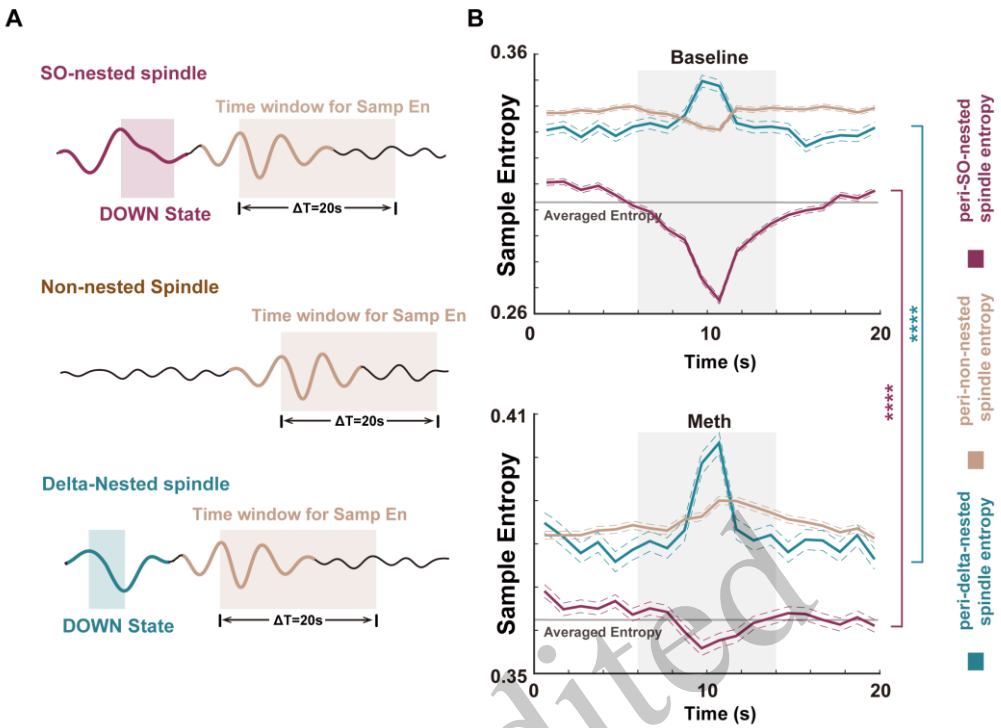

Figure 7 AME impairs the role of SO-nested spindles in maintaining sleep stability.
(A) Schematic diagram of sample entropy calculation. Spindle waves were divided into three groups: nested with delta waves, nested with slow oscillations, and non-nested spindles. To mitigate the silencing effects of SO/delta-mediated down states, sample entropy was computed within a time window consisting of the first 20 seconds following the peak of the first spindle wave after the end of the down state. For each time window, sample entropy was computed using a sliding window approach with a width of 1 second and a step size of 1 second.
(B) Average peri-event entropy for delta-nested spindles, SO-nested spindles, and non-nested spindles in baseline (top) and AME (bottom) periods. The entropy around delta-nested spindles and SO-nested spindles exhibits completely opposite activity, while non-nested spindles occupy an intermediate position. AME also induces a flatter entropy profile around SO-nested spindles. Solid lines represent the means, and the dashed line represents the standard error of the mean (SEM). The gray semi-transparent line indicates the average value of entropy. The statistical results are based on the average sample entropy around the gray box for events. The significance markers indicate the statistical results between the average peri-spindle sample entropy (for peri-delta-nested spindle entropy, n= 3494 in basal versus n=3414 in meth, unpaired t-test, $p<0.0001$; for peri-SO-nested spindle entropy, n= 13996 in basal versus n= 8861 in meth, unpaired t-test, $p<0.0001$).
* indicated $p < 0.05$, ** indicated $p < 0.01$, *** indicated $p < 0.001$, **** indicated $p < 0.0001$.

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3 Discussion

In this study, we investigated the impact of AME on the sleep state of monkeys through continuous recording of EEG-EMG data. Utilizing implanted and wearable devices with wireless technology, we longitudinally collected data in freely moving monkeys. The study refrained from imposing light-dark cycles on the monkeys' living environment, maintaining sleep under natural lighting conditions for 8-12 hours to minimize environmental interference. Basic sleep states were roughly identified with cameras, and fine staging was performed based on EEG-EMG data.

With regard to the influence of AME on sleep structure, our results indicate a significant deterioration in the depth of monkey sleep. Specifically, there were notable reductions in the length of both NREM2 and NREM3 periods. NREM3, in particular, disappeared frequently in nearly half of the trials. Building upon these

findings, we further explored whether there were disruptions in the progression of sleep following AME exposure, particularly during an intermediate phase. Analysis of sleep-transform probabilities and average duration revealed a substantial decrease in stability during NREM2(Troynikov et al., 2018). Combined with the shortened duration of NREM3, this suggests a tendency for NREM2 to transition to wakefulness in an unstable manner after AME(Shalaby et al., 2022). Previous studies, including investigations on methamphetamine abusers and non-human primate models, have consistently shown that acute or chronic exposure to methamphetamine can lead to sleep-wake behavior dysregulation and sustained disruption of sleep cycles, including reduced total sleep time, prolonged sleep-onset latency, and diminished self-reported sleep quality(ref). However, the manifestation of these effects may vary depending on the effective dose of methamphetamine.

Perez et al. initially observed in methamphetamine abusers that the impact of the drug on total sleep duration is dose-dependent, with acute sleep disturbances reliably induced only at oral doses above 0.7mg/kg(Perez et al., 2008). Therefore, we employed a 1mg/kg intravenous injection to investigate the neurophysiological mechanisms underlying AME-induced sleep disruption. Murnane et al. demonstrated in rhesus monkeys that an oral dose of 0.5mg/kg failed to consistently induce sleep disturbances(Murnane et al., 2013). Similarly, Berro et al. administered methamphetamine intravenously at doses of 0.01mg/kg, 0.1mg/kg, and 0.3mg/kg in rhesus monkeys without observing significant changes in total sleep duration(Berro et al., 2016).

The differences between these reports and the findings in our study are likely primarily due to variations in dosage. Additionally, there are differences in assessment of sleep structure. While prior studies mainly relied on activity-measuring wristbands, we employed a combination of wristbands and multi-channel sleep EEG signals for more precise sleep staging(Vrajová, et al., 2021). Furthermore, previous research has indicated that in healthy individuals exposed to acute methamphetamine, tolerance to methamphetamine's sleep-disrupting effects tends to develop gradually, with a diminishing impact approximately five days later(Comer et al., 2001). However, we did not observe this phenomenon in our study, which may also be linked to the dosage administered. It is important to note that studies investigating chronic methamphetamine exposure did not report this phenomenon, even at lower doses such as 0.01mg/kg, which could still disrupt sleep parameters(Andersen et al., 2013).

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Apart from total sleep duration, methamphetamine dosage may also dictate its effects on the NREM sleep stage. While most research on sleep cycles in humans and non-human primates primarily indicates a reduction in the duration of NREM periods, Herrmann and colleagues observed that recreational methamphetamine use did not significantly impact the duration of NREM3 periods. This discrepancy can be attributed not only to differences in species and administration routes (oral and intravenous), but more importantly to the dose dependency of methamphetamine's sleep effects(Herrmann, et al., 2017). Herrmann and colleagues primarily administered doses ranging from 0.3mg/kg to 0.5mg/kg. Considering additional studies investigating changes in sleep parameters across various doses, lower doses are unlikely to decrease the duration of NREM3 periods, while doses above 1mg/kg can reliably induce this phenomenon(Perez, et al., 2008). It is worth noting that another study using a dose of 1.3mg/kg also found a reduction in the duration of NREM3 periods and disruption of sleep structure(Berro, et al., 2022).

Building upon the impact of AME on NREM3 sleep, SO and delta oscillations emerge as distinct electroencephalographic features of the NREM3 period, garnering further attention(Diekelmann and Born, 2010). At the synaptic level, methamphetamine's influence on sleep primarily involves the monoaminergic and dopaminergic pathways(Paulus and Stewart, 2020). One of its crucial effects is the inhibition of monoamine transporters and dopamine transporters at the terminals of dopaminergic neurons, which affects the sleep process through projections from the midbrain to the frontal cortex(Brodt et al., 2023). Research indicates that during this process, there is a lengthening of the firing time of dopaminergic neurons, which potentially affects a broad spectrum of SO and delta oscillations by modulating inhibitory post-synaptic potentials; this could lead to disrupted oscillatory-mediated information exchange in the frontal cortex(Hedges et al., 2018).

Utilizing EEG electrodes positioned in the frontal lobe, we effectively captured slow oscillations (SOs) and delta oscillations originating from this brain region. Conversely, sleep-spindle waves predominantly stem from

the thalamus. During the up state of slow oscillations, neurons in the thalamic reticular nucleus are influenced by the widespread depolarization of cortical neurons, giving rise to spindle oscillations. These oscillations then propagate via thalamocortical projections to the frontal lobe cortex, thereby contributing to the maintenance of sleep stability in the brain(Brodt, et al., 2023). However, prior imaging studies have indicated a marked reduction in thalamocortical pathway activity in individuals using methamphetamine. This hindrance of the feedback process may represent one of the microstructural underpinnings for the diminished activity of spindles under the influence of methamphetamine(Andersen, et al., 2013).

Sleep-spindle waves represent a crucial functional neural oscillation for maintaining sleep stability during NREM periods(Castelnovo et al., 2016; Brockmann et al., 2020; Weng et al., 2020; Kaulen et al., 2022). Increasing spindle waves have been shown to directly improve various sleep disorders and enhance sleep quality(Joechner et al., 2023). Our study observed not only a reduction in the number of sleep spindles in cortical EEG after AME but also a functional decline characterized by shortened duration, decreased amplitude, and an overall flattened trend. Specifically, the decrease in sleep-spindle quantity was prominent during NREM2, aligning with the instability observed in this stage. This suggests a significant role for AME-induced disruptions of sleep-spindle activity, particularly in NREM2 instability(Chen et al., 2023).

The precise timing coordination between SO/delta waves and sleep spindles is a critical characteristic of NREM sleep. This process primarily takes place in the frontal cortex, where SO/delta oscillations induce spindles that propagate from the thalamus to the frontal cortex and establish coupling. Early research has shown that the efficiency and temporal accuracy of this coupling regulate various physiological functions during sleep, and its reduced precision correlates with dysfunction in the frontal-thalamic network(Helfrich et al., 2018; Muehlroth et al., 2019). methamphetamine exposure has been demonstrated to notably diminish both the volume and functionality of the thalamus(Volkow et al., 2001; Morales et al., 2015; Li et al., 2017; Warton et al., 2018).

Nesting of SO/delta oscillations with sleep-spindle waves is a critical mediator for the physiological function of spindle waves(Kim, et al., 2019). Previous research has demonstrated that the precise timing relationship between delta waves and spindles and SO waves and spindles contributes to cognitive enhancement during sleep(Miyamoto et al., 2017; Kim, et al., 2019; Hahn et al., 2020). In the competition between delta-spindle and SO-spindle nesting, our study showed an enhancement of SO-spindle nesting and a reduction of delta-spindle nesting after AME exposure. The competitive mechanism found in our study, involving stronger recruitment of spindles by SO and a doubling of the proportion of spindles involved in SO nesting, indicates a significant impact on sleep stability.

In the absence of abnormalities, SO-spindle nesting is a factor in maintaining sleep stability(Malkani and Zee, 2020; Girardeau and Lopes-Dos-Santos, 2021). Therefore, AME-induced enhancement of SO-spindle nesting is likely a pathological factor leading to the loss of normal function. This study introduces sample entropy from information theory as an evaluation metric, with increased sample entropy associated with decreased neuronal activity during deepening sleep(Ma et al., 2018; Hou et al., 2021; Liang et al., 2021; Sathyanarayana et al., 2021). Analysis of sample entropy around spindle events categorized as SO-nested, delta-nested, and non-nested spindles reveals a significant increase in sample entropy around SO-nested spindles after AME, indicating a loss of sleep-stabilizing function. Notably, baseline conditions show a clear opposite trend in sample entropy around delta-nested spindles compared to SO-nested spindles, which highlights functional differences. Delta-nested spindles are primarily implicated in cognitive function enhancement, while SO-nested spindles play a key role in sleep stability(Zhang et al., 2021).

In summary, this study of monkey sleep stability following acute methamphetamine exposure not only highlights significant changes in sleep structure but also reveals multiple key alterations at the neural oscillation level. A detailed analysis of the competition between SO-spindle and delta-spindle nesting sheds light on AME-induced enhancement of SO-spindle nesting and reduction of delta-spindle nesting. This competitive mechanism may adversely affect cognitive function, particularly through the failure of SO-spindle nesting(Kim, et al., 2019). We hope that this more comprehensive understanding will contribute to elucidating the impact of

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AME on monkey sleep stability, as it emphasizes neural oscillation changes as a critical factor in the diverse effects induced by AME.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Data availability statement

The data and custom code that support the findings from this study are available from the corresponding author upon reasonable request.

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Author contributions

X.L. proposed the research hypothesis, designed the experiments, conducted the data analysis, and drafted the paper. J.L. and S.M. collected the experimental data, with assistance from Y.W., Y.P., and X.Q. B.S. provided funding for the research. S.Z. oversaw the study design and the dataset collection.

Compliance with ethics guidelines

Xin Lv, Jie Liu, Shuo Ma, Yuhan Wang, Yixin Pan, Xian Qiu, Bomin Sun, and Shikun Zhan declare that they have no conflicts of interest.

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 (identification number: CEBSIT-2022003R01).

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Supplementary information

Figs. S1 and S2; Materials and methods

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