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Slow wave sleep deficits in the Flinders Sensitive Line rodent model of depression: effects of medial forebrain bundle deep-brain stimulation

Running title: Slow wave sleep in rodent depression model

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Introduction

Major Depressive Disorder (MDD, depression) is a common and complex mental disorder comprising many heterogeneous aspects, with inter-patient variation in onset, symptoms and treatment response. Depression is characterised by low mood, reduced motivation and diminished feelings of interest and pleasure, but encompasses a variety of other behavioural and physiological symptoms, notably sleep disturbance (Argyropoulos & Wilson, 2005; Bentley et al., 2014; Kennedy, 2008; Riemann et al., 2001). Sleep and depression share a strong, likely bi-directional relationship. Sleep deficits, manifesting primarily as insomnia, affect over 80% of patients (Yates et al., 2007). Sleep disturbance strongly affects quality of life for depressed patients and is a predictor of suicide risk (Ağargün et al., 1997; Mayers et al., 2003). Patients suffer worse subjective sleep quality alongside physiological changes to the structure of sleep (sleep architecture) and underlying processes; these include a reduction of SWS and a disinhibition of REM sleep, manifesting as reduced latency to REM sleep, prolongation of early REM periods and increased density of eye movements (Baglioni et al., 2016; Nutt et al., 2008; Riemann et al., 2001). These problems are often prodromal relative to other symptoms, and are commonly residual after treatment, a factor which is associated with the occurrence of future episodes (Baglioni et al., 2011; Fang et al., 2019; McClintock et al., 2011). Sleep is therefore a vital consideration in terms of experimental treatments for severe depression. Whereas almost all antidepressant drugs suppress REM sleep (Riemann et al., 2001; Wichniak et al., 2017), some of them actually worsen sleep (Argyropoulos and Wilson 2005). Given the role of sleep disruption in predicting relapse (Tranter et al., 2002), there is a strong argument to emphasise the importance of sleep restoration in the treatment of MDD.

A significant minority of patients – estimated up to 30% (Rush et al., 2006) — do not respond to conventional treatments. For this treatment resistant depression (TRD), experimental therapies are being developed including deep brain stimulation (DBS), by which electrical current is delivered through surgically implanted electrodes to selected brain targets (Mayberg et al., 2005; Schlaepfer et al., 2013). One such target for DBS is the medial forebrain bundle (MFB; capital letters are used here to refer to the human MFB, and lower case used for the rodent mfb), a highly connected fibre tract running between midbrain and forebrain structures, and containing, among others, dopaminergic, serotonergic and glutamatergic fibres (Coenen et al., 2011, 2012; Coenen, Schumacher, et al., 2018; Döbrösy et al., 2021). Ascending components from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and pre-frontal cortex (PFC) have been implicated in motivation and reward orientated behaviours. DBS of the superolateral branch of the MFB (slMFB, that in humans encompasses the ascending VTA projections) has been the subject of promising clinical trials in TRD patients (Bewernick, Kayser, Gippert, Coenen, et al., 2017; Bewernick, Kayser, Gippert, Switala, et al., 2017; Coenen et al., 2019; Coenen, Sajonz, et al., 2018; Fenoy et al., 2016, 2018; Schlaepfer

et al., 2013). The MFB network contains neural systems implicated in sleep, and while there is some anecdotal evidence of improved perception of sleep quality after sMFB-DBS (private correspondence, FORSEE study team), no specific investigation into the effects of MFB-DBS on sleep has been conducted.

The Flinders Sensitive Line (FSL) rat is a model of genetic predisposition to depression, exhibiting spontaneous and stress-sensitive depressive-like phenotypes (Edemann-Callesen et al., 2015; Friedman et al., 2008; Overstreet & Wegener, 2013; Thiele et al., 2016; Voget et al., 2015), including decreased latency to REM sleep (Benca et al., 1996; Shiromani et al., 1988) which suggests a suitable model for sleep-related symptoms in depression. In the FSL, mfb-DBS has been shown to have anti-depressant-like effects on behaviour (Edemann-Callesen et al., 2015; Thiele et al., 2018) and physiology (Ashouri Vajari et al., 2020). The current experiments aimed to better characterise the baseline sleep phenotype of the FSL, and investigate the effects of mfb-DBS upon sleep-related parameters. Electrophysiological recordings of the NAc, pre-limbic cortex (PrL) of the PFC, and dorsal CA1 hippocampus (CA1) were made in addition to standard ECoG/EMG recordings during sleep, before and after 24-hours of mfb-DBS. Behavioural phenotype was measured via the forced swim test (FST), in order to confirm the anti-depressant action of mfb-DBS. Previously reported REM disinhibition was replicated, while FSL rats also displayed circadian-related alterations to SWS architecture and abnormal physiology including reduced activity in the delta band, elevated gamma activity, modifications of hippocampal ripple features and spindle activity. mfb-DBS had an anti-depressant effect on behaviour in the FST and suppressed elevated gamma oscillations during SWS, but had no notable effect on sleep architecture and other oscillatory activities.

Experimental procedures

Animals and experimental design

Male FSL rats were sourced from the breeding colony maintained at the University Hospital Freiburg. Non-depressive controls were age- and sex-matched Sprague Dawley rats (Ctrl), sourced from Janvier Labs (France) (Overstreet, 2012). All animals were single housed from the start of the experimental timeline and kept under a 12h/12h light-dark cycle (Zeitgeber Time (ZT) 0 corresponding to lights-on and ZT12 to lights-off), in a temperature ($21\pm 2^{\circ}\text{C}$) and humidity ($50\pm 5\%$) controlled environment with food and drink ad libitum. The experimental timeline, showing the groups compared in analyses, is summarised in **Fig. 1**.

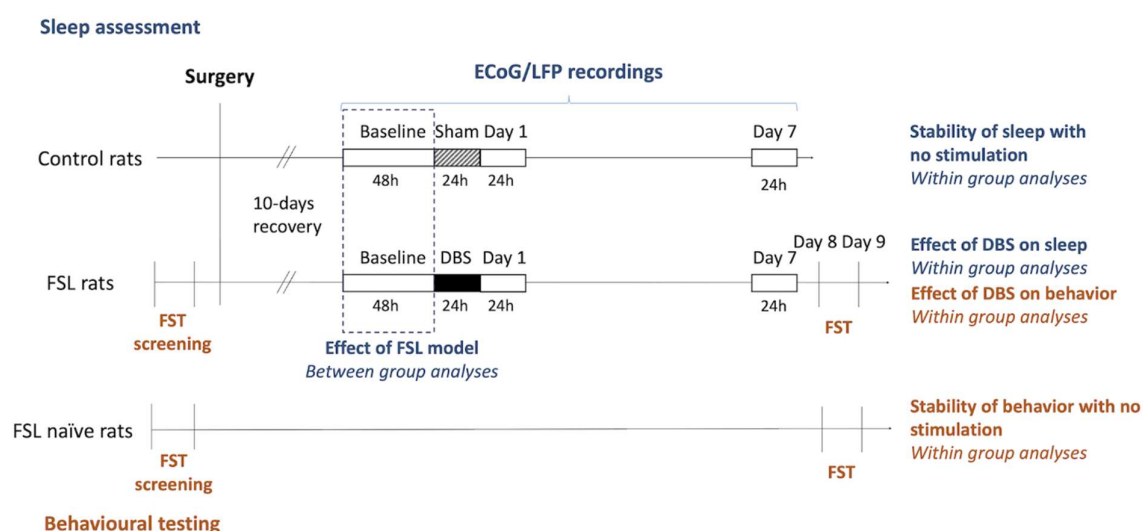


Fig. 1. Experimental timeline. FSL = Flinders Sensitive Line rat. DBS = Deep-brain stimulation.

For sleep assessment, FSL rats ($n = 6$) and control animals ($n = 5$) aged 12 weeks (350-450g) underwent surgical implantation for ECoG/LFP recordings (see below). FSL rats were implanted with mfb-DBS electrodes whereas control rats received only recording electrodes. The impact of FSL model on sleep architecture and quality was assessed comparing 48h-baseline recordings between FSL and control animals. In FSL rats, ECoG/LFP recordings were also performed one and seven days after mfb-DBS to evaluate the influence of the latter on sleep architecture and quality, in comparison with baseline recording. Control animals underwent the same sleep assessments during Day 1 and 7, without mfb-DBS, in order to evaluate the stability of sleep measurements over the time course of the experiment. In FSL animals, depressive behaviour was also analysed: a forced swim test (FST; see below) was performed before the age of 12 weeks in order to screen for spontaneous depressive-like phenotype and repeated 9 days after mfb-DBS in order to evaluate the behavioural impact of the latter. In parallel, naïve FSL rats ($n = 6$) underwent both FST evaluations (30 days apart)

in the absence of mfb-DBS to control for the stability of depressive like behaviour in this model. At the end of the experiments, animals were sacrificed and brain tissue collected for the verification of electrode placement.

The project had the approval of the veterinary board for research in animals of the University of Strasbourg (authorisation APAFIS#12113) and was carried out in accordance with the EU Directive 2010/63/EU concerning the protection of animals used for scientific purposes.

Forced Swim Test

Behavioural phenotype was measured using the FST, as previously described (Thiele et al., 2020). Briefly, animals were placed in a perspex cylinder of water (21-23°C), in which the tail did not touch the bottom nor could they escape. On day 1 of the test, the animals underwent a 15-minute period of swimming as habituation, and the following day were tested over 7 minutes. The test session was recorded from the side and manually scored by an experimenter blind to the treatment, with the amount of time spent immobile (3 of 4 limbs immobile, with no swimming or struggling) expressed as a percentage of total time.

Surgery

Surgery was performed in FSL and control rats under isoflurane anaesthesia, delivered in O₂ (Air liquide, oxygène aviation, 99.5% purity). Anaesthesia was induced with isoflurane set at 4% and maintained at 1.5% for the duration of surgery. Before incision, animals received subcutaneous injection of lidocaine (Lurocaïne, Xylovet 1%, 2 mg/kg) at the incision site, and the eyes were covered with ophthalmic gel (Lubrital) to prevent drying of the cornea. Stereotactic coordinates were taken from bregma with the skull fixed in the flat head position, with dorsal-ventral measurements taken from the level of the dura. Bipolar electrodes for DBS (constructed from 90% platinum, 10% Iridium, teflon-coated wire with 120µm diameter, World Precision Instruments, USA) were bilaterally inserted into the mfb (AP -2.7, ML ±1.7, DV -8.0). Pairs of monopolar electrodes (constructed from Tungsten wire, teflon-coated with 60µm diameter, World Precision Instruments, USA) for the recording of LFP signal were inserted into the PrL (AP +2.8, ML ±0.7, DV -3.0), NAc (AP +1.0, ML ±1.4, DV -7.1) and CA1 (AP -3.8, ML ±3.0, DV -2.0). Before insertion the tip of each electrode was coated with a fluorescent dye (Dil, Molecular Probes, Inc.) to facilitate verification of electrode placement. Two stainless steel screws (1.2mm) were inserted to the level of the dura over the frontal region and cerebellum, in order to record ECoG signal. A final screw was inserted into the skull to act as electrophysiological ground. Finally, an electrode (constructed from Tungsten wire, teflon-coated with 60µm diameter, World Precision Instruments, USA) was inserted into the nuchal muscle for the recording of electromyogram (EMG). Electrodes were connected to a connection pedestal (PlasticsOne/P1 Technologies, USA) and fixed to the skull with

Superbond (C&B) glue, and then with dental cement (Paladur). Following surgery rats received an injection of Metacam (1 mg/kg s.c.; Meloxicam, Boehringer Ingelheim).

Electrophysiological Recording

After 7 days recovery from surgery, animals were connected via flexible cable to a rotating joint (PlasticsOne/P1 Technologies, USA), allowing unrestricted movement in the home cage and incorporating both stimulation and recording channels. Before recording, all animals were habituated to the cable over 72h. Electrophysiological signals were amplified, sampled at 1kHz and digitised via LabChart software (AD Instruments, New Zealand), over 24h for each recording session, with data stored for offline analysis. Rats were continuously filmed throughout each session. 48h baseline recording was immediately followed by 24h mfb-DBS. Two further 24h periods were recorded at 1 and 7 days post-DBS. One FSL rat was excluded from post-DBS analysis after histological assessment (see below) revealed a misplaced DBS electrode.

Deep Brain Stimulation

mfb-DBS parameters, previously established as safe for chronic use in the rodent (Furlanetti et al., 2015; Thiele et al., 2018, 2020), were selected in order to model clinical application. DBS was applied continuously over 24 hours via a pulse generator (A-M Systems, USA) providing constant current stimulation, biphasic square wave with a 90 μ s pulse width at 130Hz. Prior to stimulation, the current in each electrode was individually titrated. Titration – starting at 50 μ A increasing step-wise by 50 μ A to a maximum current of 350 μ A – was initiated with the animal in a state of quiet rest, and current increased until a distinct change towards an alert state characterised by explorative, “SEEKING” behaviour was observed (Panksepp & Watt, 2011), in the absence of side effects such as dystonic movements or rotational behaviour, as per previously established procedure (Thiele et al., 2020). The titrated current was maintained for the duration of the DBS period.

Histological assessment

Animals were terminally anaesthetised with the administration of sodium pentobarbital (Dolethal, 100 mg/kg, i.p.) and transcardially perfused with 4% PFA solution. Following a 2h period of post-fixation immersion within 4% PFA, brains were suspended in a 20% sucrose solution for 48h before being frozen. 40 μ m sections were cut using a cryostat and mounted on slides in the presence of DAPI for fluorescent imaging. Electrode placement was verified visually under a fluorescence microscope. Electrodes judged to be placed outside of the target structure were excluded from relevant analyses; a summary of the positions of electrodes included in analysis is shown in **Fig. 2**.

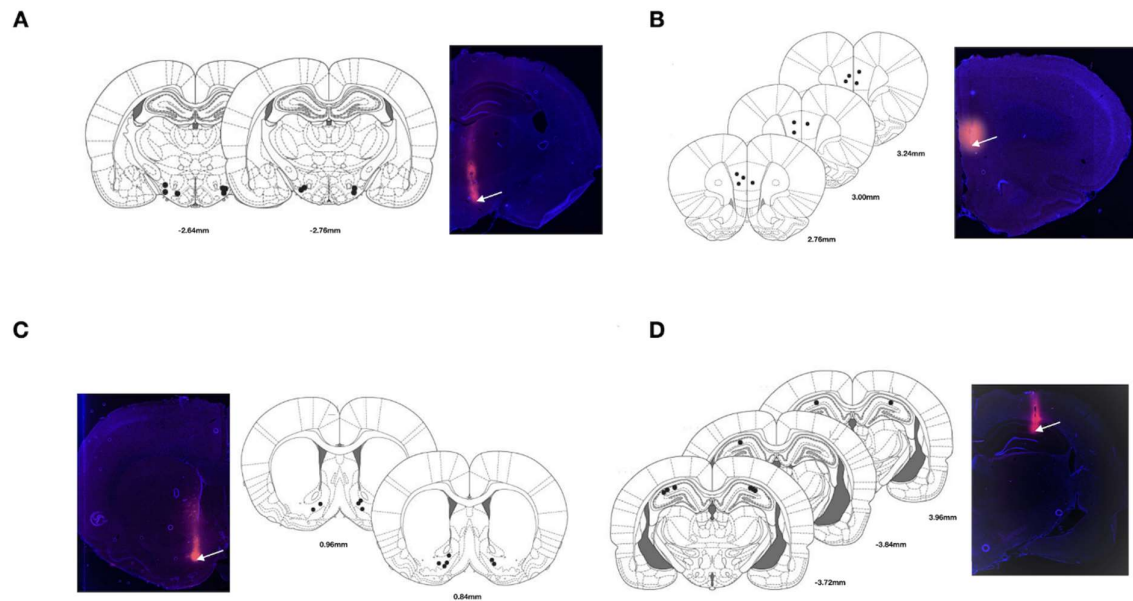


Fig. 2. Electrode placement. Positions of electrodes included in the study indicated on schematic illustration, next to example histological slide marked with fluorescent dye Dil in the (A) MFB, (B) PrL, (C) NAc and (D) CA1. White arrow indicates approximate location of electrode tip. Images adapted from Paxinos and Watson 7th edition, 2007.

Sleep Scoring

Analysis of time spent in vigilance states was performed using ProFusion software (Compumedics Ltd, Australia). ECoG and EMG signal were divided into 10s epochs, and each epoch manually scored as “wake”, “SWS” or “REM sleep”. “Wake” state was identified by typically desynchronised ECoG activity of high frequency, with concurrent EMG activity; “SWS” by characteristic slow waves of low-frequency, high-amplitude ECoG activity and low EMG; and “REM” sleep by the presence of characteristic theta waves and minimal EMG activity. Video recordings were used to verify any case of doubt. Sleep architecture measurements of each vigilance state’s duration, episode number and mean length were averaged over 3h periods. For each vigilance state, light-dark amplitude, an assessment of a circadian influence on sleep-wake behaviour, was calculated as its total duration during the light cycle – during the dark cycle.

Spectral Analysis

Signal from ECoG and deep electrodes were imported into MATLAB (Mathworks, USA) for spectral analysis using the Chronux (Bokil et al., 2010) and MATLAB Signal Processing (Mathworks, USA) toolboxes. Signals were delineated into episodes of vigilance states described above. Before processing, the data were cleaned by removing artefacts (values exceeding 3 SD from the mean, together with a 1-s window around selected artefacts). Episodes of each vigilance state during hour-long periods were concatenated, bandpass

filtered between 0.5 and 250Hz and transformed using a multitaper method (time-bandwidth product of 3, using 5 Slepian tapers with a 2s window moving at 0.1s) implemented via Chronux. Spectrograms were inspected, and spectral power averaged over classical pre-defined frequency bands (delta 0.5-4Hz, theta 5-10Hz, gamma 30-90Hz), normalised to average power over a range of frequencies (1-250Hz) and presented according to 3h ZT periods. Hippocampal ripples (100-200 Hz) were analyzed during SWS. Thresholds for beginning/end and peak of ripples were respectively 2 and 5 standard deviations, minimal inter-ripple duration was fixed to 30s and maximal ripple duration to 100 ms. The number and density of sharp-waves/ripples (SWR) were measured per 3h bins, together with ripples mean duration and amplitude. Spindles occurring during SWS were detected according to principles previously established and validated in rodents (Aguilar et al., 2019; Uygun et al., 2019). Briefly, ECoG signal was filtered using a Butterworth filter to the frequency range containing spindle activity (10-15Hz). The root mean square (RMS) of the signal was computed using a 750ms moving window, then cubed to increase the difference between noise and signal. Upper (3.5x mean cubed RMS) and lower (1.2x mean cubed RMS) thresholds were used to determine spindle peaks and boundaries, respectively. Spindle episodes between 0.5s and 10s were included. For 3h bins, spindle density (spindle episodes per minute of SWS) and average length of episodes were calculated, alongside mean and peak amplitudes during spindle episodes, normalised to a broad range of frequencies (1-250Hz).

Statistics

Statistical analyses were performed in Graphpad Prism v9.0.1. Repeated measures two-way ANOVA was performed to assess differences between FSL and control groups across ZT periods at baseline, and within FSL or control groups across experimental time points; comparisons were conducted using Fisher's post-hoc test between groups in the case of significant interaction between group and ZT period, and within groups in the case of significant effect of experimental timepoint to determine overall differences from baseline. For light-dark amplitude, groups at baseline were compared by unpaired t-test, while differences within groups in behaviour and light-dark amplitude throughout experiment timepoints were assessed with repeated measures ANOVA. In order to correct for the effects of repeated measures comparisons, Greenhouse-Geisser correction was applied where applicable. The threshold for significance was set at 0.05, and data expressed as mean \pm SEM.

Results

1. Sleep in the FSL model of depression

1.1 Sleep architecture

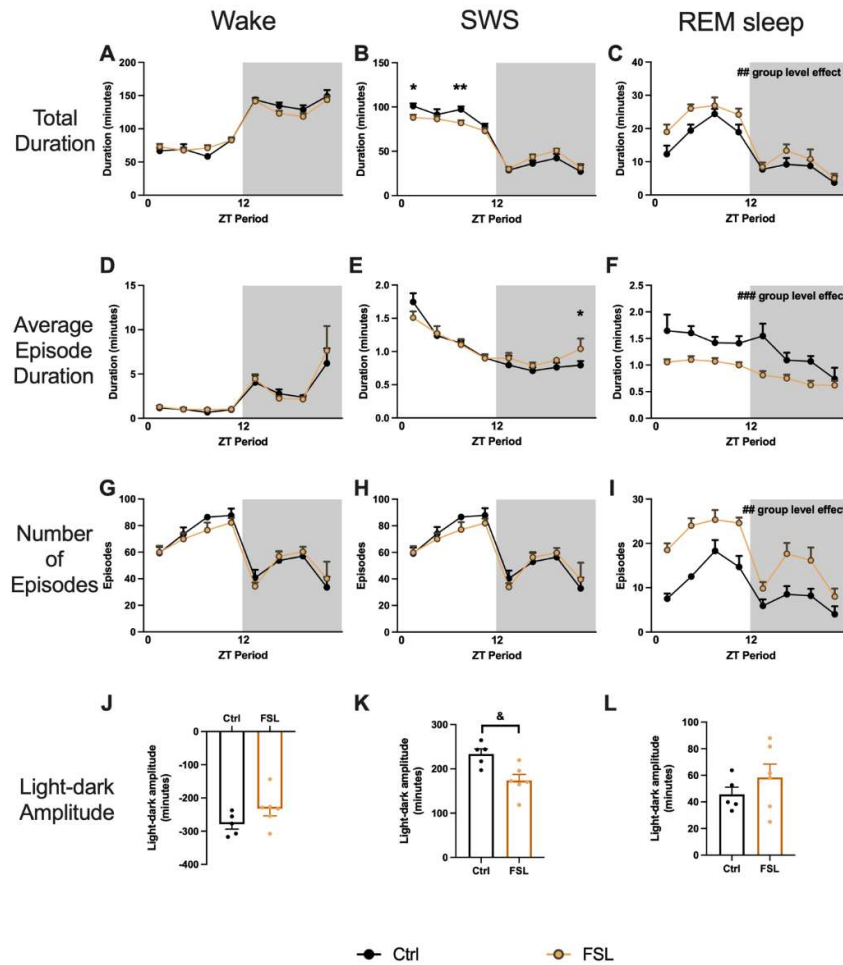


Fig. 3. Comparison of baseline sleep architecture in control and FSL rats. Sleep architecture measures in control and FSL rats over 24 hours at baseline, in 3h periods. Grey background indicates dark period. Total duration of (A) wake, (B) SWS and (C) REM sleep; mean episode duration of (D) wake, (E) SWS and (F) REM sleep; number of episodes of (G) wake, (H) SWS and (I) REM sleep; light-dark amplitude of (J) wake, (K) SWS and (L) REM sleep. Data are represented as mean + S.E.M. For all, $n = 5$ (control) and 6 (FSL). # = significant difference at group level according to two-way ANOVA, ## $p < 0.01$, ### $p < 0.005$; * = significance according to ZT-specific post-hoc comparison, * $p < 0.05$, ** $p < 0.01$; & = significance according to unpaired t-test, $p < 0.05$.

ECoG and EMG recordings were used to assess sleep in controls and the FSL rats over 48h baseline. In these measurements, FSL rats exhibited various abnormalities in sleep architecture compared to non-depressive controls. As predicted by earlier studies, FSL rats

showed clear changes to REM sleep: they consistently spent more time overall in REM sleep over 24h (two-way ANOVA, group factor $F_{(1,9)} = 10.85$, $p = 0.0093$, **Fig. 3C**), episodes of REM sleep in the FSL were more numerous (group factor $F_{(1,9)} = 22.70$, $p = 0.001$, **Fig. 3I**), but of shorter average duration (group factor $F_{(1,9)} = 22.87$, $p = 0.001$, **Fig. 3F**), describing a pattern of more fragmented REM sleep. However, the light-dark amplitude of REM sleep was not different between groups ($t = 1.063$, $p = 0.32$, **Fig. 3L**). Changes to the architecture of SWS were also present in the FSL, and were highly dependent on time: whereas the time spent in SWS during the wake-dominated dark phase was not modified in FSLs, it was reduced during the sleep-dominated light phase (group-ZT interaction, $F_{(7,63)} = 2.83$, $p = 0.012$, with significant post-hoc interactions at ZT0-3, $p = 0.017$ and ZT6-9, $p = 0.0073$, **Fig. 3B**). These light-dark cycle effects are further described by a 24.5% ($\pm 7.6\%$) mean reduction of light-dark amplitude of time spent in SWS compared to controls (unpaired t-test, $t = 3.21$, $p = 0.011$, **Fig. 3K**). Average length of SWS episodes also showed ZT-related differences, with significantly longer episodes during the final three hours of the night (group-ZT interaction, $F_{(7,63)} = 2.52$, $p = 0.024$, post-hoc interaction at ZT21-24, $p = 0.049$ **Fig. 3E**), and borderline-significantly shorter episodes during the onset of the day (post-hoc interaction at ZT0-3, $p = 0.059$, **Fig. 3E**), but no modification of SWS number of episodes was observed (group factor $F(1, 9) = 0.17$, $p = 0.69$, **Fig. 3H**). Architecture relating to wake was not different between groups, with FSL rats and controls spending similar circadian modulation of time awake (group factor $F_{(1,9)} = 0.62$, $p = 0.45$, **Fig. 3A**), with similar mean episode duration (group factor $F_{(1,9)} = 0.15$, $p = 0.71$, **Fig. 3D**), and number of episodes (group factor, $F(1, 9) = 0.08$, $p = 0.78$, **Fig. 3G**); the light-dark amplitude of time awake was not different between groups ($t = 1.70$, $p = 0.12$, **Fig. 3J**).

1.2 Oscillatory activities during sleep

SWS

FSL rats exhibited various abnormalities in oscillatory activity during SWS. Firstly, they exhibited a significant reduction in circadian modulation of delta power measured in ECoG (**Fig. 4A**), delta activity remaining low during the whole night whereas control animals showed an increase in delta power during the night in response to mounting sleep pressure (group-ZT interaction, $F(7,63) = 6.45$, $p < 0.0001$; post hoc interactions: ZT15-18, $p = 0.032$; ZT18-21, $p = 0.0066$; ZT21-24, $p = 0.038$). Relative delta power peaked in both groups in the first portion of the light period, but this peak was significantly lower in FSL rats (post-hoc comparison, ZT0-3, $p = 0.04$). In the NAc (**Fig. 4G**), the circadian pattern of delta was flatter and more closely matched between the two groups, but power was significantly lower in FSL rats overall (group factor, $F(1,9) = 12.07$, $p = 0.007$). In the PrL (**Fig. 4D**) and CA1 (**Fig. 4J**), FSL rats showed a tendency towards elevated and reduced delta power respectively, but neither of these differences reached significance (PrL group factor, $F(1, 8) = 2.970$, $p = 0.12$, CA1 group factor, $F(1, 7) = 1.60$, $p = 0.25$).

Alongside changes to delta activity, FSL rats also exhibited elevated gamma oscillations during SWS. In global ECoG signal (**Fig. 4B**), gamma power was significantly higher in FSL rats than controls overall (group factor, $F(1, 9) = 16.33$, $p = 0.0029$), and rose prominently across the night. Gamma was also significantly elevated in the FSL in the NAc (**Fig. 4H**; group factor, $F(1, 9) = 6.89$, $p = 0.028$), while activity in the PrL (**Fig. 4E**) and CA1 (**Fig. 4K**) was not significantly different between groups (group factor: PrL, $F(1, 9) = 0.53$, $p = 0.49$; CA1, $F(1, 7) = 2.31$, $p = 0.17$).

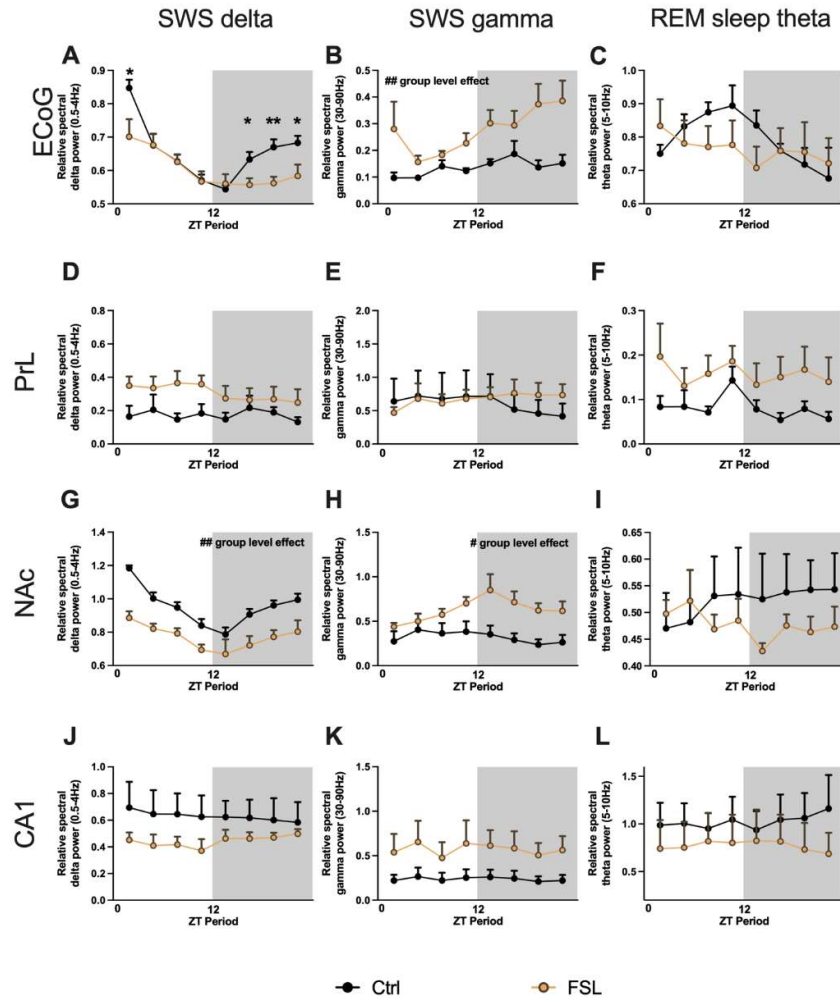


Fig. 4. Comparison of baseline spectral activity in control and FSL rats. Spectral activity in control and FSL rats over 24 hours at baseline, in 3h periods. Grey background indicates dark period. ECoG SWS delta (A), gamma (B) and REM sleep theta (C) activity; PrL SWS delta (D), gamma (E) and REM sleep theta (F) activity; NAc SWS delta (G), gamma (H) and REM sleep theta (I) activity; CA1 SWS delta (J), gamma (K) and REM sleep theta (L) activity. Data are represented as mean + S.E.M. For ECoG, PrL and NAc, $n = 5$ (control) and 6 (FSL); for CA1, $n = 4$ (control) and 5 (FSL). # = significant difference between groups according to two-way ANOVA, # $p < 0.05$, ## $p < 0.01$; * = significant difference between groups according to ZT-specific post-hoc comparison, * $p < 0.05$, ** $p < 0.01$.

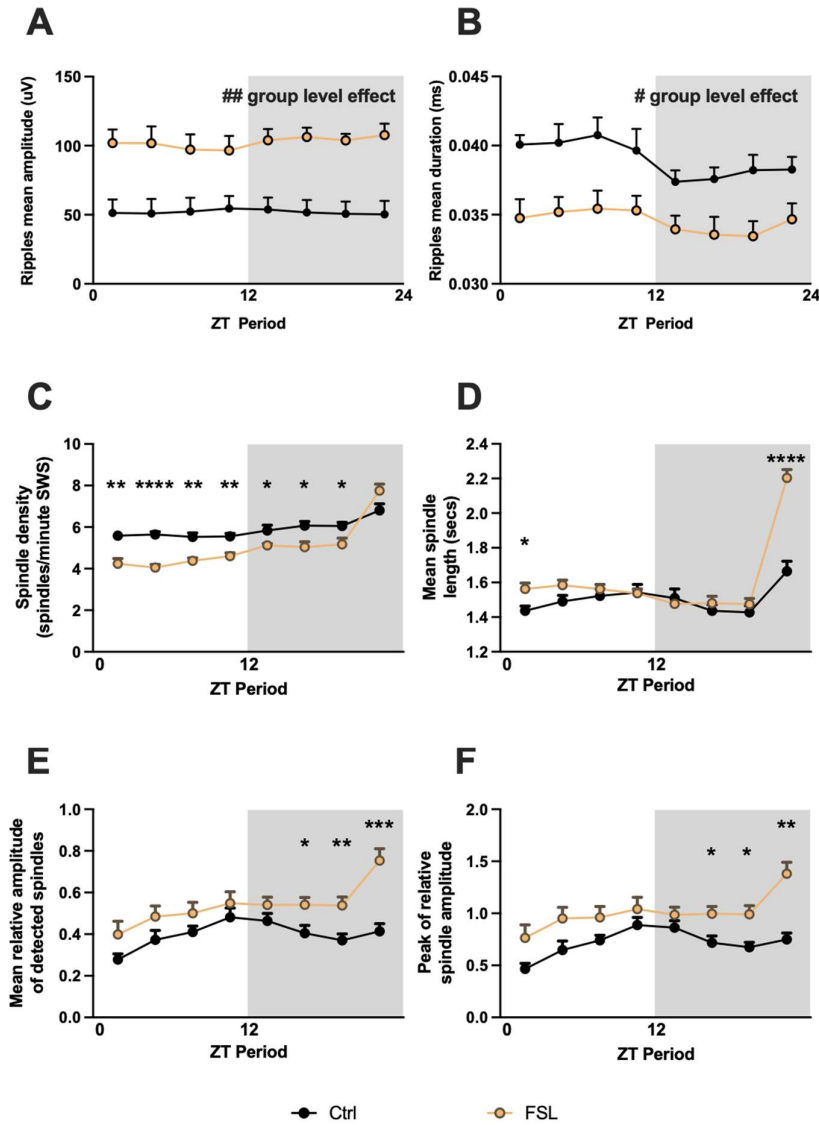


Fig. 5. Comparison of baseline hippocampal ripples and ECoG spindles in control and FSL rats. Sharp wave ripples mean amplitude (A) and duration (B), and spindle density (C), episode length (D), mean amplitude (E) and peak amplitude (F) over 24 hours at baseline, in 3h periods. Grey background indicates dark period. Data are represented as mean + S.E.M. For hippocampal ripples, $n = 4$ (control) and 5 (FSL); For spindles, $n = 5$ (control) and 6 (FSL). # = significant difference between groups according to two-way ANOVA, # $p < 0.05$, ## $p < 0.01$. * = significant difference according to post-hoc comparisons after significant two-way ANOVA interaction, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

The number and density of SWR measured during SWS were not different between FSL rats and control animals (data not shown). However, FSL rats displayed a higher amplitude (**Fig. 5A**; group factor, $F_{(1, 7)} = 16.26$, $p = 0.0050$) and a lower duration (**Fig. 5B**; group factor, $F_{(1, 7)} = 9.16$, $p = 0.019$) of ripples compared to control rats, across all the 24h (no group \times time interactions).

FSL rats exhibited altered density of spindles during SWS compared to controls, with reduced density in all but the final 3 hours of the day/night cycle (**Fig 5C**; group x time interaction $F_{(7, 63)} = 18.89$, $p < 0.0001$; post-hoc differences between groups at ZT0-3, $p = 0.002$; ZT3-6, $p < 0.0001$; ZT6-9, $p = 0.0016$; ZT9-12, $p = 0.0019$; ZT12-15, $p = 0.049$; ZT15-18, $p = 0.0012$; ZT18-21, $p = 0.036$; ZT21-24, $p = 0.066$). Additionally, the length of episodes was significantly higher than in controls at the onset of the light cycle and the final three hours of the night (**Fig 5D**; group x time interaction $F_{(7, 63)} = 52.45$, $p < 0.0001$; post-hoc differences between groups at ZT0-3, $p = 0.018$; ZT21-24, $p < 0.0001$). The amplitude of spindles was elevated in the FSL, with both the mean (**Fig 5E**; group x time interaction $F_{(7, 63)} = 8.22$, $p < 0.0001$; post-hoc differences between groups at ZT15-18, $p = 0.026$; ZT18-21, $p = 0.0093$; ZT21-24, $p = 0.0009$) and peak (**Fig 5F**; group x time interaction $F_{(7, 63)} = 6.65$, $p < 0.0001$; post-hoc differences between groups at ZT15-18, $p = 0.017$; ZT18-21, $p = 0.011$; ZT21-24, $p = 0.0012$) amplitudes rising significantly compared to controls during the night.

REM sleep

Despite architectural changes, FSL rats showed no changes in oscillatory activity during REM sleep, and especially in the prominent theta activity. Measurements of theta were not significantly different between groups in ECoG signal (**Fig. 4C**; group factor, $F_{(1, 9)} = 0.17$), $p = 0.69$), in the PrL (**Fig. 4F**; group factor, $F_{(1, 9)} = 2.40$, $p = 0.16$), NAc (**Fig. 4I**; group factor, $F_{(1, 9)} = 0.49$, $p = 0.50$) or in the hippocampus CA1 (**Fig. 4L**; group factor, $F_{(1, 7)} = 0.45$, $p = 0.53$). No differences in any structure were found in other frequency bands investigated (data not shown).

2. Effects of mfb-DBS

2.1 Behaviour

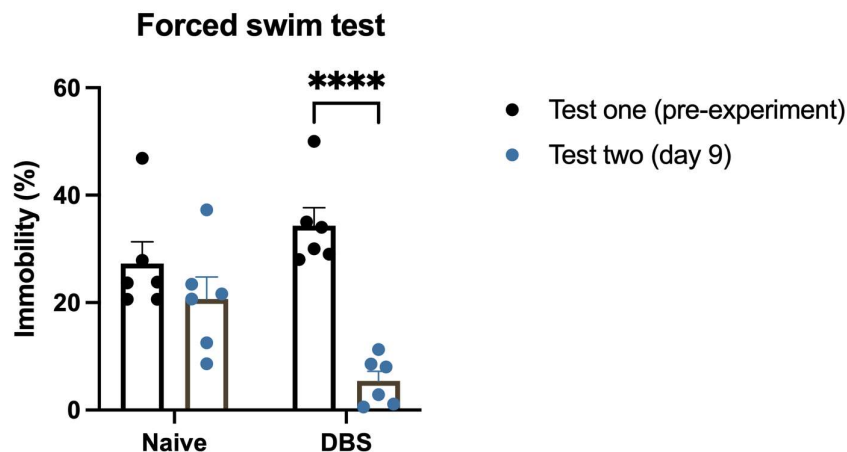


Fig. 6. Effect of mfb-DBS on the forced swim test. Immobility as measured in the forced swim test in test one (pre-experiment phenotype screening) and test two (experimental day 9), in unstimulated naïve FSL rats and FSL rats with mfb-DBS. Data are represented as mean + S.E.M. For each, $n = 6$. * = significance indicated according to two-way ANOVA with Fisher's post-hoc comparisons, $p < 0.001$.

When tested 9 days after mfb-DBS, FSL rats showed a significant reversal of depressive-like phenotype reflected in the reduction of immobility in the FST, compared to an unimplanted cohort which showed no significant change in behaviour (group-treatment interaction, $F_{(1, 10)} = 21.4$, $p < 0.001$; test 1 vs test 2 comparison, DBS group $t = 8.46$, $p < 0.0001$; naïve group, $t = 1.92$ $p = 0.084$; **Fig. 6**). Within-group assessment of control rats receiving no stimulation showed no significant differences in sleep architecture measures or electrophysiological signal over time (data not shown). Therefore, sleep results obtained during baseline, Day 1 and Day 7 were pooled and included as a dotted line in **Fig. 7, 8 and 9** (showing within-group data in the FSL) for reference. Data from FSL group at different experimental timepoints were compared in within-group analyses in order to evaluate the effect of mfb-DBS on these parameters and also compared against the average of control data over the three experimental timepoints in order to assess if DBS is able to normalise modulations due to FSL model.

2.2 Sleep architecture

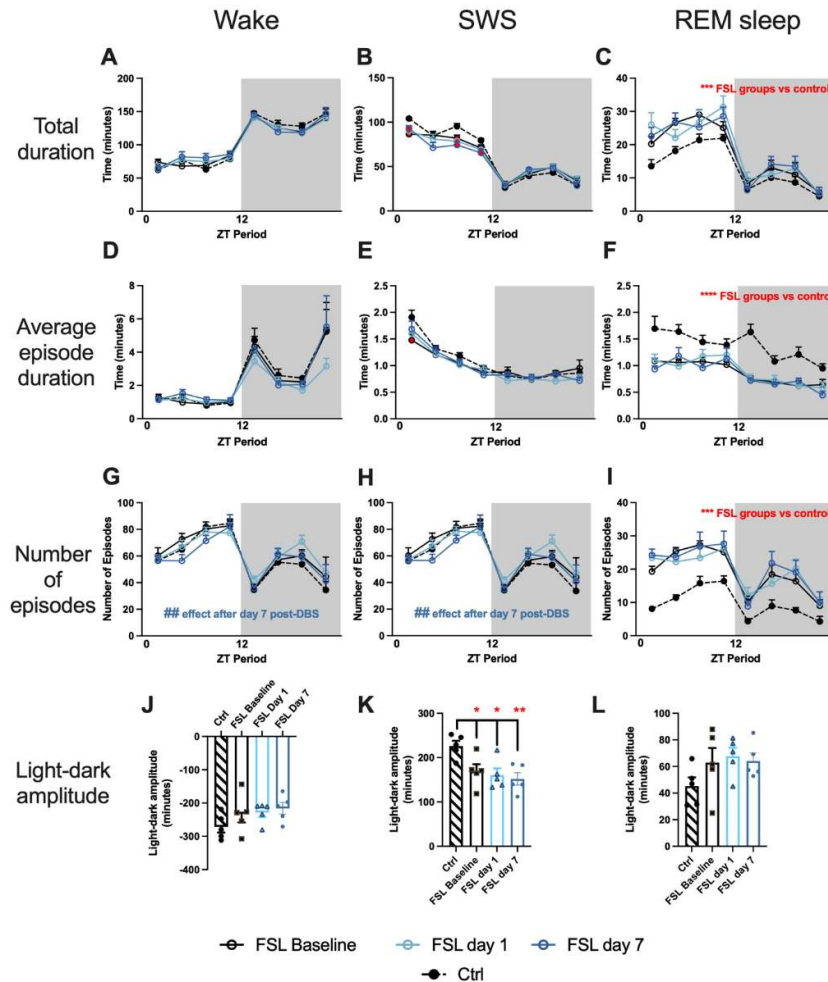


Fig. 7. Effect of mfb-DBS on the time course of sleep in the FSL, compared with pooled data of control rats. Sleep architecture in FSL rats at baseline (black), 1 (light blue) and 7 (dark blue) days after mfb-DBS represented over 24 hours expressed in 3h periods. Grey background indicates dark period. Dashed line represents pooled data from unstimulated non-depressive controls. Total duration of wake (A), SWS (B) and REM sleep (C); mean episode duration of wake (D), SWS (E) and REM sleep (F); number of episodes of wake (G), SWS (H) and REM sleep (I); light-dark amplitude of wake (J), SWS (K) and REM sleep (L). Data are represented as mean + S.E.M. For all, n = 5 (control) and 5 (FSL). ## = significant overall difference between experimental time points within the FSL according to two-way ANOVA, $p < 0.01$. Colour of significance marker indicates which experimental timepoint is different from FSL baseline. Red markers indicate difference between FSL group and pooled control group, $p < 0.05$. * = significant overall difference between each FSL experimental timepoint and control group, *** $p < 0.005$, **** $p < 0.001$.

Sleep architecture showed little change after mfb-DBS in FSL rats. Within-group assessments showed neither change in FSL rats after mfb-DBS in the overall duration (**Figs. 7A, B, C**), mean duration of episodes (**Figs. 7D, E, F**) of any vigilance states (experimental timepoint factor: wake total duration, $F_{(1.63, 6.53)} = 1.34$, $p = 0.32$; wake mean episode duration, $F_{(1.91, 7.64)} = 2.93$, $p = 0.12$; SWS total duration, $F_{(1.42, 5.69)} = 2.20$, $p = 0.20$; SWS mean episode duration, $F_{(1.36, 5.43)} = 1.20$, $p = 0.34$; REM sleep total duration, $F_{(1.81, 7.23)} = 0.37$, $p = 0.69$; REM sleep mean episode duration, $F_{(1.92, 7.68)} = 1.57$, $p = 0.27$) nor in light-dark amplitude in any vigilance state (**Fig. 7J, K, L**; repeated measures one-way ANOVA: wake, $F_{(1.94, 7.74)} = 0.43$, $p = 0.66$; SWS, $F_{(1.32, 5.29)} = -0.012$, $p > 0.99$; REM, $F_{(1.37, 5.49)} = 0.20$, $p = 0.75$). Although time spent in vigilance states was not changed, the number of wake and SWS episodes were significantly reduced at 7 days post-DBS (number of wake episodes, experimental time point factor: $F_{(2, 8)} = 9.69$, $p < 0.01$, post-hoc baseline vs day 7, $p < 0.01$, **Fig. 7G**; number of SWS episodes, experimental time point factor: $F_{(2, 8)} = 10.73$, $p < 0.01$, post-hoc baseline vs day 7, $p < 0.01$, **Fig. 7H**).

Comparisons between the FSL group at different experimental timepoints and the pooled data of controls showed no change between experimental stages in measures of REM sleep, with significant differences at baseline in REM sleep (more time spent overall, shorter but more numerous episodes) maintained at days 1 and 7 post-DBS (**Figs. 7C, F, I**). In SWS, differences in total duration between FSL baseline and controls were maintained at 1 and 7 days post-DBS (**Fig. 7B**), but the significant difference in average episode duration at the start of the light cycle between FSLs at baseline and controls (post-hoc comparison baseline vs control, ZT0-3: $p < 0.05$, **Fig. 7E**) was diminished at day 1 ($p = 0.14$) and day 7 ($p = 0.29$), suggesting potential normalisation. Overall, while these data provide some evidence of normalisation of fragmentation of SWS, the effect of mfb-DBS on sleep architecture appears to be minimal, with no apparent influence on the architecture of REM sleep, and no normalisation of the phenotype seen in the FSL rats.

2.3 Oscillatory activities during sleep

SWS

Analysis of post-stimulation global ECoG signal suggested small changes to SWS delta power (**Fig. 8A**). In within-group comparisons between baseline and post-DBS timepoints within the FSL rats, no significant changes were observed (experimental timepoint factor, $F_{(1.2, 5.99)} = 0.87$, $p = 0.41$). However, comparisons between the FSL group at different experimental time points and the averaged control group suggested some normalisation may have occurred at certain circadian times (two-way ANOVA, experimental group-ZT interaction $F_{(21, 119)} = 3.85$, $p < 0.0001$). Post-hoc comparisons show that during ZT0-3, FSL rats were significantly different to controls at baseline ($p < 0.05$) and day 1 ($p < 0.05$), but not at day 7 ($p = 0.45$); during ZT15-

18, significantly different at baseline ($p < 0.05$), but not at day 1 ($p = 0.07$) or day 7 ($p = 0.27$); and at ZT18-21, significantly different at baseline ($p = 0.0075$) and day 1 ($p < 0.01$) but not at day 7 ($p = 0.057$). These changes suggest a normalising of delta activity during the early stages of sleep 7 days after mfb-DBS. In deep structures, delta power was not significantly modified by mfb-DBS stimulation in FLS rats (**Fig. 8D, G, J**).

Analysis of high frequency oscillations during SWS revealed effects of mfb-DBS on gamma oscillations. In global ECoG signal (**Fig. 8B**), analysis within the FSL group over experimental timepoints demonstrated that DBS resulted in suppression of the elevated gamma observed at baseline during the night, both at day 1 (experimental time point-ZT period interaction, $F_{(2.86, 11.44)} = 4.86$, $p < 0.05$; post-hoc differences between baseline and day 1 significant at ZT12-15 ($p < 0.05$), ZT18-21 ($p < 0.05$) and ZT21-24 ($p < 0.05$)) and to a lesser extent at day 7 (post-hoc differences between baseline and day 7 significant at ZT18-21 ($p < 0.05$). Comparison between FSL animals at different experimental time points and the pooled mean of unstimulated controls also suggested a normalising effect of mfb-DBS on SWS gamma power at one day post-DBS during certain parts of day-night cycle (experimental group-ZT interaction $F_{(21, 112)} = 4.35$, $p < 0.0001$). Whereas gamma remained slightly higher than controls during the day (post-hoc comparisons at day 1 vs control: ZT0-3, $p = 0.21$; ZT3-6, $p = 0.0004$; ZT6-9, $p = 0.15$; ZT9-12, $p < 0.01$), the significant increase of gamma during the night in the FSL at baseline was clearly attenuated at day 1 post-DBS (post-hoc comparisons at day 1 vs control: ZT12-15, $p = 0.77$; ZT15-18, $p = 0.20$; ZT18-21, $p = 0.13$; ZT21-24, $p = 0.10$). At day 7 post-DBS, this effect had diminished to the extent that FSL animals were different from controls at all ZT periods in the day and all but ZT15-18 in the night (post-hoc comparisons at day 7 vs control: ZT0-3, $p < 0.05$; ZT3-6, $p < 0.01$; ZT6-9, $p < 0.05$; ZT9-12, $p < 0.05$; ZT12-15, $p < 0.05$; ZT15-18, $p = 0.089$; ZT18-21, $p < 0.05$; ZT21-24, $p < 0.01$).

In the PrL (**Fig. 8E**), within-group analysis of FSL rats suggested an increase in gamma power at 7 days post-mfb-DBS (experimental time point factor, $F_{(2,10)} = 7.72$, $p < 0.01$; post-hoc baseline vs day 7, $p < 0.05$). However, PrL gamma activity in the FSL (not different from controls at baseline) was not different from the pooled control data at any point in the experiment (experimental group factor, $F_{(3, 16)} = 1.46$, $p = 0.26$). Gamma power did not significantly change after DBS within the FSL group in the NAc (**Fig. 8H**; experimental time point factor, $F_{(1.56, 7.78)} = 1.05$, $p = 0.38$). Comparisons between FSL groups and pooled controls did not show the significant difference between groups (group factor, $F_{(3,17)} = 2.003$, $p = 0.16$) that had been observed during analysis of baseline, which may suggest some normalisation. In the CA1, no significant differences were observed within the FSL (**Fig. 8K**; experimental time point factor, $F_{(1.42, 5.68)} = 1.22$, $p = 0.34$). Overall, normalisation of the increased SWS gamma observed in FSL rats compared to control animals was apparent in global ECoG signal,

particularly one day after mfb-DBS, with local increases in gamma activity in the PrL after 7 days.

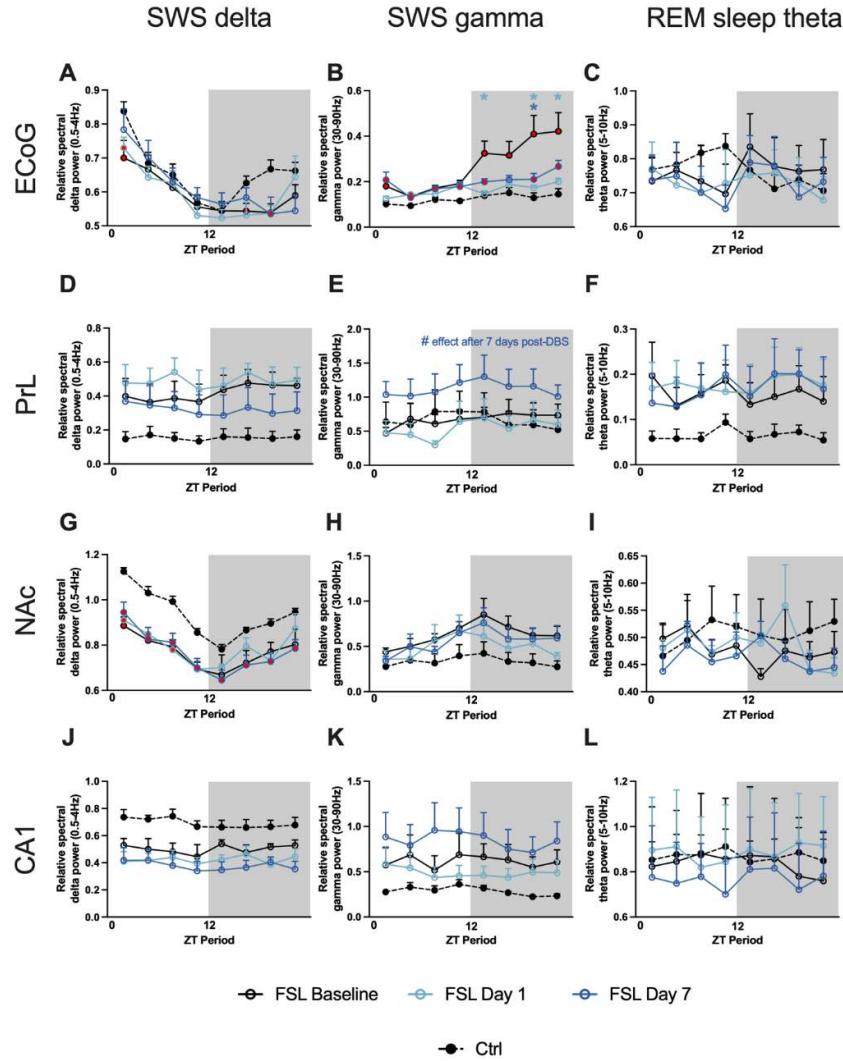


Fig. 8. Effect of mfb-DBS on the time course of spectral activity in the FSL, compared with pooled data of control rats. Spectral activity in FSL rats over 24 hours at baseline (black), 1 (light blue) and 7 (dark blue) days after mfb-DBS, represented in 3h periods. Grey background indicates dark period. Dashed line represents pooled data from unstimulated non-depressive controls. ECoG SWS delta (A), gamma (B) and REM sleep theta (C) activity; PrL SWS delta (D), gamma (E) and REM sleep theta (F) activity; NAc SWS delta (G), gamma (H) and REM sleep theta (I) activity; CA1 SWS delta (J), gamma (K) and REM sleep theta (L) activity. Data are represented as mean \pm S.E.M. For ECoG, PrL and NAc, $n = 5$ (control) and 5 (FSL); for CA1, $n = 4$ (control) and 5 (FSL). # = significant overall difference between experimental timepoints within the FSL group according to two-way ANOVA, $p < 0.05$. * = significant difference at specific ZT period within the FSL group according to post-hoc comparison after significant interaction of ZT x experimental timepoint, $p < 0.05$. Colour of significance marker indicates which experimental timepoint is different from FSL baseline. Red markers in 7A, B

and G indicate significant difference between FSL and control at ZT according to post-hoc comparison after significant interaction of ZT x group, $p < 0.05$; clear marker in these graphs indicates no significant difference between FSL and control at these ZT points.

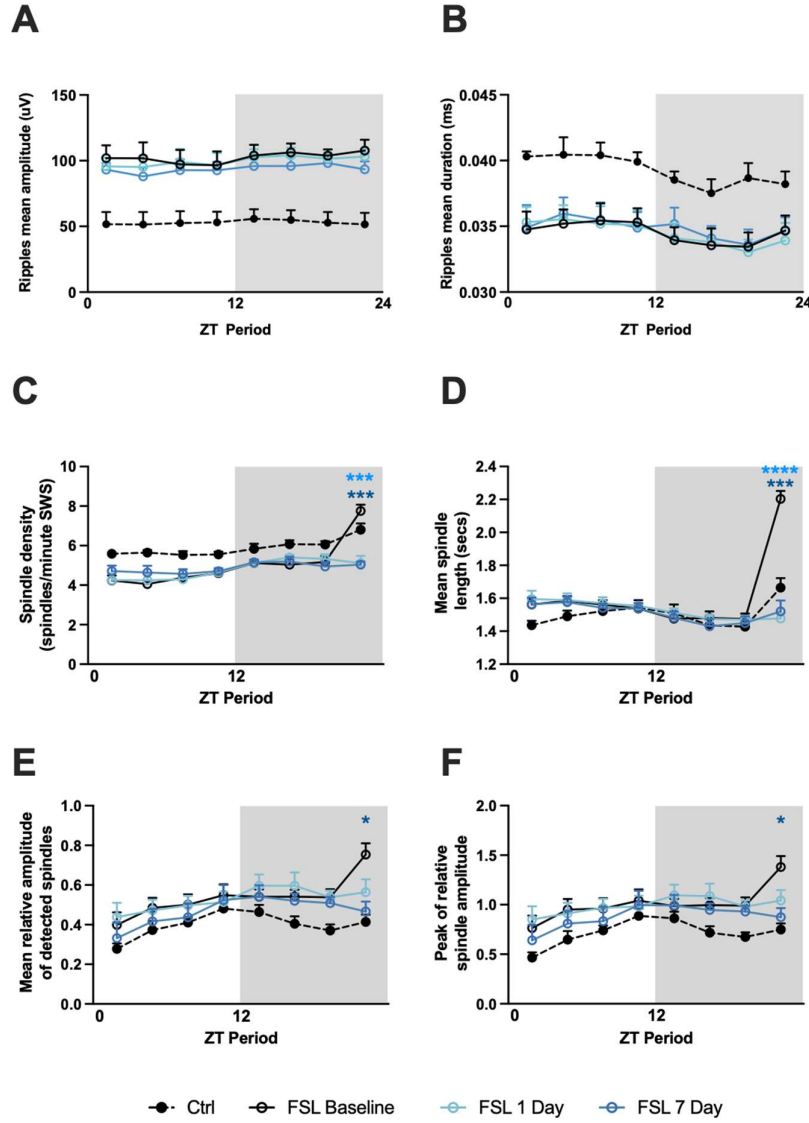


Fig. 9. Effect of mfb-DBS on the time course of hippocampal ripples and spindle activity in the FSL, compared with pooled data of control rats. Ripples mean amplitude (A) and duration (B), and ECoG spindles density (C), mean duration (D), mean amplitude (E) and peak amplitude (F) over 24 hours at baseline (black), 1 (light blue) and 7 (dark blue) days after mfb-DBS, represented in 3h periods. Grey background indicates dark period. Dashed line represents pooled data from unstimulated non-depressive controls. Data are represented as mean \pm S.E.M. For hippocampal ripples, $n = 4$ (control) and 5 (FSL); for spindles, $n = 5$ (control) and 5 (FSL). * = significant difference according to post-hoc comparisons after significant two-way ANOVA interaction, * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

Mfb-DBS stimulation had no effect on hippocampal ripples amplitude (**Fig. 9A**; experimental timepoint factor, $F_{(1,2)} = 1.50$, $p = 0.28$) or duration (**Fig. 9B**; experimental timepoint factor, $F_{(1,2)} = 2.55$, $p = 0.14$) in FSL animals. Mfb-DBS had ZT-specific effects on both the quantity and physiology of spindles during SWS, with all changes evident only during the final 3h period of the active dark phase. Spindle density and average length of episodes were reduced at both 1 day and 7 days post-DBS (**Fig 9C**; spindle density, group x time interaction $F_{(2.13, 8.23)} = 16.25$, $p = 0.0013$; post-hoc differences between FSL baseline and 1 day post-DBS at ZT21-24, $p = 0.0021$; between FSL baseline and 7 days post-DBS at ZT21-24, $p = 0.0002$. **Fig 9D**; mean episode length, group x time interaction $F_{(3.16, 12.18)} = 59.10$, $p < 0.0001$; post-hoc differences between FSL baseline and 1 day post-DBS at ZT21-24, $p = 0.0001$; between FSL baseline and 7 days post-DBS at ZT21-24, $p = 0.0012$). Spindle amplitude, elevated between ZT15-24 of the dark period at baseline, was reduced in the final 3h of the dark cycle at 7 days post-DBS only (**Fig 9E**; mean amplitude, group x time interaction $F_{(2.68, 10.35)} = 4.67$, $p = 0.029$; post-hoc comparison between FSL baseline and 7 days post-DBS at ZT21-24, $p = 0.03$; **Fig 9F**; peak amplitude, group x time interaction $F_{(2.82, 10.86)} = 3.78$, $p = 0.046$; post-hoc comparison between FSL baseline and 7 days post-DBS at ZT21-24, $p = 0.042$).

REM

No changes were observed in FSL rats after mfb-DBS in REM sleep theta oscillations in either global ECoG (**Fig. 8C**; experimental time point factor, $F_{(1.12, 4.46)} = 0.72$, $p = 0.45$), the PrL (**Fig. 8F**; $F_{(1.24, 4.95)} = 1.31$), $p = 0.32$), NAc (**Fig. 8I**; $F_{(1.75, 7.02)} = 0.38$, $p = 0.68$) nor CA1 hippocampal signal (**Fig. 8L**; $F_{(1.67, 6.67)} = 1.21$, $p = 0.35$). No other changes in any structure were observed in other frequency bands during REM after mfb-DBS (data not shown).

Discussion

While sleep anomalies are strongly associated with depression, it is not known how the experimental treatment of sIMFB-DBS affects these symptoms. The current study demonstrated abnormalities in the FSL rat in REM sleep architecture, the distribution of SWS and related delta activity, as well as elevated high-frequency gamma oscillations in ECoG signal and the NAc. 24h mfb-DBS produced a behavioural anti-depressant effect, while normalising ECoG gamma oscillations and early-sleep delta activity during SWS without influencing SWS or REM sleep architecture.

The FSL as a model for sleep disturbances in depression

Reported REM sleep disinhibition in the FSL (Benca et al., 1996; Shiromani et al., 1988), is replicated in our data in the form of increased time spent in REM sleep, with shorter, more numerous episodes. However, our results also suggest the presence of previously unreported changes to both the quantity and quality of SWS. More precise methodology, including shorter sleep scoring epochs and specific frequency band analysis may explain differences with previous reports. Both of the main regulatory mechanisms of sleep – homeostatic and circadian processes – are implicated by these results. The circadian influence on time spent in SWS was flattened in the FSL, while previous work has shown phase-advanced temperature regulation during normal light conditions and an accelerated effect of circadian rhythm on drinking behaviour without light cues (Shiromani et al., 1991; Shiromani & Overstreet, 1994). Changes to delta activity distribution may reflect deficiencies of processes relating to sleep homeostasis (Borbély et al., 2016; Borbély & Wirz-Justice, 1982). Such altered distribution has been linked with depressive symptomatology (Goldschmied et al., 2019; Landsness et al., 2011) and treatment response (Ehlers et al., 1996; Nissen et al., 2001), and may predict recurrence of depressive episodes (Kupfer et al., 1990). As far as we are aware, such altered distribution of delta activity has not been previously reported in any other animal models of depression. Given that SWS, slow oscillations and circadian changes have been identified in human patients with depression (Borbély et al., 1984; Nutt et al., 2008; Riemann et al., 2001), the range of sleep abnormalities present in the FSL may represent stronger face validity of the model in relation to sleep than previously acknowledged, and may be useful as a specific model for depression presenting with these characteristics.

Slow wave sleep as a state of dysfunction in depression

While quantitative changes to REM sleep in depression are prominent, our data suggest the underlying physiology may not be qualitatively different, with no difference in theta activity in ECoG and CA1 signal between FSL and control animals. SWS, on the other hand, shows changes to both architecture and oscillatory activity, highlighting its potential importance as a facet of the disorder. SWS and delta activity are bidirectionally associated with both

homeostatic drive for sleep (Borbély et al., 2016), and crucial mechanisms relating to neuronal plasticity such as synaptic downscaling (Huber et al., 2007; Tononi & Cirelli, 2003, 2006). Deficiencies in mechanisms of sleep homeostasis, reflected by a reduction in delta activity, may prevent optimal SWS, impairing the processes of synaptic downscaling, while impaired plastic processes may in turn contribute to abnormal delta activity; it is therefore possible that one deficit initiates the other, or positive feedback occurs between the mechanisms, exacerbating the problem. In addition, whereas synaptic downscaling is also found in REM sleep (Vyazovskiy et al., 2008; Niethard & Born, 2019), disturbances seen in REM sleep in FSL rats could also participate to those impairments.

The observed elevation of gamma oscillations may also be a sign of reduced SWS quality related to these impaired processes, rising during the dark cycle as sleep pressure builds, when delta would be expected to build (as seen in the non-depressive control group). Gamma oscillation generation mostly relies on GABAergic interneuron population activity (Buszaki and Wang 2012; Fee et al., 2017). These interneurons may participate to the initiation and maintenance of the up-state phase of slow oscillations (Niethard et al., 2018), being fundamental to maintaining excitatory/inhibitory (e/i) balance (Steriade et al 1996, Hasenstaub et al 2005, Kuki et al 2015). Here, elevated gamma power may therefore reflect - either as a causative factor or marker - pathophysiology which prevents the generation of adequate slow oscillation, impacting important processes of synaptic plasticity. Impaired functioning of these circuits has been previously implicated in depression (Fee et al., 2017; Thompson et al., 2015). As well as in global signal, such dysfunction was also observed locally in the NAc, a structure in which gamma oscillations are sensitive to stress (Iturra-Mena et al., 2019), and which has a role in emotional and reward-related memory processing during sleep (Perogamvros & Schwartz, 2012). Abnormal gamma oscillations during various behavioural states have been reported in human patients with depression and in animal models, and have been associated with treatment resistance and suicidality (Arikan et al., 2018, 2019; Fitzgerald & Watson, 2018). In terms of sleep, high frequency EEG oscillations during SWS have been previously associated with insomnia, with depressed insomniacs specifically exhibiting elevated power in a higher frequency range (45-125Hz), with this activity negatively correlated with perception of sleep quality (Perlis et al., 1997, 2001). Abnormal gamma oscillations may therefore represent a feature of reduced SWS quality in depression, and provide further evidence for the role of altered gamma activity in depressive pathology. Further research into the presence of elevated SWS gamma activity in human patients and other models for depression is required to further elucidate this relationship.

During the whole 24h period, we observed an alteration of hippocampal ripple and ECoG spindle activity parameters during SWS. Given the critical role of these oscillations during sleep in memory consolidation (Rasch & Born 2013), such modifications of brain oscillatory activity

could underlie cognitive deficits that are part of the symptomatology of depression (*DSM-V*) and that have already been described in the FSL model (Overstreet, 1993, Thiele et al, 2016). Furthermore, while spindle deficits are not classically considered a feature of sleep disturbance in depression, reduction in spindle density has been previously reported in depressed and at-risk populations (de Maertelaer et al., 1987; Lopez et al., 2010). Overall, our results suggest various abnormalities of SWS may comprise the depressive phenotype.

mfb-DBS anti-depressant treatment modulates features of SWS without affecting REM sleep architecture

mfb-DBS had an anti-depressant effect on behaviour in the FST. In this study, FSL rats receiving DBS were compared with naive counterparts, which may have influenced the behaviour in final tests. However, anti-depressant effects in the FST have been previously reported after acute and chronic applications of mfb-DBS, including when compared to sham-stimulated animals (Bregman et al., 2015; Edemann-Callesen et al., 2015; Furlanetti et al., 2015; Dandekar et al., 2017, 2019), suggesting no confounding effect of implantation. The anti-depressant effects of MFB/mfb-DBS are thought to be mediated, in part, via modulation of ascending mesocortical and mesolimbic projections to frontal and striatal nuclei (Döbrössy et al., 2021; Schlaepfer et al., 2013) which also have roles in sleep (Monti, 2011; Monti & Monti, 2007; Perogamvros & Schwartz, 2012). However, our study shows that mfb-DBS produces anti-depressant changes in behaviour without altering sleep architecture. This suggests that REM sleep suppression is not a necessary prerequisite for the anti-depressant effect. By contrast, even if mfb-DBS did not influence the modification of SWS circadian distribution observed in the FSL model, it improved SWS quality in normalising SWS gamma power. As high frequency oscillations during SWS are suggested to be negatively correlated with perception of sleep quality (Perlis et al., 2001), suppression of such activity could also provide a biological substrate for improved sleep quality. Subjective sleep perception may change independently of sleep architecture changes: depressed patients perceive lower sleep quality than control subjects despite suffering similar objective levels of disturbance (Mayers et al., 2003; Perlis et al., 1997), and report improved sleep after treatment even when sleep architecture contradicts this (Mayers & Baldwin, 2005). This would concur with anecdotal evidence of patients 'self-reporting during clinical trials of sIMFB-DBS, but must be verified in formal investigations.

Alongside effects on gamma activity, evident immediately following DBS, our results also provided some evidence of normalisation of delta activity during the early stages of sleep as a possible longer term effect. Normalisation of the distribution of delta activity has been previously identified as a potential marker of successful anti-depressant treatment (Ehlers et al., 1996), and may represent a reversal of the SWS abnormalities associated with depressive symptoms (Borbély et al., 1984; Nutt et al., 2008; Riemann et al., 2001). Given the potential

relationship between gamma and delta activity during SWS (Fee et al., 2017; Niethard et al., 2018), the short term changes to gamma may precipitate longer term changes to delta after mfb-DBS.

Finally, although some beneficial effects of mfb-DBS have been observed in a memory task in FSL rats (Thiele et al., 2018), our results show only narrow, ZT-specific effects on spindle activity and no effect of mfb-DBS on hippocampal oscillatory activity. Further research would be necessary to clarify the relationship between these brain activities during SWS and cognitive performances in the FSL model and the influence of mfb-DBS.

In the presented work, mfb-DBS was applied for a period of 24h, with technical limitations preventing recording during continuous stimulation, whereas in the clinical setting treatment is chronic and continuous. Previous studies have suggested a dynamic nature of response to mfb-DBS, with NAc dopaminergic response evolving over minutes to hours (Ashouri Vajari et al., 2020; Klanker et al., 2017, Bregman et al., 2015). However, there is no definitive understanding on when DBS response becomes 'chronic'. However, 24h was sufficient to produce anti-depressant-like and physiological effects, providing evidence for potential mechanisms. Effects of 24h DBS on gamma activity appeared to decay after one week; determining whether continuous stimulation prolongs the effect on gamma and whether this may enhance any subsequent normalisation of delta activity are key questions in determining the potential clinical effects of treatment.

The influence of mfb-DBS observed on behaviour and specific aspects of sleep may suggest more selective modulation of circuits involved in mood and sleep compared with systemic pharmacological anti-depressants. MFB-DBS may theoretically interact directly with inhibitory interneuron circuits, while serotonergic modulators have also been shown capable of influencing gamma oscillations (Döbrössy et al., 2021; Jakobs et al., 2019; Puig et al., 2010). Further research is therefore required to elucidate the mechanisms by which mfb-DBS influences these oscillations.

The presented data emphasise the fundamental importance of SWS deficits in affective disorders, demonstrating their presence alongside REM disinhibition in a validated model of depression, and illustrating abnormalities in the quantity, circadian distribution, and physiology of SWS. Elevated gamma activity may be of particular relevance due to its connection with other aspects of depressive pathophysiology. 24h mfb-DBS was able to produce an anti-depressant behavioural effect and normalise SWS gamma activity without altering sleep architecture, suggesting specific, independent modulation of circuits believed to share many biological substrates. This suggests a potential positive effect on sleep which must be validated in human patients, while modulation of gamma oscillations may represent an anti-depressant mechanism common with other treatments.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

The authors declare that animals were handled in accordance with ethical requirements enacted by the French authorities and the Strasbourg university who approved the present project (APAFIS #12113).

AUTHOR CONTRIBUTIONS

LL, MD, WG designed the study. LD, WG performed the experiments and analyzed the data. FF analyzed the data. WG, FF wrote the manuscript. MD, PB, LL corrected the manuscript.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding authors.