Received: 20 October 2021 | Revised: 30 March 2022 | Accepted: 5 April 2022

DOI: 10.1111/jsr.13615

RESEARCH ARTICLE

Down-phase auditory stimulation is not able to counteract pharmacologically or physiologically increased sleep depth in traumatic brain injury rats

Carlos G. Moreira[1] | Pascal Hofmann[1] | Adrian Müllner[1] | Christian R. Baumann[1,2,3] | Varun R. Ginde[1] | Sedef Kollarik[1] | Marta M. Morawska[1] | Daniela Noain[1,2,3]

[1]Department of Neurology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

[2]University Center of Competence Sleep & Health Zurich (CRPP), University of Zurich, Zurich, Switzerland

[3]Neuroscience Center Zurich (ZNZ), Zurich, Switzerland

Correspondence
Daniela Noain, Department of Neurology, University Hospital Zurich, Wagistrasse 14, CH-8952 Schlieren, Switzerland.
Email: daniela.noain@usz.ch

Funding information
This project was funded by the Swiss National Science Foundation (project no. 163056, CRB), the Clinical Research Priority Program "Sleep and Health" of the University of Zurich (CRB), the Neuroscience Center Zurich with the patronage of Rahn & Bodmer banquiers (DN), and the Synapsis Foundation for Alzheimer Research through an earmarked donation of the Armin & Jeannine Kurz Stiftung (DN).

Summary

Modulation of slow-wave activity, either via pharmacological sleep induction by administering sodium oxybate or sleep restriction followed by a strong dissipation of sleep pressure, has been associated with preserved posttraumatic cognition and reduced diffuse axonal injury in traumatic brain injury rats. Although these classical strategies provided promising preclinical results, they lacked the specificity and/or translatability needed to move forward into clinical applications. Therefore, we recently developed and implemented a rodent auditory stimulation method that is a scalable, less invasive and clinically meaningful approach to modulate slow-wave activity by targeting a particular phase of slow waves. Here, we assessed the feasibility of down-phase targeted auditory stimulation of slow waves and evaluated its comparative modulatory strength in relation to the previously employed slow-wave activity modulators in our rat model of traumatic brain injury. Our results indicate that, in spite of effectively reducing slow-wave activity in both healthy and traumatic brain injury rats via down-phase targeted stimulation, this method was not sufficiently strong to counteract the boost in slow-wave activity associated with classical modulators, nor to alter concomitant posttraumatic outcomes. Therefore, the usefulness and effectiveness of auditory stimulation as potential standalone therapeutic strategy in the context of traumatic brain injury warrants further exploration.

KEYWORDS
closed-loop auditory stimulation, down-phase modulation, posttraumatic outcomes, sleep modulation, sleep slow waves, traumatic brain injury

1 | INTRODUCTION

Traumatic brain injury (TBI) is a major public health concern that very often presents with disabling symptoms, including cognitive decline

Carlos G. Moreira and Pascal Hofmann shared authorship.

(Dikmen et al., 2017) persisting several months and up to years after injury. TBI presents a highly heterogeneous neuropathology, but one prevalent pathological hallmark is diffuse axonal injury (DAI) that results from complex cytoskeletal changes leading in severe cases to primary axotomy (Gennarelli et al., 1998). In DAI, amyloid precursor protein (APP) accumulates in axonal bulbs, for which APP reactivity is regarded as a robust quantifiable marker in models of TBI (Büchele et al., 2016; Johnson et al., 2013). Recently, slow-wave sleep enhancement (SE) has emerged as a promising candidate to improve TBI symptoms and pathological outcome (Iliff et al., 2014; Martinez-Vargas et al., 2012; Morawska et al., 2016; Ren et al., 2013), conceivably increasing the clearance of posttraumatic protein aggregates (Christensen et al., 2020) and therefore reducing DAI. Our previous results showed that increased levels of slow-wave activity (SWA; electroencephalographic power in the delta frequency range between 0.5 and 4 Hz) in sleep-enhanced (by administration of sodium oxybate; SO) and sleep-restricted (with prominent rebound sleep; SR) TBI rats presented with significantly reduced APP accumulation, i.e. alleviated DAI, and displayed preserved posttraumatic cognitive ability (Morawska et al., 2016). However, pharmacological agents aiming at enhancing SWA (Mednick et al., 2013; Walsh, 2009), even though therapeutically effective, do not preserve natural sleep architecture and can lead to dependency and misuse. Additionally, they lack specificity as non-sleep-mediated pathways might be triggered and ultimately tamper the findings (Wendt et al., 2014), raising the question of whether the previously observed pharmaco-related alleviating effects on TBI outcomes were indeed mediated by sleep. Therefore, steering away from pharmacotherapy and implementing tailored and more specific approaches to modulate slow waves is imperative in the context of preclinical TBI.

In fact, the development and evaluation of alternative scalable and non-invasive approaches for manipulation of sleep slow waves are in high demand not only given the putative beneficial impact of modulated slow waves on neurological disease (Kang et al., 2009; Morawska et al., 2016, 2021) but also on healthy brain functions, including memory (Ngo et al., 2013; Rasch & Born, 2013; Stickgold, 2013). One such approach of clinical interest is closed-loop auditory stimulation (CLAS) of slow waves during non-rapid eye movement (NREM) sleep. Auditory stimulation of slow oscillations during NREM sleep was successfully implemented by presenting sound triggers real-time locked with the phase of slow waves (Ngo et al., 2015). Targeting the ascending phase of slow waves enhances their amplitude and entrainment, with consequent impact on memory performance and restorative functions in both health and disease (Leminen et al., 2017; Ngo et al., 2013; Ong et al., 2016; Papalambros et al., 2017), whereas targeting the down-phase of slow waves results in a decrease of SWA, which was associated with impaired behavioural performance (Fattinger et al., 2017; Moreira et al., 2021). However, to test the preclinical applicability and the degree of therapeutic potential of CLAS in a model of TBI, there are a few critical aspects we must explore further. Importantly, the effectiveness of CLAS onto the injured brain has not been determined. Moreover, the comparative modulatory strength and usefulness of CLAS in relation to classical SWA modulatory methods, such as pharmacotherapy or sleep deprivation, compels further investigation. Gathering such insights shall shed light regarding the potential suitability of CLAS implementation in preclinical and clinical environments.

Therefore, we conceived three main objectives for this study: first, exploring the methodological feasibility of CLAS in our rat model of TBI; second, quantifying the modulatory effectiveness of CLAS in the context of brain injury; and third, determining the comparative strength of down-phase CLAS with classical pharmacological- (SO) or physiological- (sleep restriction followed by SR) SWA modulatory strategies. For the latter, we assessed the antagonistic effect of down-phase CLAS over SO- and SR-treated healthy and TBI rats, and subsequently evaluated respective co-effects over behavioural and posttraumatic neuropathological outcomes. We demonstrated here that down-phase CLAS is feasible and effective in modulating sleep slow waves in the context of rodent TBI, and measured its response size within the frame of two previously employed techniques for manipulation of SWA. Follow-up studies where auditory stimulation is targeted to the up-phase of the slow waves shall test the adequacy and effectiveness of CLAS as a treatment alternative for TBI in preclinical and clinical domains.

2 | MATERIALS AND METHODS

2.1 | Animals, surgeries and husbandry

We used 76 young-adult male Sprague–Dawley rats (Charles River, Italy) weighing 250–300 g and group-housed them in standard IVC cages (T2000) prior to interventions. In all animals, we surgically implanted electrodes for continuous recording of electroencephalography and electromyography (EEG/EMG) as previously described (Büchele et al., 2016). Briefly, we inserted four stainless-steel miniature screws (Hasler, Switzerland), constituting one differential derivation for each hemisphere, following specific stereotactic coordinates: the anterior electrodes were implanted 3 mm posterior to bregma and 2 mm lateral to the midline, and the posterior electrodes 6 mm posterior to bregma and 2 mm lateral to the midline. For monitoring of muscle tone, we inserted into the rats' neck muscle a pair of gold wires that served as EMG electrodes (Figure 1a). Following surgery, we let animals recover for a minimum of 14 days, with food and water ad libitum. The animal-room temperature was maintained at 22–23°C, and animals were kept on a 12-hr light–dark cycle. All procedures were approved by the veterinary office of the Canton Zurich (licence ZH231/2015), and conducted in accordance with national and cantonal regulations for care and use of laboratory animals.

2.2 | Experimental design

We performed the experiment in multiple batches of six–eight animals each, allocated evenly to 12 experimental groups (SHAM: placebo + mockCLAS or downCLAS, SE + mockCLAS or downCLAS, and

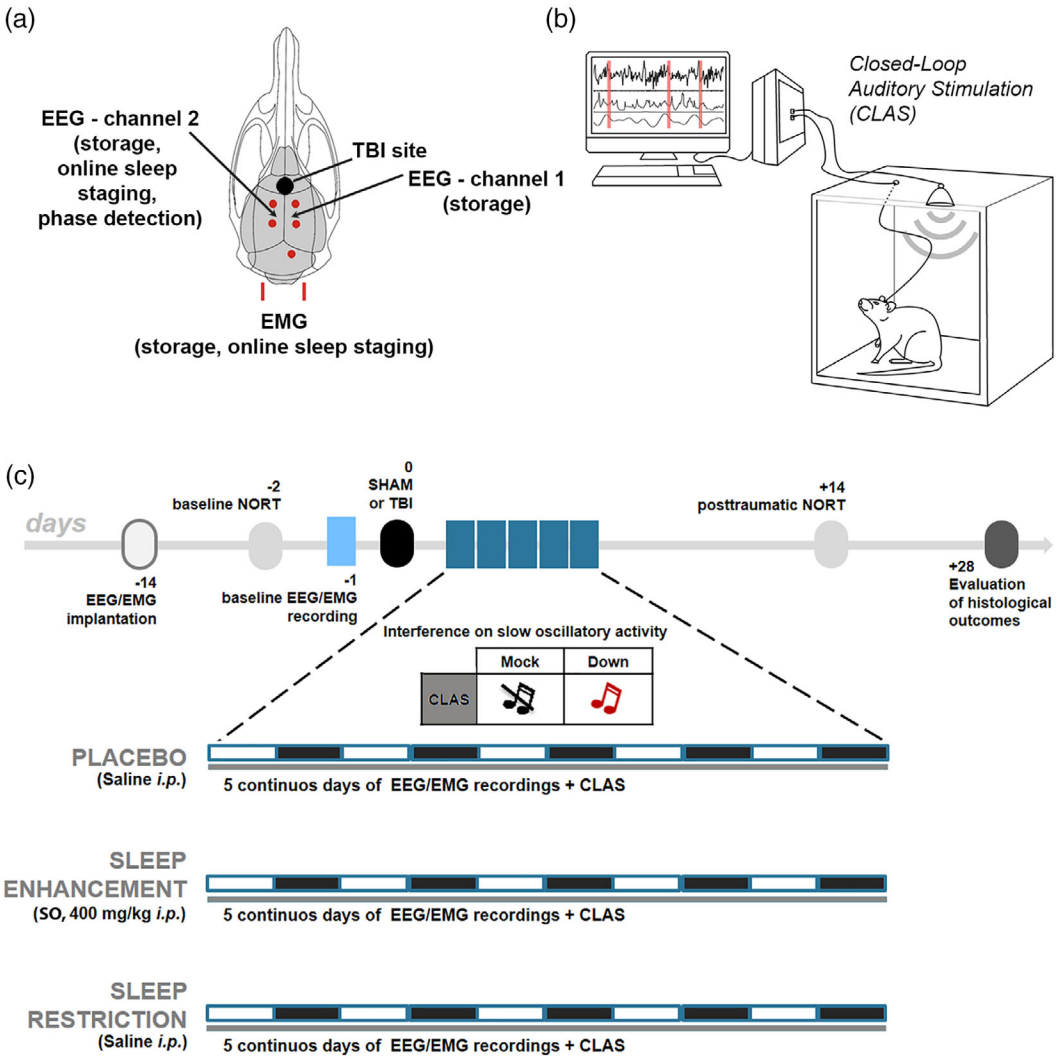

FIGURE 1 Methodology and experimental design. (a) Schematic representation of the electroencephalogram (EEG) and electromyogram (EMG) channels' placement in relation to the TBI site in the rat skull. (b,c) Diagram of the closed-loop auditory stimulation (CLAS) setup where SHAM and TBI rats were housed for EEG/EMG recording and real-time processing for CLAS delivery during 5 continuous days starting 1 day after SHAM or TBI surgeries (day 0). During the 5 days of treatment post-trauma, mock CLAS and down-phase CLAS animals additionally underwent a control condition ("placebo", saline i.p.) or slow-wave activity (SWA) enhancing regimes consisting of twice daily SO administration ("sleep enhancement" or "SO", Xyrem® 400 mg kg^{-1}, i.p.) or 6 hr per day of gentle handling ("sleep restriction" or "SR", also receiving twice daily saline i.p. to control for injection-unspecific effects). NORT, novel-object recognition test; NREMS, non-rapid eye movement sleep; SO, sodium oxybate; SR, sleep restriction; TBI, traumatic brain injury

SR + mockCLAS or downCLAS; TBI: placebo + mockCLAS or downCLAS, SE + mockCLAS or downCLAS, and SR + mockCLAS or downCLAS; Table 1). Following the first novel-object recognition test (NORT), we transferred the animals, individually, to custom-made acrylic-glass cages (26.5 × 42.5 × 43.5 cm) positioned inside of a sound-attenuated chamber, for 24-hr undisturbed EEG/EMG recording (baseline, BL; Figure 1b). Later, the animals underwent closed-skull TBI induction or SHAM surgery (see "TBI induction"). On the following day, we initiated a 5-day auditory stimulation protocol in combination with pharmacological sleep induction or a sleep restriction regimen. We stopped all sleep modulation procedures at the end of the 5th day. Duration of treatment was determined by our previous studies in this model (Büchele et al., 2016), in which we detected a cognitive deficit at 7 days post-TBI, leaving a possible window for interventions of 5 days. Two weeks following TBI or SHAM surgeries, the animals were re-tested in the NORT and killed 2 weeks later for collection of their brains (Figure 1c).

2.3 | TBI induction

We induced TBI as previously described (Büchele et al., 2016; Morawska et al., 2016). Briefly, rats were deeply anaesthetised with 2.5% isoflurane and positioned on a foamy platform. To mark the

TABLE 1 Experimental groups of the study

	Placebo		SO (400 mg kg^{-1}, i.p.)		SR (6 hr per day)	
SHAM	Mock (90° flag)	8	Mock (90° flag)	4	Mock (90° flag)	6
	Down-phase (270°)	8	Down-phase (270°)	5	Down-phase (270°)	7
TBI	Mock (90° flag)	7	Mock (90° flag)	4	Mock (90° flag)	7
	Down-phase (270°)	8	Down-phase (270°)	5	Down-phase (270°)	7

Note: SHAM and TBI animals underwent either placebo- (saline, i.p., twice daily), SE- (SO, 400 mg kg^{-1}, i.p., twice daily) or SR- (gentle handling 6 hr per day starting on the second hour of the light-period + saline, i.p., twice daily) interventions. Each treatment condition was combined with either mock CLAS (90°, only flags with no sound delivery) or down-phase CLAS (270°, pink noise triggers). The right column within each treatment indicates the number of rats included in each group.
Abbreviations: SO, sodium oxybate; SR, sleep restriction; TBI, traumatic brain injury.

impact area, 2 mm anterior to bregma, we made a 0.5–0.7-cm scalp incision over the midline in the frontal area and protected the skull using a 1-mm-thick metal shield. A 2500 g stainless-steel rod was mounted on a sliding stand and held 25 cm away from the point of impact on a 70° angle of inclination (Figure 1a). To induce TBI, the rod was released from its elevated position towards the protective shield. The single impact of the heavy rod over the protected skull was termed as the TBI "hit." SHAM animals underwent the exact same procedures except the rod was not released; therefore no "hit" occurred. A single i.p. injection of buprenorphine (0.05 mg kg^{-1}) was given on the day of TBI induction and in the two subsequent days, and regularly monitored. In our mild diffuse closed-skull TBI model — consisting of a widespread mild cellular and white matter damage with no focal point induced by diffused forces impacting the closed head — induction is considered effective, or not, based on several criteria: (i) there is no skull fracture observed immediately after the "hit." Any animal presenting open-skull TBI did not meet the model criteria, for which was excluded from further experiments and immediately killed (in the case of the present study, 0 subjects were excluded); (ii) the animals recover without remarkable posttraumatic sequelae, such as paresis or hemiparesis, abnormal levels of activity, or altered balance and reflexes, as assessed by a neurological severity score (Büchele et al., 2016); and (iii) the animals present a progressively worsened cognitive ability starting from day 7 after TBI, as assessed by the NORT (Büchele et al., 2016), among other deficits.

2.4 | Pharmacological modulation and sleep restriction protocol

The sleep modulation procedures started the day after TBI or SHAM surgeries and lasted 5 days (Figure 1c). All animals received two intraperitoneal injections per day (1 hr after lights-ON and 1 hr after lights-OFF). We treated the control (placebo) and SR groups with saline, whereas the SE group was treated with SO (400 mg kg^{-1}; Xyrem®, UCB Pharma; Lettieri & Fung, 1979; Morawska et al., 2016). We performed sleep restriction by gentle handling (6 hr daily, starting at 2 hr after lights ON; Morawska et al., 2016; Tobler & Jaggi, 1987). We granted 1 week for treatment-washout before assessment of cognitive performance.

2.5 | Evaluation of posttraumatic outcomes associated to slow waves modulation: NORT and quantification of APP axonal varicosities

We used standard procedures to evaluate cognitive ability and posttraumatic expression of DAI in SHAM and TBI rats (Ennaceur & Delacour, 1988; Gentleman et al., 1993; McAllister, 2011; Meythaler et al., 2001; Morawska et al., 2016). For details, please see Supplementary Materials.

2.6 | EEG/EMG recording and pre-processing

In order to verify the effect of auditory stimulation on SWA, we conducted bilateral tethered EEG/EMG recordings (differential mode) during 24 hr, to serve as BL, and throughout the subsequent 5 treatment-days (Figure 1c), and run our stimulation paradigm as previously described (Moreira et al., 2021). Briefly, following confirmation of impedances below 5 kΩ, we recorded EEG/EMG signals using SYNAPSE software (TDT, USA): the data were sampled at 610.35 Hz, amplified (bandwidth 0.1–285 Hz; PZ5 NeuroDigitizer preamplifier, TDT, USA) following an anti-aliasing low-pass filter (285 Hz, corresponding to 45% of sampling frequency), synchronously digitised (RZ2 BIOAMP processor, TDT, USA), and stored locally (WS-8 workstation, TDT, USA). We filtered real-time EEG between 0.1 and 36.0 Hz (2nd-order biquad filter, TDT, USA), and EMG between 5.0 and 525.0 Hz (2nd-order biquad filter and 40-dB notch filter centred at 50 Hz; TDT, USA), and fed the signals to real-time detection algorithms for NREM staging and phase detection.

2.7 | Online NREM sleep staging

Parallel rule-based NREM sleep staging and phase detection features were continuously running alongside EEG/EMG recording (Figure S1a), i.e. sound triggers were presented at every instance the stimulatory truth function compounding these features was reached. As previously specified (Moreira et al., 2021), a classifier compounds two major decision nodes for online NREM sleep staging: power in EEG and power in EMG. Briefly, we computed high-beta (20–30 Hz) and

delta (0.5–4 Hz) bands' root mean square (rms) from the left EEG derivation. NREM episodes were identified once the ratio rms_delta/rms_high_beta, hereinafter referred as NREMratio and the rms_EMG, crossed predefined thresholds, established following the BL recording. REM_ratio and rms_EMG thresholds, suggestive of sustained NREM sleep, were extracted individually as follows: 24 hr EEG/EMG data from BL recording were automatically scored (SPINDLE, ETH Zurich, Switzerland; Miladinović et al., 2019) and fed to a custom-written MATLAB (ver. R2016b) script; a strict estimate for the NREM_ratio threshold during NREM sleep was established as +1.0 SD over the mean (representing the 84.1% percentile) of the NREM_ratio of all identified NREM epochs; similarly, rms_EMG was delimited to values −1.0 SD below the mean (15.9% percentile) of the rms_EMG of those same NREM epochs. These two values marked the transition into consolidated NREM sleep in each subject and were inputted to our customised SYNAPSE® (TDT, USA) algorithm for phase-locked auditory stimulation (Figure S1b).

2.8 | Phase-locked CLAS

We divided the animals into two different phase-targeted stimulation approaches: down-phase stimulation targeting the slow-wave trough (270°); and mock stimulation (with no delivery of sound as the speaker was disconnected) flagging the slow waves' positive peak (90°). Auditory stimuli consisted of free-field clicks of pink 1/f noise (30 ms duration, 35 dB SPL, 2 ms rising and falling slopes) delivered via built-in speakers (MF1 Multi-field magnetic speakers, TDT) installed 50 cm above the centre of the floor-area. During CLAS, SYNAPSE® combines our NREM sleep staging feature with a phase-detector introduced in Moreira et al. (2021). In short, a runtime very-narrow bandpass filter (TDT, USA) for EEG phase detection isolated the 1-Hz component of each subjects' left EEG channel, including a threshold rule to avoid stimulation during very-low-amplitude slow waves (Figure S1c). We predetermined slow wave's 0° as the rising zero crossing, 90° to the positive peak and 270° to the slow-wave trough. At every identified positive zero-crossing on the filtered signal, the phase detector resets to 0°, and calculates any of the selected target-phases based on the number of elapsed sample points succeeding the zero crossing. This method offered the chance to recognise the phase of slow waves consistently across conditions, independently of the target-phase. For down-phase stimulation — the only regimen with delivery of sound — the average time-delay across the groups was 32.9 ± 3.4 ms (mean ± SEM), calculated from the shift between the identification of the target phase (270°) to the global maxima in the phase distribution plot.

2.9 | EEG/EMG scoring

We scored all recording files using the online computational tool SPINDLE (Miladinović et al., 2019) for animal sleep data (https://sleeplearning.ethz.ch/). In short, European Data Format (.edf) files, consisting of two parietal EEG and one nuchal EMG channels, were uploaded to SPINDLE to retrieved vigilance states with 4-s epoch resolution. The algorithm classified three vigilance states: wakefulness; NREM sleep and REM sleep. Wakefulness was defined based on high or phasic EMG activity for more than 50% of the epoch duration, and low-amplitude but high-frequency EEG. NREM sleep was characterised by reduced or no EMG activity, increased EEG power in the frequency band < 4 Hz, and the presence of slow oscillations. REM sleep was defined based on high theta power (6–9 Hz frequency band) and low muscle tone. Additionally, unclear epochs containing data outliers or signal perturbations related to environmental interference rather than changes in brain state were labelled as artefacts in wakefulness, NREM or REM sleep.

2.10 | Post-processing of EEG and EMG, online NREM sleep staging, sound triggers and evoked-response potentials

Time spent in NREM sleep was determined as an absolute number of minutes for BL and stimulation period. For the same days, we extracted measures of global spectral responses in the delta frequency by processing the left-hemisphere EEG signal with a custom MATLAB routine (ver. R2016b). Briefly, the EEG signal was resampled at 300 Hz, and multiplied with a basic Fermi window function $f(n) = (1 + e^{(5 - n/50)})^{(-1)}$ to gradually attenuate the first and last 2 s ($n = 600$). Next, we filtered the signal between 0.5 and 48 Hz using low- and high-pass zero-phased equiripple FIR filters (Parks-McClellan algorithm; applied in both directions [*filtfilt*]; order high = 1880, order low = 398; −6 dB [half-amplitude] cut-off: high pass = 0.28 Hz, low-pass = 49.12 Hz). We performed spectral analysis of consecutive 4-s epochs (FFT routine, Hamming-window, 2 s overlap, resolution of 0.25 Hz), and normalised the power estimate of each frequency-bin in relation to the total spectral power (0.5–30 Hz). Additionally, relative light- and dark-periods and 24-hr delta-power were calculated as the average of all corresponding equally-sized NREM-epoch bins (12 bins during light-period, and 6 bins during dark-period), and the 5 stimulation days were subsequently grand-averaged to obtain the power for the entire stimulation period per animal (Figure S1d).

2.11 | Statistical analysis

We present all data as mean ± standard error of the mean (SEM), or median in violin plots. We performed statistical analyses using Prism 8.0 (GraphPad, CA, USA), SPSS Statistics 26.0 (IBM, NY, USA), and Matlab R2016b and R201-9a (Natick, MA, USA). Two-way repeated-measures analysis of variance (RM-ANOVA, adjusted using the Greenhouse–Geisser correction when necessary) or mixed-models were used to assess *treatment*TBI* interactions, *TBI* or *treatment main* effects (mockCLAS versus downCLAS). We tested all datasets for normality using the Anderson–Darling test (omnibus K2 test for sample sizes > 7) or the Shapiro–Wilk test (for sample sizes ≤ 7). As two-way

ANOVA is robust to violations of normality, our run tests were considered valid when a few groups failed to pass normality tests, whereas all *t*-tests passed normality tests and did not require transformations or non-parametric analysis. The significant hypothesis-driven main-effects or interactions were subjected to post hoc assessments using Dunnett's or Šidák's multiple comparisons tests. Four significance levels, $^{\dagger}p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, were considered in all statistical conclusions.

3 | RESULTS

3.1 | Down-phase CLAS reduces SWA in non-sleep-modulated animals, but does not counteract SO- and SR-triggered enhancement of SWA

Non-pharmacological SWA-modulatory techniques of low-invasiveness, such as CLAS, are desirable in clinical contexts where slow waves have been suggested to play important roles. However, whether the effectiveness and strength of CLAS is comparable to classical SWA-modulatory methods remains unknown. To explore the capacity of down-phase CLAS, or lack thereof, to counter-modulate the effects of SO and SR on SWA, we tested their combination in a preclinical model of TBI with known sensitivity to modulation of slow waves (Morawska et al., 2016). We analysed NREM sleep proportion and SWA during the 5 days of combined modulation in SHAM and TBI animals (Figure 1; Table 1).

The SO and SR did not alter 24-hr NREM sleep amount in relation to placebo-treated animals (two-way ANOVA, *treatment* *TBI interaction; $F_{2,27} = 0.62$, $p = 0.547$; Figure 2a). By combining down-phase CLAS, NREM sleep amount remained unchanged, both in SHAM and TBI animals (two-way ANOVA for each subplot, *treatment*TBI* interaction; placebo: $F_{1,24} = 0.44$, $p = 0.513$; SE: $F_{1,12} = 0.71$, $p = 0.417$; SR: $F_{1,17} = 0.13$, $p = 0.727$; Figure 2b). When inspecting localised differences in NREM sleep time-course, we found no effect of down-phase CLAS on hourly NREM sleep amount in any sleep modulated group, in both SHAM (two-way mixed-models for each subplot, *treatment* main factor; placebo: $F_{1,8} = 1.67$, $p = 0.232$; SE: $F_{1,8} = 3.61$, $p = 0.094$; SR: $F_{1,8} = 0.44$, $p = 0.526$; Figure 2c) and TBI animals (placebo: $F_{1,8} = 2.10$, $p = 0.185$; SE: $F_{1,8} = 2.15$, $p = 0.181$; SR: $F_{1,8} = 0.12$, $p = 0.734$; Figure 2d).

Confirming our previous results (Morawska et al., 2016), both SHAM and TBI rats without auditory stimulation (mock CLAS) presented a significant enhancement in SWA after SO administration (one-sample *t*-test SO versus BL; SHAM: light-period: $^{\dagger}p = 0.054$; dark-period: $^{*}p = 0.017$; 24 hr: $^{**}p = 0.007$; TBI: light-period: $^{\dagger}p = 0.069$; dark-period: $p = 0.134$; 24 hr: $^{\dagger}p = 0.093$; Table 2). Likewise, SR subjects without auditory stimulation showed higher SWA levels following 6-hr SR, with most marked differences taking place during the dark-period (one-sample *t*-test SR versus BL; SHAM: light-period: $p = 0.207$; dark-period: $^{*}p = 0.018$; 24 hr: $p = 0.296$; TBI: light-period: $p = 0.448$; dark-period: $^{**}p = 0.003$; 24 hr: $p = 0.291$; Table 2). No change in SWA was observed in placebo-treated animals (one-sample *t*-test placebo versus BL; SHAM: light-period: $p = 0.371$; dark-period: $p = 0.277$; 24 hr: $p = 0.691$; TBI: light-period: $^{\dagger}p = 0.099$; dark-period: $p = 0.222$; 24 hr: $p = 0.875$; Table 2).

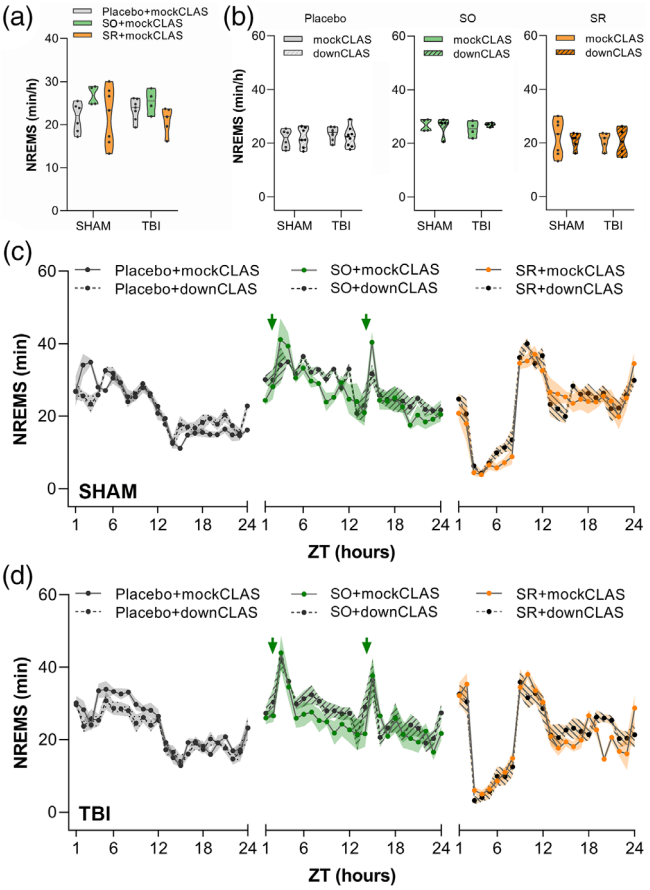

FIGURE 2 Down-phase closed-loop auditory stimulation (CLAS) does not disrupt the unaltered 24-hr NREM sleep proportions upon SO or SR in SHAM and TBI rats. (a) Mean NREM sleep amount per hour over 5 days of sleep modulation in all animals under mock CLAS, (b) and in comparison with downCLAS. (c) Time-course of NREM sleep amount for SHAM animals. (d) Time-course of NREM sleep amount for TBI animals. Green arrows mark the time of SO i.p. administration. NREMS, non-rapid eye movement sleep; SO, sodium oxybate; SR, sleep restriction; TBI, traumatic brain injury

Down-phase CLAS, on the other hand, successfully reduced SWA in placebo-treated animals in relation to mock CLAS placebo-treated counterparts, independently of TBI induction (two-way ANOVA, CLAS condition as main factor: light-period: $F_{1,27} = 15.29$, $^{***}p < 0.001$; dark-period: $F_{1,27} = 0.82$, $p = 0.372$; 24 hr: $F_{1,27} = 11.14$, $^{**}p = 0.002$; Šidák correction for multiple comparisons; Figure 3a), with the difference being mainly driven by the effect observed during the light-period. In SE animals, SWA enhancement was not impacted by down-phase CLAS over 12 hr or 24 hr (two-way ANOVA, CLAS condition as main factor: light-period: $F_{1,14} = 1.03$, $p = 0.328$; dark-period: $F_{1,14} = 0.03$, $p = 0.865$; 24 hr: $F_{1,14} = 0.17$, $p = 0.690$; Šidák correction for multiple comparisons; Figure 3b). An identical null effect was observed onto the SWA modulatory effect of SR (two-way ANOVA, CLAS condition as main factor: light-period: $F_{1,23} = 0.96$, $p = 0.337$; dark-period: $F_{1,23} = 1.29$, $p = 0.269$; 24 hr: $F_{1,23} = 1.48$, $p = 0.235$; Šidák correction for multiple comparisons; Figure 3c).

TABLE 2 Percentage of change of mean-SWA in relation to own BL and correspondent *p*-values (one-sample *t*-tests) during joint sleep-modulation + CLAS, per light-, dark- and 24-hr periods, in SHAM and TBI animals

		SHAM				TBI			
		mock-		down-		mock-		down-	
	CLAS	% change from BL	*p-value*	% change from BL	*p-value*	% change from BL	*p-value*	% change from BL	*p-value*
Placebo	Light-	5.09	*0.371*	−8.54	*0.107*	10.96	***†0.099***	−15.51	****0.017***
	Dark-	−14.37	*0.277*	−17.45	****0.036***	−10.86	*0.222*	−24.51	****0.026***
	24 hr	−1.52	*0.691*	−13.87	****0.011***	−0.94	*0.875*	−19.26	*****0.006***
SO	Light-	20.25	***†0.054***	9.26	*0.413*	18.16	***†0.069***	13.96	****0.036***
	Dark-	40.94	****0.017***	53.18	*****0.005***	49.38	*0.134*	31.62	*0.144*
	24 hr	26.38	*****0.007***	22.88	***†0.084***	27.8	***†0.093***	24.22	****0.025***
SR	Light-	−12.23	*0.207*	−24.67	***†0.096***	−7.26	*0.448*	−14.19	*0.144*
	Dark-	47.7	****0.018***	34.42	***†0.05***	33.8	*****0.003***	19.05	*0.155*
	24 hr	11.34	*0.296*	−4.24	*0.74*	8.16	*0.291*	0.39	*0.961*

Note: All significant *p*-values appear in bold (†$p < 0.1$, *$p < 0.05$, **$p < 0.01$).
Abbreviations: BL, baseline; CLAS, closed-loop auditory stimulation; SO, sodium oxybate; SR, sleep restriction; TBI, traumatic brain injury.

Altogether, although these results suggest that down-phase CLAS is effective per se in reducing SWA in both healthy and TBI animals, its counteracting strength is insufficient when combined with SWA-enhancing agent SO or following SR.

3.2 | Down-phase CLAS exerts no major changes on SE and SR-associated improvement in cognitive performance and neuropathological status associated with boosted slow waves after TBI

We previously observed preserved cognitive outcomes following SO administration and 6-hr sleep restriction in TBI animals (Morawska et al., 2016). To assess whether down-phase CLAS is able to alter such beneficial effects in TBI rats, we tested all animals' performance in the NORT 14 days after TBI or SHAM interventions. The analysis of the placebo-treated groups showed that SHAM animals, both under mock CLAS or down-phase CLAS groups, performed well at discriminating the novel object (one-sample *t*-test in comparison with *chance level* = 0.5; mockCLAS, *$p = 0.031$; downCLAS, *$p = 0.011$). On the contrary, TBI animals treated with placebo, under both mockCLAS or downCLAS, did not identify the novel object above *chance level* (mockCLAS, $p = 0.156$; downCLAS, †$p = 0.100$; Figure S2a). Animals in the SO group were, unfortunately, insufficient for statistical analysis (Figure S2b). As expected, SR preserves the cognitive ability in TBI animals (downCLAS, **$p = 0.006$), and this effect appears to be slightly weakened upon down-phase CLAS (downCLAS, †$p = 0.087$; Figure S2c).

In order to estimate DAI extent, we killed the animals 28 days after TBI and removed the brains for immunohistochemical staining of APP^+ axonal bulbs (Figure 4a,b) in the anterior portion of the corpus callosum. Brain tissue from placebo + mockCLAS TBI animals presented significantly higher estimates of APP-reactive axonal bulbs in comparison to placebo + mockCLAS SHAM animals (two-way ANOVA; *treatment*TBI* interaction, $F_{2,30} = 5.51$, **$p = 0.009$; *SHAM*TBI* from placebo + mockCLAS group, ***$p < 0.001$). In TBI animals under mock CLAS, SO administration (*placebo*SO*, **$p = 0.004$) and SR (*placebo*SR*, **$p = 0.006$) led to a lower number of axonal bulbs (Figure 4c). Down-phase stimulation did not impact APP^+ bulb estimates in placebo-treated TBI animals (two-way ANOVA; *TBI* main-factor, $F_{1,27} = 31.38$, ***$p < 0.001$; Figure 4d), or halted DAI reduction associated with SO (two-way ANOVA; *treatment*TBI* interaction, $F_{1,14} = 0.72$, $p = 0.410$; Figure 4e) or SR (two-way ANOVA; *treatment*TBI* interaction, $F_{1,23} = 0.62$, $p = 0.438$; Figure 4f).

The combined interpretation of these results suggest that down-phase CLAS was unable to counteract the beneficial effect of SO or SR onto posttraumatic DAI, confirming the weak antagonistic coeffect of down-phase CLAS at the histopathological level when combined with SWA-enhancing treatments.

4 | DISCUSSION

The original aims of this study were to assess the feasibility and comparative strength of CLAS in relation to classical SWA modulatory techniques as well as its ability to affect functional outcomes in a known SWA-sensitive model of TBI. For that, we delivered down-phase targeted CLAS to disrupt pharmacologically or physiologically enhanced SWA in a rat model of TBI. Overall, our results indicate that, although able to reduce SWA in both healthy and TBI animals in control condition, down-phase targeted CLAS was not capable of reversing SWA increase exerted by administration of SO or during SR following 6 hr of sleep restriction. In consequence, behavioural performance and histopathological findings previously found to be associated with increased SWA after TBI were only marginally affected by down-phase CLAS. These results indicate that CLAS is capable of modulating SWA in the context of neurological disease during a regular sleep pattern but not under conditions of strongly enhanced SWA.

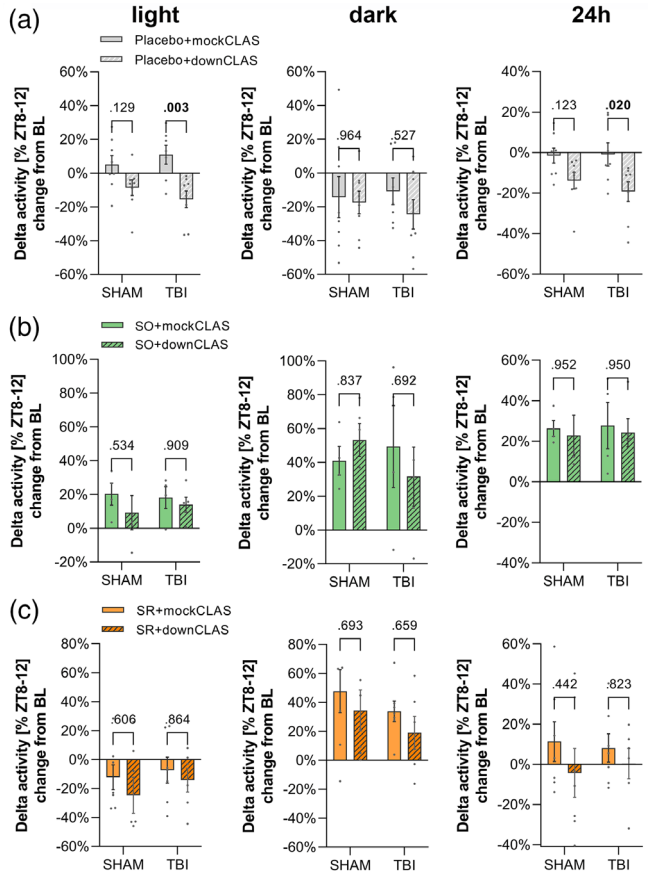

FIGURE 3 Down-phase closed-loop auditory stimulation (CLAS) is unable to alter slow-wave activity (SWA) enhancement following SO administration and partial SR in SHAM and TBI rats. (a–c) Five-day mean SWA change from BL, per light-, dark- and 24-hr periods, representing all days of sleep modulation and CLAS (two-way ANOVA followed by Šidák correction for multiple comparisons, further details in Table 2), for placebo-, SO- and SR-treated animals, respectively. NREMS, non-rapid eye movement sleep; SO, sodium oxybate; SR, sleep restriction; TBI, traumatic brain injury

Firstly, we investigated whether down-phase CLAS is methodologically feasible and effective in a rodent model of TBI. We have previously shown that the TBI model used in this work is compatible with electrophysiological recordings of vigilance states (Büchele et al., 2016), and that CLAS is feasible and effective in modulating SWA and behaviour in healthy rats (Moreira et al., 2021). Here, our results demonstrate a reduction of SWA upon down-phase CLAS compared with mock condition in a similar manner among SHAM and TBI rats. This indicates that CLAS is able to modulate both healthy and diseased brain's slow waves in a comparable fashion. This finding is congruent with the fact that posttraumatic slow waves appear unaltered in this model, except for a transient increase of power towards the 7th day after trauma (Noain et al., 2018). Overall, our results provide first proof-of-principle information regarding the viability of CLAS as a candidate to manipulate slow waves in a scalable and low-invasive manner in preclinical models of brain disorders. Additionally, the relatively easy applicability of CLAS — based on the use of existing implanted EEG/EMG electrodes — offers advantages over other sleep-modulating attempts, such as transcranial magnetic or current stimulations in humans, or electric or optical stimulation of neurons in animals. All of these are associated with strong electric artefact generation during sleep recordings, impractical implementation and questionable safety during chronic exposure (Annarumma et al., 2018; Bergmann et al., 2016).

Once down-phase CLAS feasibility in the context of TBI was determined, we explored the following two open questions: Can down-phase CLAS counter-modulate SWA increase exerted by pharmacological (SO) or physiological (forced increase in sleep pressure) means in healthy and TBI animals? And, will such counteraction be reflected by behavioural and histopathological TBI outcomes known to be responsive to SWA changes? Our findings indicate a comparable SWA profile in SHAM and TBI animals under mock CLAS or down-phase CLAS, independently of them being in the SE or SR groups. Therefore, it is conceivable that CLAS has a comparably weak strength to be able to antagonise SWA increases arising from powerful pharmacological or physiological regimens (Marshall et al., 2006; Massimini et al., 2007; Salfi et al., 2020). This relatively ineffective response may help explain the lack of fully reproducible modulatory effects of CLAS in human subjects, where a wide range of outcomes have been reported (Fehér et al., 2021). In addition, the absence of SWA alterations upon combination of SO or SR with CLAS was also associated with largely unchanged behavioural and histopathological posttraumatic outcomes. Others also found that measures of functional outcomes in healthy and diseased individuals present inconsistent results across studies upon CLAS application (Fehér et al., 2021), indicating that further exploration of the modulatory response to CLAS both in animals and humans is warranted. Standardising CLAS approaches, pipelines and algorithms, as well as advancing the exploration of the mechanisms underlying its modulatory effect shall benefit the response reproducibility and strength of CLAS in future applications.

If down-phase CLAS would have displayed strong SWA-modulatory capability comparable to that of SO or SR, we would have been able to achieve another goal: challenging SO and SR-associated SWA increases with down-phase CLAS to explore the consequent alterations at behavioural and histopathological levels could have delivered proof that SWA increases triggered by SO or SR were responsible for the ameliorated posttraumatic outcomes, and not chemical properties of SO or unspecific stress-related brain metabolism changes related to sleep restriction (Morawska et al., 2016). The weak antagonistic action of down-phase CLAS onto pharmacologically and physiologically boosted SWA did not allow drawing such conclusions.

Overall, our study proves the technical feasibility of CLAS in a preclinical rodent model of TBI with evident SWA modulatory action on its own, but at the same time a lack of sufficiently effective antagonistic effect in combination with powerful pharmacological and physiological methods for SWA enhancement. Follow-up studies shall evaluate the capability of up-phase CLAS to enhance SWA and its behavioural and histopathological correlates in the context of rodent TBI. Moreover, considering the known ability of CLAS to modulate memory-related spindles besides slow waves, up-phase CLAS could additionally be explored as a direct therapeutic targeting posttraumatic memory decline.

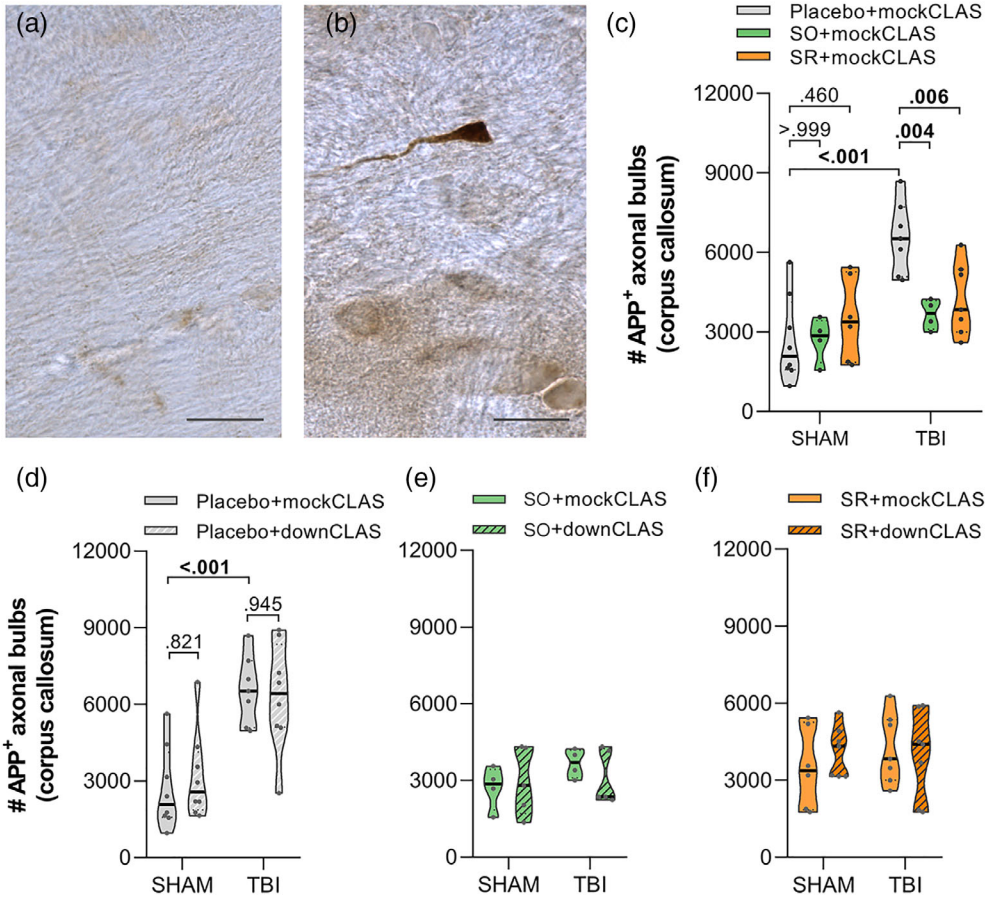

FIGURE 4 Both SO + mockCLAS and SR + mockCLAS TBI animals presented reduced DAI in comparison to placebo + mockCLAS, with down-phase CLAS not offering any counteraction. (a,b) Representative pictures of corpus callosum without and with the presence of APP^{+} axonal bulbs. (c) Estimate of axonal bulbs in SHAM and TBI animals under mock CLAS (two-way ANOVA followed by Dunnett's multiple comparisons test). (d) Placebo-treated animals showed increased APP immunoreactivity following TBI. (e,f) APP estimates were found reduced to SHAM levels after both sleep enhancement (SE) and SR protocols, including in groups under down-phase CLAS. APP, amyloid precursor protein; CLAS, closed-loop auditory stimulation; DAI, diffuse axonal injury; SO, sodium oxybate; SR, sleep restriction; TBI, traumatic brain injury

AUTHOR CONTRIBUTIONS

CGM, CRB and DN designed the study. CGM, VRG, SK, MMM and DN performed the animal experiments. CGM, PH and AM executed the immunohistochemistry and stereology studies. CGM, PH, AM and DN curated and analysed the data. CGM, PH, CRB and DN interpreted the results and wrote the manuscript. All authors corrected and approved the final version of the manuscript.

ACKNOWLEDGEMENTS

The authors thank Mr Mark Hanus, Mr Myles Billard, Dr Sophie Masneuf, Mr Maurizio Scandella, Ms Dnyanada Sahasrabudhe, Dr Virginia Meskenaite, Ms Inês Dias and Dr Joachim Buhmann's group for their time and valuable technical support. Open Access Funding provided by Universitat Zurich. [Correction added on 27 May 2022, after first online publication: CSAL funding statement has been added.]

CONFLICT OF INTERESTS

The authors state no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available in repository or from the corresponding author. (Following the guidelines, data will be upload to a repository following acceptance).

ORCID

Carlos G. Moreira https://orcid.org/0000-0003-3197-3668
Christian R. Baumann https://orcid.org/0000-0003-3417-1978
Daniela Noain https://orcid.org/0000-0001-5482-7933

REFERENCES

Annarumma, L., D'Atri, A., Alfonsi, V., & De Gennaro, L. (2018). The efficacy of transcranial current stimulation techniques to modulate resting-state EEG, to affect vigilance and to promote sleepiness. *Brain Sciences*, *8*, 137.

Bergmann, T. O., Karabanov, A., Hartwigsen, G., Thielscher, A., & Siebner, H. R. (2016). Combining non-invasive transcranial brain stimulation with neuroimaging and electrophysiology: Current approaches and future perspectives. *NeuroImage*, *140*, 4–19.

Büchele, F., Morawska, M. M., Schreglmann, S. R., Penner, M., Muser, M., Baumann, C. R., & Noain, D. (2016). Novel rat model of weight drop-induced closed diffuse traumatic brain injury compatible with electrophysiological recordings of vigilance states. *Journal of Neurotrauma*, *33*, 1171–1180.

Christensen, J., Wright, D. K., Yamakawa, G. R., Shultz, S. R., & Mychasiuk, R. (2020). Repetitive mild traumatic brain injury alters glymphatic clearance rates in limbic structures of adolescent female rats. *Scientific reports*, *10*(1), 1–9.

Dikmen, S., Machamer, J., & Temkin, N. (2017). Mild traumatic brain injury: Longitudinal study of cognition, functional status, and post-traumatic symptoms. *Journal of Neurotrauma*, *34*, 1524–1530.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research*, *31*, 47–59.

Fattinger, S., de Beukelaar, T. T., Ruddy, K. L., Volk, C., Heyse, N. C., Herbst, J. A., Hahnloser, R. H. R., Wenderoth, N., & Huber, R. (2017). Deep sleep maintains learning efficiency of the human brain. *Nature Communications*, *8*, 15405.

Fehér, K. D., Wunderlin, M., Maier, J. G., Hertenstein, E., Schneider, C. L., Mikutta, C., Züst, M. A., Klöppel, S., & Nissen, C. (2021). Shaping the slow waves of sleep: A systematic and integrative review of sleep slow wave modulation in humans using non-invasive brain stimulation. *Sleep Medicine Reviews*, *58*, 101438.

Gennarelli, T. A., Thibault, L. E., & Graham, D. I. (1998). Diffuse axonal injury: An important form of traumatic brain damage, *4*, 202–215.

Gentleman, S. M., Nash, M. J., Sweeting, C. J., Graham, D. I., & Roberts, G. W. (1993). Beta-amyloid precursor protein (beta APP) as a marker for axonal injury after head injury. *Neuroscience Letters*, *160*, 139–144.

Iliff, J. J., Chen, M. J., Plog, B. A., Zeppenfeld, D. M., Soltero, M., Yang, L., Singh, I., Deane, R., & Nedergaard, M. (2014). Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. *The Journal of Neuroscience*, *34*, 16180–16193.

Johnson, V. E., Stewart, W., & Smith, D. H. (2013). Axonal pathology in traumatic brain injury. *Experimental Neurology*, *246*, 35–43.

Kang, J. E., Lim, M. M., Bateman, R. J., Lee, J. J., Smyth, L. P., Cirrito, J. R., Fujiki, N., Nishino, S., & Holtzman, D. M. (2009). Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science*, *326*, 1005–1007.

Leminen, M. M., Virkkala, J., Saure, E., Paajanen, T., Zee, P. C., Santostasi, G., Hublin, C., Müller, K., Porkka-Heiskanen, T., Huotilainen, M., & Paunio, T. (2017). Enhanced memory consolidation via automatic sound stimulation during non-REM sleep. *Sleep*, *40*(3), zsx003. https://doi.org/10.1093/sleep/zsx003

Lettieri, J. T., & Fung, H. L. (1979). Dose-dependent pharmacokinetics and hypnotic effects of sodium gamma-hydroxybutyrate in the rat. *The Journal of Pharmacology and Experimental Therapeutics*, *208*, 7–11.

Marshall, L., Helgadóttir, H., Mölle, M., & Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature*, *444*, 610–613.

Martinez-Vargas, M., Rojo, F. E., Tabla-Ramon, E., Navarro-Argüelles, H., Ortiz-Lailzon, N., Hernández-Chávez, A., Solis, B., Tapia, R. M., Arredondo, A. P., Morales-Gomez, J., Gonzalez-Rivera, R., Nava-Talavera, K., & Navarro, L. (2012). Sleep deprivation has a neuroprotective role in a traumatic brain injury of the rat. *Neuroscience Letters*, *529*, 118–122.

Massimini, M., Ferrarelli, F., Esser, S. K., Riedner, B. A., Huber, R., Murphy, M., Peterson, M. J., & Tononi, G. (2007). Triggering sleep slow waves by transcranial magnetic stimulation. *Proceedings of the National Academy of Sciences*, *104*, 8496–8501.

McAllister, T. W. (2011). Neurobiological consequences of traumatic brain injury. *Dialogues in Clinical Neuroscience*, *13*, 287–300.

Mednick, S. C., McDevitt, E. A., Walsh, J. K., Wamsley, E., Paulus, M., Kanady, J. C., & Drummond, S. P. (2013). The critical role of sleep spindles in hippocampal-dependent memory: A pharmacology study. *The Journal of Neuroscience*, *33*, 4494–4504.

Meythaler, J. M., Peduzzi, J. D., Eleftheriou, E., & Novack, T. A. (2001). Current concepts: Diffuse axonal injury-associated traumatic brain injury. *Archives of Physical Medicine and Rehabilitation*, *82*, 1461–1471.

Miladinović, Đ., Muheim, C., Bauer, S., Spinnler, A., Noain, D., Bandarabadi, M., Gallusser, B., Krummenacher, G., Baumann, C., Adamantidis, A., Brown, S. A., & Buhmann, J. M. (2019). SPINDLE: End-to-end learning from EEG/EMG to extrapolate animal sleep scoring across experimental settings, labs and species. *PLoS Computational Biology*, *15*, e1006968.

Morawska, M. M., Buchele, F., Moreira, C. G., Imbach, L. L., Noain, D., & Baumann, C. R. (2016). Sleep modulation alleviates axonal damage and cognitive decline after rodent traumatic brain injury. *The Journal of Neuroscience*, *36*, 3422–3429.

Morawska, M. M., Moreira, C. G., Ginde, V. R., Valko, P. O., Weiss, T., Büchele, F., Imbach, L. L., Masneuf, S., Kollarik, S., Prymaczok, N., Gerez, J. A., Riek, R., Baumann, C. R., & Noain, D. (2021). Slow-wave sleep impacts synucleinopathy and regulates proteostatic processes in Parkinson mice. *Science Translational Medicine*, *13*, eabe7099.

Moreira, C. G., Baumann, C. R., Scandella, M., Nemirovsky, S. I., Leach, S., Huber, R., & Noain, D. (2021). Closed-loop auditory stimulation method to modulate sleep slow waves and motor learning performance in rats. *eLife*, *10*, e68043. https://doi.org/10.7554/elife.68043

Ngo, H. V., Miedema, A., Faude, I., Martinetz, T., Mölle, M., & Born, J. (2015). Driving sleep slow oscillations by auditory closed-loop stimulation-a self-limiting process. *The Journal of Neuroscience*, *35*, 6630–6638.

Ngo, H.-V. V., Martinetz, T., Born, J., & Mölle, M. (2013). Auditory closed-loop stimulation of the sleep slow oscillation enhances memory. *Neuron*, *78*, 545–553.

Noain, D., Büchele, F., Schreglmann, S. R., Valko, P. O., Gavrilov, Y. V., Morawska, M. M., Imbach, L. L., & Baumann, C. R. (2018). Increased sleep need and reduction of Tuberomammillary histamine neurons after rodent traumatic brain injury. *Journal of Neurotrauma*, *35*, 85–93.

Ong, J. L., Lo, J. C., Chee, N. I. Y. N., Santostasi, G., Paller, K. A., Zee, P. C., & Chee, M. W. L. (2016). Effects of phase-locked acoustic stimulation during a nap on EEG spectra and declarative memory consolidation. *Sleep Medicine*, *20*, 88–97.

Papalambros, N. A., Santostasi, G., Malkani, R. G., Braun, R., Weintraub, S., Paller, K. A., & Zee, P. C. (2017). Acoustic enhancement of sleep slow oscillations and concomitant memory improvement in older adults. *Frontiers in Human Neuroscience*, *11*, 109. https://doi.org/10.3389/fnhum.2017.00109

Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiological Reviews*, *93*, 681–766.

Ren, Z., Iliff, J. J., Yang, L., Yang, J., Chen, X., Chen, M. J., Giese, R. N., Wang, B., Shi, X., & Nedergaard, M. (2013). Hit & Run' model of closed-skull traumatic brain injury (TBI) reveals complex patterns of post-traumatic AQP4 dysregulation. *Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism*, *33*, 834–845.

Salfi, F., D'Atri, A., Tempesta, D., De Gennaro, L., & Ferrara, M. (2020). Boosting slow oscillations during sleep to improve memory function in elderly people: A review of the literature. *Brain Sciences*, *10*, 300.

Stickgold, R. (2013). Parsing the role of sleep in memory processing. *Current Opinion in Neurobiology*, *23*, 847–853.

Tobler, I., & Jaggi, K. (1987). Sleep and EEG spectra in the Syrian hamster (Mesocricetus auratus) under baseline conditions and following sleep deprivation. *Journal of Comparative Physiology. A*, *161*, 449–459.

Walsh, J. K. (2009). Enhancement of slow wave sleep: Implications for insomnia. *Journal of Clinical Sleep Medicine: JCSM: Official publication of the American Academy of Sleep Medicine*, *5*, S27–S32.

Wendt, G., Kemmel, V., Patte-Mensah, C., Uring-Lambert, B., Eckert, A., Schmitt, M. J., & Mensah-Nyagan, A. G. (2014). Gamma-hydroxybutyrate, acting through an anti-apoptotic mechanism, protects native and amyloid-precursor-protein-transfected neuroblastoma cells against oxidative stress-induced death. *Neuroscience*, *263*, 203–215.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Moreira, C. G., Hofmann, P., Müllner, A., Baumann, C. R., Ginde, V. R., Kollarik, S., Morawska, M. M., & Noain, D. (2022). Down-phase auditory stimulation is not able to counteract pharmacologically or physiologically increased sleep depth in traumatic brain injury rats. *Journal of Sleep Research*, *31*(6), e13615. https://doi.org/10.1111/jsr.13615