"Paradoxical wakefulness" induced by psychedelic 5-methoxy-N,N-dimethyltryptamine in mice

Benjamin JB Bréant[1], José Prius Mengual[1], David M Bannerman[2],

Trevor Sharp[3], Vladyslav V Vyazovskiy[1]

1. Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford UK

2. Department of Experimental Psychology, University of Oxford, Oxford UK

3. Department of Pharmacology, University of Oxford, Oxford UK

Abstract

Vigilance states – waking, slow-wave sleep (SWS) and paradoxical sleep, are thought to be controlled by several cortical and subcortical neuromodulatory circuits, among which the serotonergic (5-HT) system plays an important role. Recently, serotonergic psychedelics have attracted attention as potent antidepressants. While they are known to induce profound changes in subjective experience, the immediate and delayed effects of psychedelics on classical signatures of sleep-wake states remain under-investigated. To address this, we performed chronic electrophysiological recordings in the cortex of freely moving adult male mice following an injection of a short-acting psychedelic 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT). The most noticeable effect of 5-MeO-DMT administration was the suppression of paradoxical sleep and an acute induction of a mixed state of vigilance, characterised instead by prominent SWS-like slow waves on the EEG and LFP in awake, moving animals. We posit that the occurrence of this state in mice, which we refer to as "paradoxical wakefulness", may be a rodent equivalent of the altered state of consciousness induced by psychedelics in humans.

Introduction

Three vigilance states have been described in mammals using behavioural and electrophysiological criteria: wakefulness and two stages of sleep, slow wave sleep (SWS), also referred to as NREM sleep, and paradoxical sleep (PS), often referred to as REM sleep.[1–5] Wakefulness is typically a behaviourally active state, as reflected by movement, accompanied by high electromyographic (EMG) activity and electroencephalographic (EEG) low amplitude signals rich in theta (5 – 10 Hz) and higher frequencies.[6] During quiet wakefulness or after sleep deprivation, fast theta-activity decreases significantly and slow frequencies show an increase even in behaviourally awake animals.[7,8] Sleep, however, is marked by an immobility of the animal and reduced responsiveness to external stimuli. As EEG activity is typically correlated with arousal and behaviour,[5] the main electrophysiological feature of SWS is the occurrence of EEG slow oscillation (0.5 – 4 Hz) of high amplitude.[5,9–11] PS is instead defined by wake-like EEG dominated by theta-frequency activity but with a complete muscle atonia interrupted by transient twitches of the limbs, ears and whiskers and accompanied by rapid eye movements.[1,2,4] Similar to wakefulness, PS is not a uniform, homogenous state, but is characterised by prominent differences in neural activity between brain regions and cortical layers.[12–14]

Serotonin (5-HT) is considered one of the key neuromodulators involved in the regulation of wakefulness and sleep.[15–17] Because 5-HT neurons are typically active during wakefulness, they are thought to play a role in promoting arousal.[18–20] However, reduced electrical activity of the 5-HT neurons or even complete silence during PS suggest that 5-HT is not necessary for the activated state of the cortex.[15,17] The 5-HT system is also associated with many aspects of cognition and emotion, as well as peripheral physiology, including thermoregulation and metabolism.[21–26] It is widely acknowledged that the 5-HT system plays

an important role in mood disorders, including anxiety and depression, although the precise mechanisms are still a matter of considerable debate.[22,25,27,28] Furthermore, selective serotonin reuptake inhibitors (SSRIs) are widely used as antidepressants, and are often accompanied with marked suppression of PS in both humans and rodents.[29–32]

Psychedelics are a fascinating class of drugs known to profoundly alter sensory and behavioural experiences in humans, which is thought to be mediated through the 5-HT system.[33–36] They were first studied as adjuncts to psychotherapy in a range of psychiatric disorders and there is currently a new wave of interest in their potential therapeutic effects.[37–39] In humans, the administration of psychedelics can induce transient dissociated states of vigilance, confusion or thought disorder.[33,36,40,41] In mice, the potency of a psychedelic can be correlated with head-twitch responses, defined as brisk movements of the head and are often used as a marker of the acute effect of the drug.[42] 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) is a fast-acting psychedelic drug found naturally in the venom of the *Bufo alvarius* toads from the Arizona Desert and the bark of the South American plant *Dictyoloma incanescens* DC.[43] Its behavioural effects in mice are noticeable within 5-10 min after administration and have a half-life of 12-19 min.[43–46] They are thought to be primarily mediated through the 5-HT_{2A} and 5-HT_{1A} receptor and include head twitch responses, and changes in vocal behaviour and brain plasticity.[47,48]

Interestingly, as with SSRIs, it has been shown that psychedelic drugs such as psilocybin transiently suppress PS.[29,32,49,50] In addition, it was recently shown that injection of psilocin causes mice to rapidly alternate between shallow SWS episodes and short wake episodes.[50] These findings, taken together with the proposed role of the 5-HT system in arousal-promotion, may suggest that the suppression of PS induced by psychedelic drugs is due to an acute disruption of the core mechanism of global vigilance state control. However, it has also been noted that administration of psychedelic drugs results in disrupted and desynchronised

EEG,[50–53] indicating that it is not merely global state control which is affected by psychedelics but also state quality. Surprisingly, the possibility that psychedelic drugs cause the occurrence of an altered state of vigilance has received less attention. In this study, we report that the acute administration of the psychedelic compound 5-MeO-DMT to mice induces a profoundly altered state of vigilance which paradoxically expresses features of SWS during wakefulness.

Methods

Animal Husbandry

Adult male C57BL/6J mice (n = 15, 9 to 15 weeks old) were kept singly housed in individual Plexiglas cages (20.3cm x 32cm x 35cm) placed inside ventilated sound-attenuated Faraday chambers (Campden Instruments, Loughborough, UK), under a 12-12 hour light-dark cycle (9 am - 9 pm). The recording room was maintained at 22 ± 1 °C and 50 ± 20 % humidity. Food and water were provided *ad libitum* throughout the experiment. A subset of animals were not implanted and were used to study the effects of injection on running wheel activity (n=4) or feeding behaviour (n=4). All procedures were performed under a UK Home Office Project License and conformed to the Animals (Scientific Procedures) Act 1986.

Surgeries

Procedures were performed based on established protocols for device implantation in mice.[50,54] Prior to surgeries, mice were habituated to mash and jelly food, and housed in individually ventilated cages. Surgeries were performed under isoflurane anaesthesia (4 % induction, 1 – 2 % maintenance). EEG screws were implanted above the right frontal cortex (2 mm anteroposterior, 2 mm mediolateral), right occipital cortex (anteroposterior 3.5 mm, mediolateral 2.5 mm), and left cerebellum for reference (Figure 1A). In a subset of animals (n = 6) laminar probes (A1x16-3mm-100-703-Z16, NeuroNexus) were implanted, in addition to

EEG electrodes as above, in the primary visual cortex (-3.4 mm anteroposterior, -2 mediolateral) and referenced to the cerebellum screw. EMG wires were inserted into the left and right nuchal muscle. Dental acrylic (Super Bond, Prestige Dental, Bradford, UK) was used to fix the implanted electrodes to the skull and to protect the exposed wires (Simplex Rapid, Kemdent, Swindon, UK). Analgesics were administered immediately before surgery (5 mg/kg metacam and 0.1 mg/kg vetergesic, subcutaneous) and for at least three days following surgery (metacam, oral). Mice were kept in individual, ventilated cages and monitored at least twice a day until baseline levels of well-being were scored for three consecutive days. They were then moved to their home Plexiglass cages for a week of habituation.

Experimental Design

The first experiment aimed at investigating the effects of the compound on brain activity. In this experiment, after one day of baseline recording, the animals (n = 10) underwent a crossover design in which they received an injection of either vehicle (saline) or 5 mg/kg 5-MeO-DMT (1 mg/ml saline, Beckley Psytech) at random on day 1, followed by two days of recovery, then the other injection on day 4, after which they were allowed two further days of recovery (Figure 1B). Each solution was prepared fresh before the experiment and administered by intraperitoneal injection at light onset (ZT0). The volume of injected solution was identical between vehicle and the drug. 5-MeO-DMT was selected for its fast-acting and short-lasting properties as well as its relatively short half-life when compared to other psychedelics.[43,46] The concentration of 5-MeO-DMT was chosen based on previous reports of behavioural effects.[45,48] The implanted animals were then left undisturbed for the remainder of the day, and EEG, LFP and MUA recording was performed continuously.

Behavioural paradigm

In the 2nd experiment, to assess the effects of the compound on exploratory behaviour and feeding, animals (n = 4) received a bowl of 12 sugar pellets (Sucrose Tab/Fruit Punch 14MG, 5TUT - 1811324, TestDiet). On day 0, the bowl was introduced without prior habituation 10 minutes after dark onset (ZT12). On day 1, the animals received either a vehicle (saline) or a 5 mg/kg 5-MeO-DMT (1mg/ml saline, Beckley Psytech) injection at dark onset (ZT = 12) at random, followed by two days of recovery, then the other injection on day 4. The bowl of sugar pellets was placed on the cage floor 10 minutes after the injections. Video recording was acquired for one hour following the injection and analysed offline.

In the 3rd experiment, to investigate the effects of 5-MeO-DMT on behaviour, animals (n = 4) were given access to running wheels as an assay for locomotor activity. Installation of the setup and habituation was performed based on established protocols.[55] In a crossover design, the animals received either vehicle (saline) or a 5 mg/kg 5-MeO-DMT (1mg/ml saline, Beckley Psytech) injection at dark onset (ZT12) at random on day 1, followed by two days of recovery, then the other injection on day 4. The animals were placed in the running wheels 10 minutes following each injection and were left otherwise undisturbed to assess spontaneous activity. Video recording was acquired for one hour following the injection and analysed offline.

Data Acquisition

Electrophysiological signals were acquired using a multichannel neurophysiology recording system (Tucker-Davis Technologies Inc., Florida, USA). Signals were acquired and processed online using the software package Synapse (Tucker-Davis Technologies Inc., Florida, USA). All signals were amplified (PZ5 NeuroDigitizer preamplifier, Tucker-Davis Technologies Inc., Florida, USA), filtered online (0.1 – 128 Hz) and stored with a sampling rate of 305 Hz. Signals

were read into Matlab (Mathworks) and filtered with a zero-phase Chebyshev type filter (0.5 - 120 Hz for EEG, 10 – 45 Hz for EMG), then resampled at 256 Hz.

Vigilance states were scored manually by visual inspection of the signals (4 second epoch resolution) using the software SleepSign (Kissei Comtec, Nagano, Japan) (Figure 1C). Blinding was not possible, as 5-MeO-DMT led to prominent alterations of the EEG signals, as described below. During vehicle condition, wake was characterised by low amplitude irregular EEG signals alongside high EMG activity. Following a 5-MeO-DMT injection, wake was scored relying primarily on high EMG activity and visual observation of behaviour. SWS was readily identifiable by the presence of high amplitude EEG slow waves (0.5 Hz - 4 Hz) and low EMG amplitude. If SWS was interrupted by movement for less than five 4-second epochs, these epochs were scored as brief awakenings. PS periods were identifiable by a reduced slow wave activity, increased theta power (6 - 10 Hz), especially in the occipital derivation, and readily distinguishable from waking by low EMG levels and sleep-wake context, as an epoch of PS cannot occur immediately following an epoch of wakefulness (Figure 1C). We defined consolidated episodes of SWS as periods lasting longer than a minute and consolidated episodes of PS as episodes lasting longer than 30 seconds to accommodate for their respective transition periods.

Slow-wave detection

To investigate the incidence of local field potential (LFP), slow waves and corresponding neuronal activity, signals from one representative animal were analysed as previously.[56] The LFP signal was first bandpass filtered between 0.5 and 4 Hz (stopband edge frequencies 0.2–8 Hz) with MATLAB filtfilt function exploiting a Chebyshev Type II filter design,[10,54,57] and waves were detected as positive deflections of the filtered LFP signal between two consecutive negative deflections below the zero-crossing. Only LFP waves with a peak amplitude larger

than mean plus one standard deviation of the amplitude across all detected waves were included in subsequent analyses. Subsequently, all slow waves were aligned to their positive peak, and the corresponding average profile of neuronal spiking was computed.

Statistical analysis

Analyses were performed using Matlab. Normality was assessed using the Shapiro-Wilk normality test using the *swtest* function. Multiple paired t-tests were conducted using the *ttest* function if parametric conditions were fulfilled, followed by a calculation of Cohen's d using *computeCohen_d.* Otherwise, non-parametric Wilcoxon tests were conducted using the *ranksum* function. All tests were conducted with a $p < 0.05$ significance threshold. Following convention for power spectra analysis, no correction for multiple comparisons was applied. Results from the statistical analyses are reported in the supplementary tables.

Results

5-MeO-DMT delays paradoxical sleep onset but does not change daily sleep dynamics

The injection of 5-MeO-DMT induced behavioural changes characteristic for psychedelics, as manifested in the occurrence of head twitches (Supplementary Video 1, 01:53). It did not induce statistically significant changes in SWS onset ($t(6) = 2.07$, $p = 0.08$, $d = -0.78$, Supplementary Table 1) (Figure 2A). However, PS onset was significantly delayed by on average 44.1±10.3 minutes, with a large effect size ($t(6) = 3.81$, $p = 0.008$, $d = -1.44$, Supplementary Table 1) (Figure 2A). To further analyse the effects of the compound on sleep architecture, we quantified the total amount of time spent in each vigilance state during the 24h following the injections and found no significant differences (Figure 2B, Supplementary Table 2). We also quantified the amount of each state as well as the incidence of sleep interruptions

over the same period and did not find any notable differences between 5-MeO-DMT and vehicle conditions (Supplementary Figure 1, Supplementary Tables 3-7).

5-MeO-DMT acutely alters brain activity during waking

We then focussed on EEG signals, which are traditionally used for determining vigilance states. A striking observation was that following 5-MeO-DMT injection in all mice, EEG slow-wave activity normally characteristic of SWS was accompanied by EMG signals typical of the awake state (Figure 3A). Visual observations of the mice after injection of 5-MeO-DMT revealed that they were unequivocally awake demonstrating normal exploratory behaviour (Supplementary Video 1, 01:28; 01:40), grooming (Supplementary Video 1, short bouts: 01:31; 02:21; long bouts: 03:23) and walking (Supplementary Video 1; 04:45; 05:01), while brain activity in both derivations was characteristic of SWS (Figure 3A). Neither EEG nor EMG activity in subsequent SWS and PS were noticeably affected by the administration of 5-MeO-DMT as compared to saline controls (Figure 3A).

Plotting the EEG spectrograms following vehicle and 5-MeO-DMT revealed that marked effects of the compound on cortical activity lasted less than an hour in all cases (representative example: Figure 3B-C). Specifically, we observed that while all animals were awake after the injection of 5-MeO-DMT, theta-frequency activity was replaced by slow frequencies for approximately 45 min before returning to levels comparable to vehicle (Figure 3C). Further quantitative analyses determined that in the frontal derivation the injection of 5-MeO-DMT resulted in a significant increase in EEG slow wave activity (SWA) and in the frequencies between 12 – 14 Hz (Figure 4A, Supplementary Table 9). In the occipital derivation, there was a significant increase in SWA and a significant decrease in theta frequency power (Figure 4B, Supplementary Table 8).

The possibility remains that the slowing down of the EEG after 5-MeO-DMT merely reflects abnormal or artefactual brain signals, unrelated to physiological slow-wave activity, typical for sleep. To address this possibility, we carefully inspected the local field potentials (LFP) and corresponding multi-unit activity (MUA), which were recorded in a subset of animals in the primary visual cortex (V1). As well known, slow waves during physiological sleep are accompanied by the occurrence of OFF periods – generalised periods of synchronised neuronal silence, when the recorded populations of neurons do not emit action potentials, typically lasting 100-200 ms.[58,59] As expected, after vehicle condition, LFPs and MUA showed well known signatures of wakefulness and SWS, where the latter was characterised by frequent OFF periods during LFP slow waves (Figure 5A-B, Supplementary Video 2). In contrast, OFF periods were rare during PS (Figure 5C, Supplementary Video 2). 5-MeO-DMT injection resulted in the occurrence of prominent LFP slow waves during wakefulness, resembling those occurring in SWS, and invariably accompanied by neuronal OFF periods (Figure 5D, Supplementary Video 2). To further characterise the relationship between LFPs and neuronal activity during sleep and after 5-MeO DMT, we detected individual LFP slow waves in both conditions and calculated the corresponding average MUA. We observed that in both SWS (Figure 6A) and wake after 5-MeO-DMT (Figure 6B), the average LFP slow wave was accompanied by a strong suppression of MUA.

The effects of 5-MeO-DMT on brain activity are transient

The effects of 5-MeO-DMT on brain activity were short-lasting. One hour after injection, the differences between vehicle and 5-MeO-DMT condition in waking had dissipated except for the 3.25 Hz bin (Figure 4C-D). No significant differences were found in the frequencies higher than 30Hz, either immediately or an hour after the injection (Supplementary Figure 2).

EEG power during SWS in the first 2 hours following the injection of 5-MeO-DMT was characterised by a significant decrease in the frequencies between 6 and 20 Hz in the frontal and occipital derivations (Supplementary Figure 3A-B, Supplementary Table 9). These changes were no longer apparent in the period 2-4 hours post-injection in the frontal derivation, suggesting that the effects were short-lasting (Supplementary Figure 3C). Occipital derivation still showed a significant difference between the two signals 2-4 hours after the injection (Supplementary Figure 3D) which was no longer apparent afterwards.

Despite large inter-individual variability, sparse significant differences in EEG during PS were observed in the frontal derivation (Supplementary Figure 3E). A shift in the theta frequency range was also observed in the occipital derivation towards lower frequencies following 5-MeO-DMT injection (Supplementary Figure 3F) The strong inter-individual variation was no longer observed in the next 2 hours during which some significant differences can be noticed sparsely between the two conditions in both derivations (Supplementary Figure 3G-H, Supplementary Table 10). An analysis of higher frequencies showed only negligible differences in the EEG power for frequencies over 60 Hz (Supplementary Figure 3)

Effects of 5-MeO-DMT on behaviour

The classical approach to define states of vigilance is based on the electrophysiological criteria, such as the levels of EMG and spectral composition of EEG and LFP signals. While our data unequivocally demonstrate that 5-MeO-DMT induces a mixed state of vigilance, which we provisionally refer to as “paradoxical wakefulness”, the possibility remains that we merely induced an abnormal state, characterised by behavioural deficits or locomotor abnormalities, such as those typical for “serotonin syndrome” (SS).[60] To further address this, we performed two additional experiments in unimplanted mice, which were injected with the same dose of the drug or vehicle and were provided access to the running wheel or given a sugar pellet.

These experiments were performed at dark onset, when laboratory mice are typically awake spontaneously, and would be expected to display normal, species-specific behaviours. We hypothesized that these interventions would reveal abnormalities in locomotor, sensory and motivational aspects of wakefulness, if they were induced by 5-MeO-DMT.

Firstly, in this additional cohort of mice, our visual observations confirmed that as in implanted animals (see above), the behaviour of the animals was entirely normal for the entire period of observation after the injection, corresponding to the persistence of "paradoxical wakefulness". Specifically, we never observed hyperactivity, flat body posture, cataplexy, hind limb abduction or body tremors.

Typically, mice stayed awake after injection, and during this time their behaviour was dominated by exploratory behaviour, grooming and nesting. All these were invariably observed in all animals, including both controls and 5-MeO-DMT injected animals. However, we noticed that the 5-MeO-DMT-injected animals showed less interaction with their environment than the vehicle group, and exhibited somewhat altered behaviour, characterised by repeated exploration of the bedding immediately under the animal using their forelimbs (Supplementary Video 3-4).

To characterise the sensory and motivational component of wakefulness, we presented the animals with a plastic cup of sugar pellets 10 minutes after the injection, corresponding to the time when the effects of the 5-MeO-DMT on brain activity have fully developed. We observed that invariably the vehicle group approached and explored or interacted with the cup and its content within less than a minute after it was presented, retrieving the first pellet on average after 0.20±0.08 minutes (12.3±4.7 seconds, individual animals = 0.02 minutes, 0.22 minutes, 0.18 minutes, 0.40 minutes, Supplementary Video 3). In contrast, after an injection of 5-MeO-DMT, the animals took markedly longer (21.16±7.2 minutes, individual animals = 4.8 minutes, 37.4 minutes, 24.8 minutes, 14.6 minutes) before they started exploring the cup. The

animals only engaged with the cup and attempted to eat the pellets well after 30 min of observation (Supplementary Video 3).

Finally, we set out to address whether 5-MeO-DMT affects locomotor activity. To this end, we provided animals with running wheels, which mice are well known to use spontaneously.[55] For both the vehicle and the 5-MeO-DMT condition, no interaction with the wheel was observed within the first 5-10 minutes after the injection as in both conditions the behaviour of the animals was dominated by grooming. When placed on the running wheel, the animals did not manifest any notable differences in body posture between the conditions. The animals either stayed on the wheel with no attempt at running, ran, or got off the wheel (Supplementary Video 4). In the following time interval, both groups showed repeated spontaneous climbing and running with no noticeable differences in this behaviour. However, a notable difference was found in the total amount of running wheel activity, where vehicle injected animals completed 310.7±179.8 full wheel revolutions over the 30-minute period (individual animals: 1.9, 203.7, 830.6 and 206.7 revolutions respectively), at an average speed of 23.5±8.3 revolutions per minute (rpm), while only 67.0±37.6 revolutions (individual values: 179.5, 20.8, 34.2 and 33.4) were performed by 5-MeO-DMT injected mice within the 30 min period, corresponding to average running speed of 11.03±4.4 rpm (Supplementary Video 4).

Discussion

The results presented in this paper report the acute effects of 5-MeO-DMT injection on vigilance states, spontaneous behaviour and brain activity in freely moving mice. 5-MeO-DMT induced a prominent change in EEG and LFP during wakefulness, as reflected in the occurrence of sleep-like slow waves associated with neuronal OFF-periods, while theta-frequency activity was markedly suppressed. Despite sleep-like patterns of brain activity, the behaviour of the animal was typical for wakefulness, as reflected in the occurrence of grooming, exploring and

running, and no abnormalities in body posture were noted. The effects were short-lasting, and dissipated within an hour, consistent with the kinetics of 5-MeO DMT.

Paradoxical Wakefulness

The highly unusual features of the state induced by the psychedelic 5-MeO DMT in mice lead us to dub it "paradoxical wakefulness" (PW), as it was a hybrid state, precisely opposite to the characteristics of paradoxical sleep (PS). Specifically, while in PS high brain activity dominated by theta-rhythm is accompanied with low EMG tone, PW was instead accompanied with SWS-like cortical activity in actively moving mice, with elevated EMG activity. Furthermore, theta-frequency activity was profoundly suppressed, while combined LFP and MUA recordings during PW revealed an occurrence of OFF-periods typical for slow waves during SWS (Figure 6, Supplementary Video 2).

While the relationship between SWS, PS and PW remains to be clarified, we speculate that the occurrence of PW may contribute to the delay in PS typically observed after administration of psychedelics such as LSD, psilocybin and psilocin.[49,50] One possibility is that PW alters the homeostatic mechanism responsible for the regulation of sleep/wake cycle, diminishing the propensity for PS.[61,62] While it is well recognised that wakefulness is a highly dynamic and heterogenous state, often having subtle features of SWS, for example during quiet state or after sleep deprivation, the state we refer to as PW is an unusually extreme case of state dissociation, akin to parasomnias.[63] The occurrence of slow-wave activity during normal waking behaviour has been reported before, as well as periodic alternation of distinct sub-states of wakefulness in conjunction with spontaneous locomotor activity in mice.[7,64] Our current observations further highlight an underappreciated capacity to manifest hybrid states, such as PW, although whether PW can occur spontaneously and under what conditions, remains to be determined. At any rate, the existence of such a paradoxical brain state during wake supports

the notion that SWA, thought of as a key defining feature and a hallmark of SWS in human and rodent sleep research, is not a feature exclusive to sleep, and its causal link with states of consciousness, sleep depth or sleep functions must be reconsidered.[12,65,66]

5-MeO-DMT profoundly alters electrophysiological markers of vigilance states

As PW was associated with markedly increased SWA, it remains to be investigated whether it is homeostatically regulated or contributes to the dissipation of sleep pressure, given the well-recognised notion that SWA during SWS reflects preceding sleep-wake history.[10,67] Likewise, it remains unknown whether the network oscillatory activity during PW arises from the same cellular and network mechanism as the slow oscillation during sleep.[10,64,65]

We observed the profound suppression of theta activity in the occipital derivation during active wakefulness, when it is typically present in exploring animals. Theta activity is mediated through the hippocampus where rhythmical activity has been linked to waking behaviour, notably spatial and temporal processing.[68–71] Intriguingly, human psychedelic experience is associated with altered processing of time and space.[33] We therefore hypothesise that suppression of theta activity during PW would be associated with a disruption of the processing of spatial and temporal information.

Studies in rodents suggest psychedelic drugs such as LSD alter the firing rates of hippocampal neurons.[72] Taken together, the alteration of EEG activity pattern observed here after injection of 5-MeO-DMT could be due to reorganisation within neuronal networks. Cortical activity patterns are tuned by changing concentrations of a wide range of neuromodulators, such as acetylcholine, 5-HT, and norepinephrine.[73–75] Whether the state described here is accompanied by altered levels of other wake- or arousal-promoting neuromodulators remains to be determined.

Changes in sleep architecture

We did not observe significant changes in SWS stability, contrary to other studies addressing the effects of psychedelics.[50] We hypothesise this could be due to the short-lasting effects of 5-MeO-DMT. While PS is delayed by the injection of 5-MeO-DMT, the rebound shown in supplementary Figure 1C several hours later is likely due to PS homeostasis [61,62].[74] We did not find any difference in the total time spent in PS 24 hours after the injection of 5-MeO-DMT. As psychedelics are reported to have enduring effects on brain plasticity and mood effects, it would be interesting to analyse whether these are accompanied by changes in the sleep/wake cycle in the days or weeks following the injection, and whether longer-acting psychedelics, such as LSD and short-acting compounds, such as 5-MeO-DMT, differ in this respect. Furthermore, it is important to optimise the route of delivery of the drug, as an acute intraperitoneal injection may be stressful and alter both the immediate and delayed effects of the compounds.

Changes in behaviour

We observed that following the injection of 5-MeO-DMT, mice started expressing behaviour typically associated with potent psychedelic drugs such as head twitches (Supplementary Video 1, 01:53). The injection of high doses of drugs capable of potentiating the 5-HT system is typically associated with a so-called serotonin syndrome (SS), manifested in a wide range of abnormal behavioural and physiological responses.[60] In rodents, SS consists mostly of hind limb abduction and head weaving, but also in rare cases backward walking, straub tail, tremors, low body posture, hyperactivity and decrease in body temperature.[60] We did observe backward walking (Supplementary Video 1, 01:11) and some other subtle behavioural alterations, yet by and large the behaviour of the animals after 5-MeO-DMT was normal. Specifically, our experiments with running wheel activity did not reveal abnormalities in locomotor activity, and

we never observed hind limb abduction or flat body posture (Supplementary Video 3). In contrast, we observed the typical repertoire of normal waking behaviours, such as walking, grooming or exploration (Supplementary Video 1-2), which are typically associated with fast cortical activity and hippocampal theta oscillations.[69]

Conclusion

In conclusion, we found that the injection of 5-MeO-DMT induced a hybrid state of vigilance showing unequivocal features of wakefulness, such as active behaviour and high muscle tone, while brain activity was typical of slow wave sleep. This paradoxical association of wake and sleep characteristics mirroring the one observed during paradoxical sleep supports the notion that wake and sleep are not uniform, mutually exclusive phenomena. We hypothesise that PW represents a rodent equivalent of an altered state of consciousness typical of a psychedelic experience in humans.

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Figures

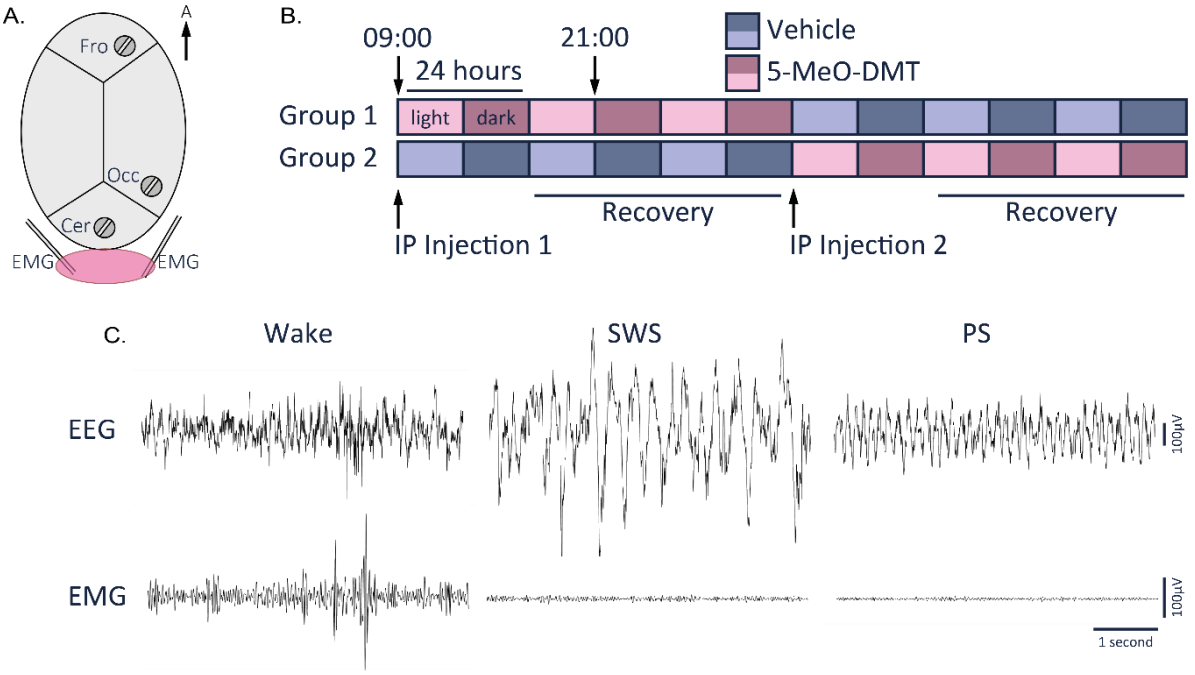

Figure 1. Experimental protocol

A. Drawing of a skull of a mouse viewed from the top. The arrow points toward the anterior of the animal. Each screw is represented by a barred circle in the frontal (Fro) and occipital (Occ) part of the skull and above the cerebellum (Cer). The nuchal muscles are represented in pink. **B.** Diagram of the experimental plan. The colour on each group line represents the substance animals from that group received on the injection day. **C.** Raw EEG and EMG traces of one animal representative of wakefulness, SWS and PS.

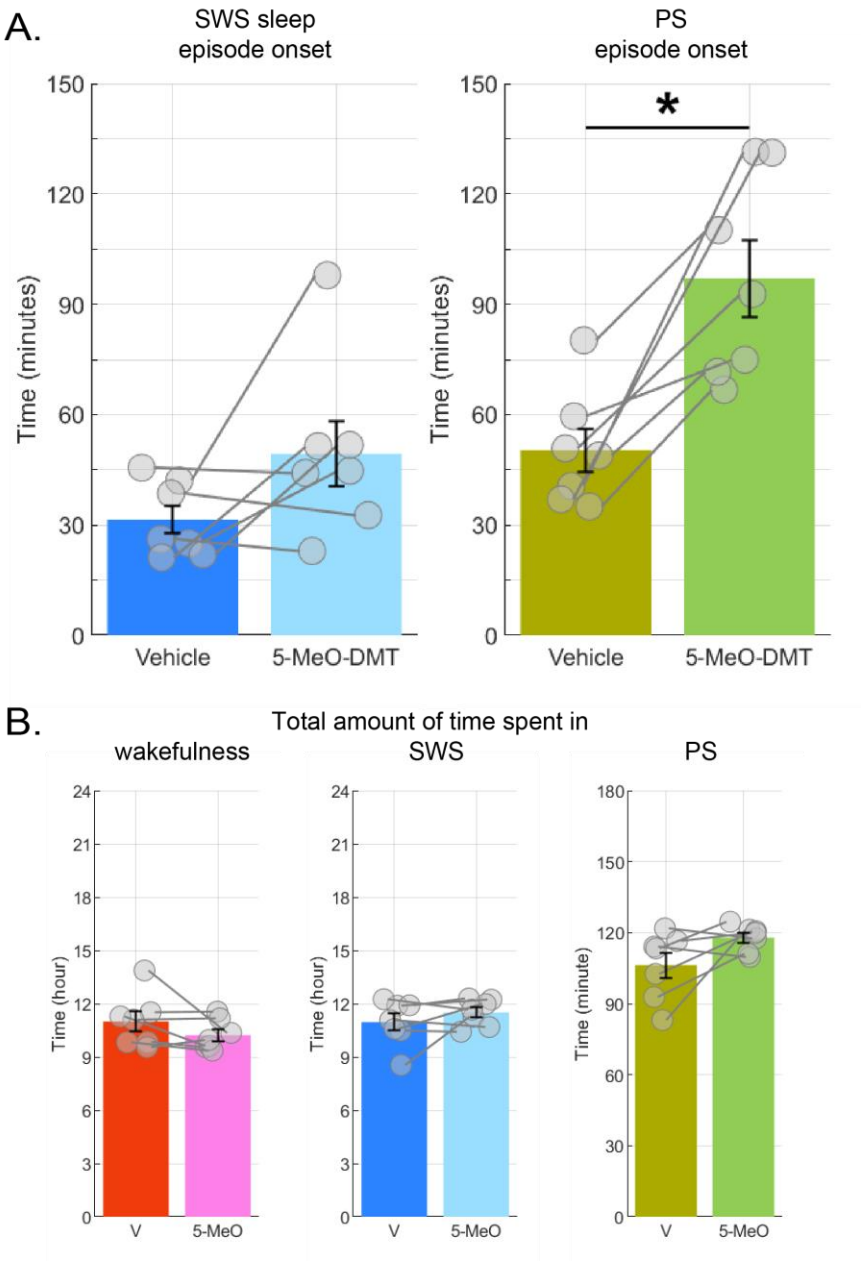

Figure 2. Changes in global sleep architecture.

A. Mean onset of first consolidated SWS and PS episode with SEM for the Vehicle and 5-MeO-DMT condition. A circle represents a single animal, a line connects its values in the Vehicle and 5-MeO-DMT condition. The star denotes significant results ($t(6) = 3.81$, $p = 0.008$, $d = -1.44$) **B.** Mean and SEM of the total amount of time spent in Wakefulness, SWS, or PS. A circle represents a single animal, a line connects its values from the Vehicle to the 5-MeO-DMT condition.

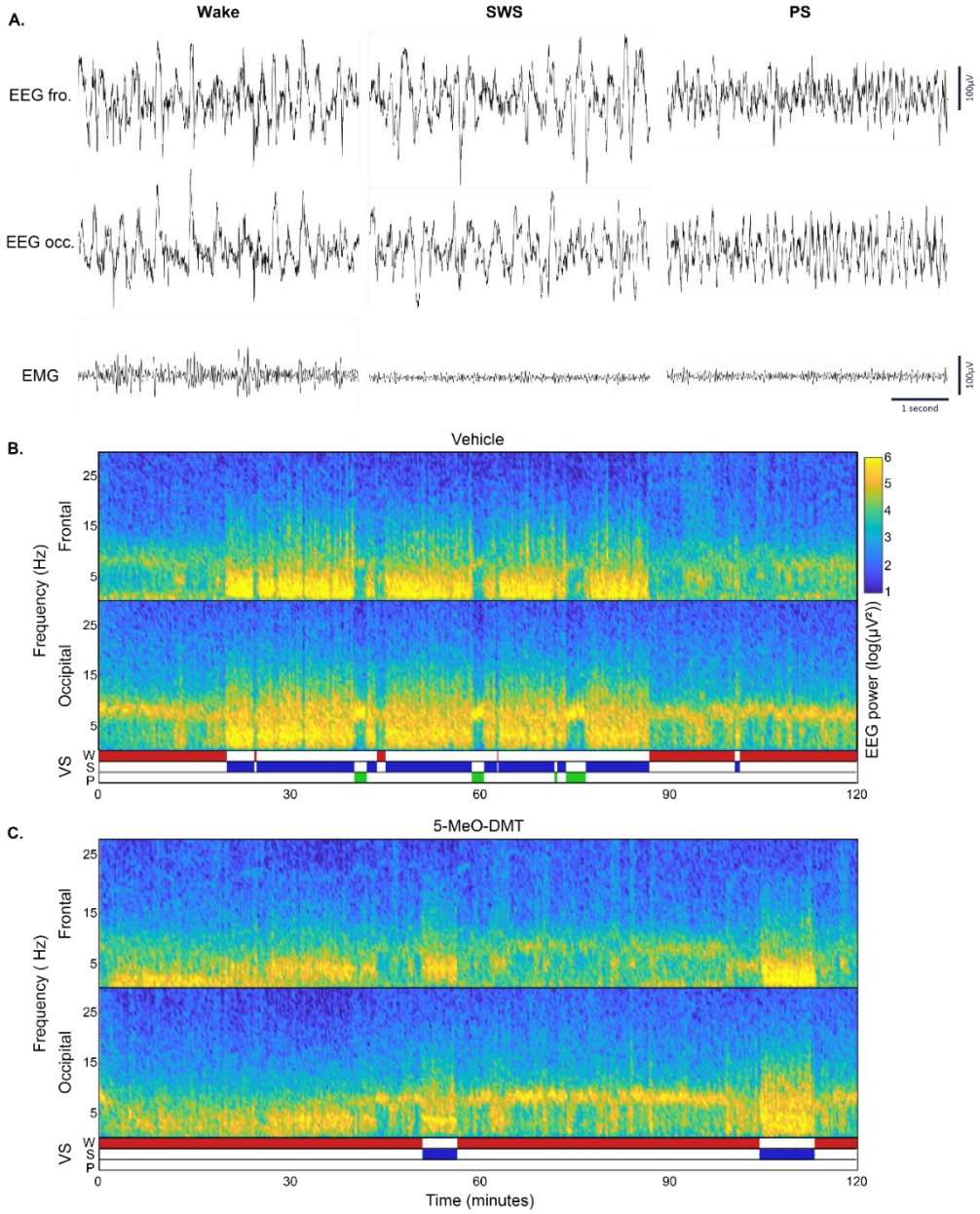

Figure 3. Raw traces and power spectra.

A. Representative raw traces from the EEG frontal (fro.), occipital (occ.) and EMG derivations following a 5-MeO-DMT injection for wakefulness, sleep and PS over 5 seconds. **B.** Representative power spectra of one animal of the frontal and occipital derivation, with the vigilance states (VR, Wakefulness: W, SWS: S, PS: P) for 120 minutes following a vehicle injection. **C.** Representative power spectra of one animal of the frontal and occipital derivation, with the vigilance states (VR, Wakefulness: W, SWS: S, PS: P) for 120 minutes following a 5-MeO-DMT injection.

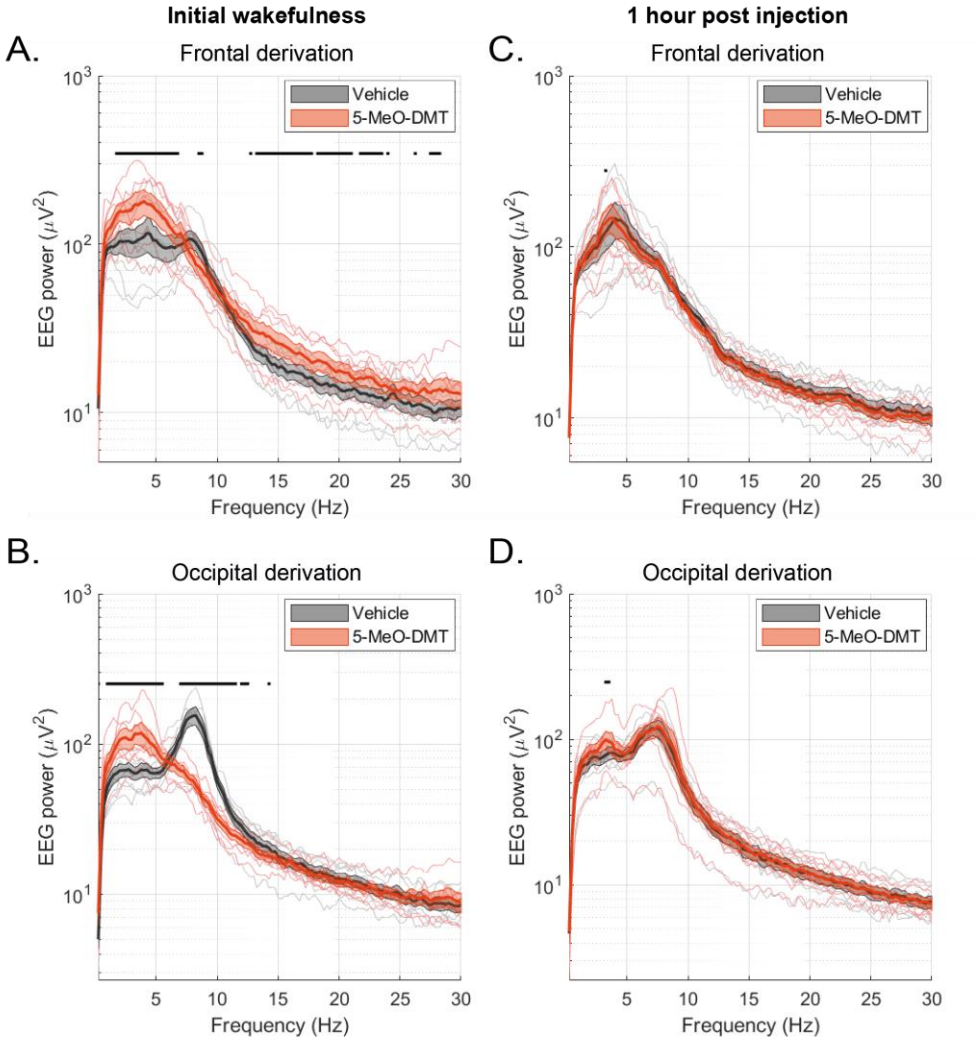

Figure 4. Absolute EEG power during wakefulness.

See Supplementary Table 9 for the statistical analyses. **A-B.** Mean and SEM of the EEG power immediately after a 5-MeO-DMT injection (coloured) or a Vehicle injection (black) during the initial episode wakefulness in the frontal (A.) and occipital (B.) Derivation. The horizontal lines denote a significant result at the given frequency bins. Each thin coloured line represents one animal. **C-D.** Mean and SEM of the EEG power 1 hour after a 5-MeO-DMT injection (coloured) or a Vehicle injection (black) during wakefulness in the frontal (C.) and occipital (D.) Derivation. The horizontal lines denote a significant result at the given frequency bins. Each thin coloured line represents one animal.

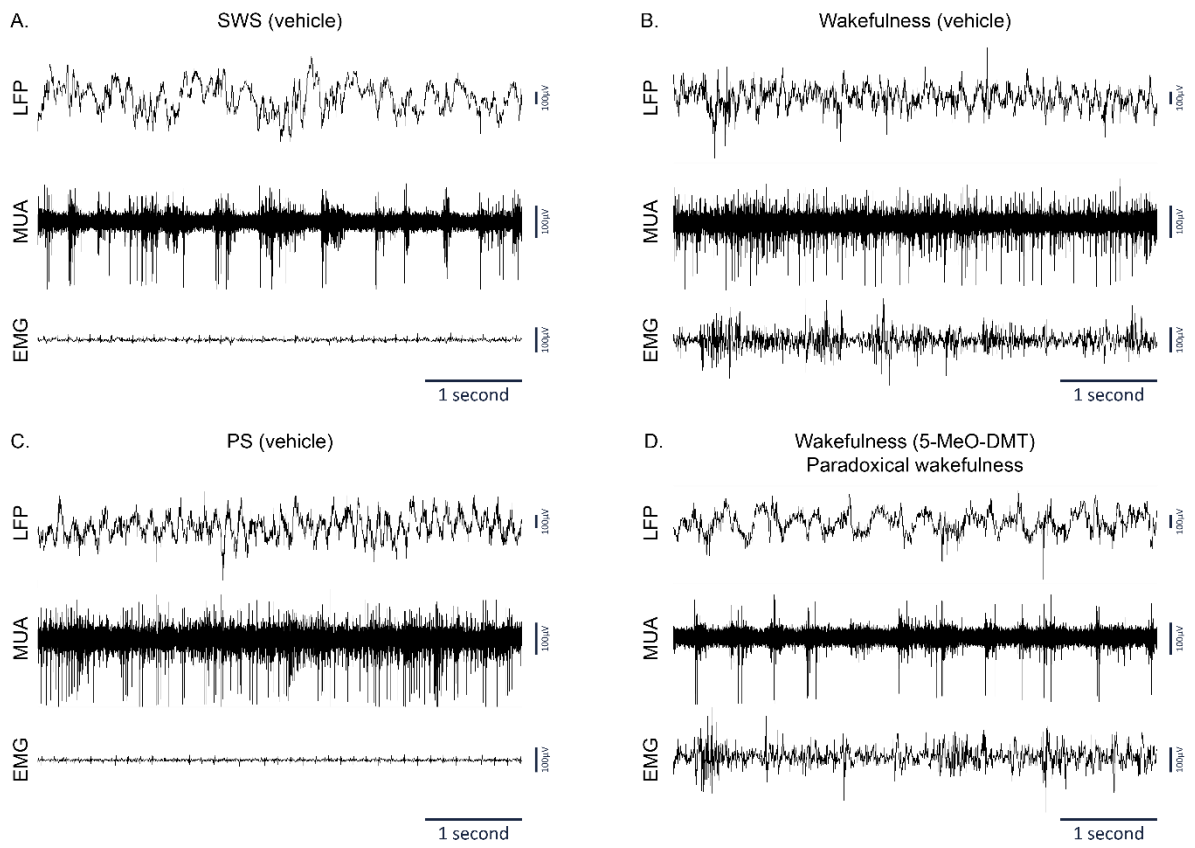

Figure 5. Intracortical activity during all vigilance states

A. Representative LFP and corresponding MUA in the primary visual cortex and nuchal EMG during SWS following a vehicle injection over 5 seconds. **B.** Representative LFP and corresponding multi-unit activity (MUA) in the primary visual cortex and nuchal EMG 5 minutes after a vehicle injection over 5 seconds. **C.** Representative LFP and corresponding MUA in the primary visual cortex and nuchal EMG during PS following a 5-MeO-DMT injection over 5 seconds. **D.** Representative LFP and corresponding MUA in the primary visual cortex and nuchal EMG 5 minutes after a 5-MeO-DMT injection over 5 seconds.

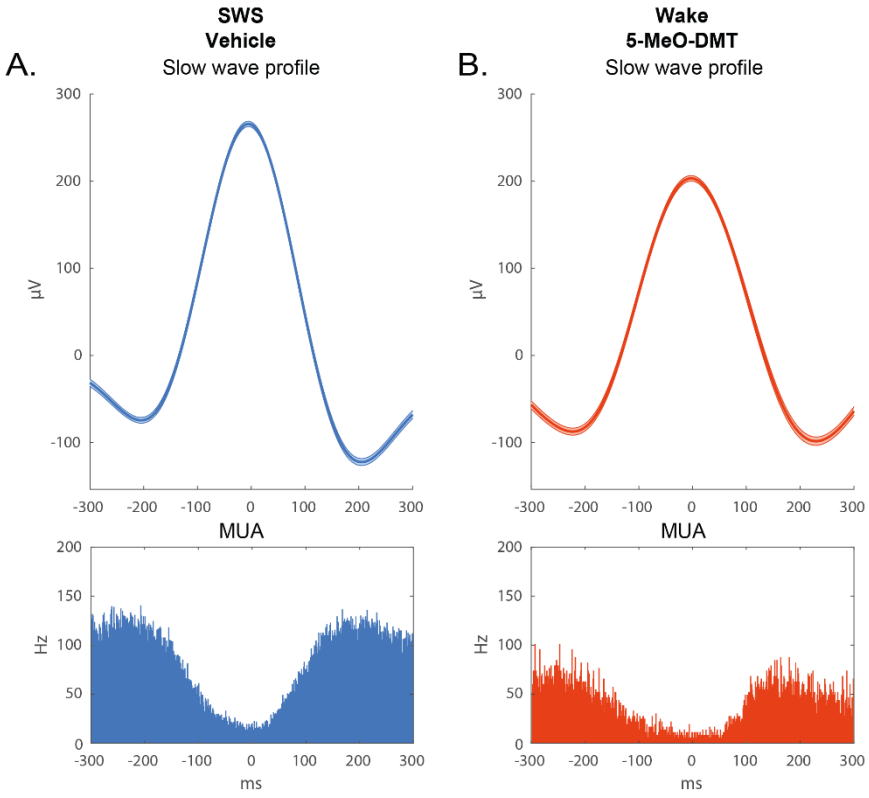

Figure 6. Slow wave and corresponding MUA

A. Average slow wave and SEM (top) and corresponding MUAs (bottom) for automatically detected slow waves occurring during 30 minutes of SWS happening after a vehicle injection.

B. Average slow wave and SEM (top) and corresponding MUAs (bottom) for automatically detected slow waves happening during all episodes of wake within 30 minutes after an injection of 5-MeO-DMT.