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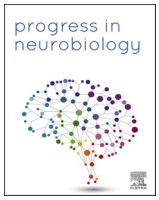

Bidirectional control of generalized absence epilepsy networks via real-time direct depolarization of thalamocortical neurons

Tatiana P. Morais [a,1], Hannah L. Taylor [a,1], Olivér Nagy [b,*], Ferenc Mátyás [c], Francois David [d], Vincenzo Crunelli [a,2,*], Magor L. Lőrincz [b,2,*]

[a] Neuroscience Division, School of Bioscience, Cardiff University, Museum Avenue, Cardiff CF10 3AX, UK
[b] Department of Physiology, University of Szeged, Szeged 6720, Hungary
[c] Institute of Experimental Medicine, Budapest, Hungary
[d] Center for Interdisciplinary Research in Biology, Collège de France, Paris, France

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ABSTRACT

Absence seizures (ASs), characterized by bilateral spike-and-wave discharges (SWDs), are a hallmark of idiopathic generalized epilepsies. We investigated the role of thalamocortical (TC) neurons in the generation and termination of ASs using optogenetic techniques in freely behaving GAERS rats, a well-established AS model. We demonstrate that direct depolarization of ChR2-transfected TC neurons in the ventrobasal thalamic nuclei during quiet wakefulness (QW) reliably elicits ethosuximide-sensitive ASs that have similar duration and frequency to those of spontaneous ASs, while showing little and no effect during active wakefulness (AW) and slow wave sleep (SWS), respectively. Light-stimulation of TC neurons fails to elicit ASs during AW, QW and SWS in non-epileptic control (NEC) rats, whereas it could evoke short ASs in Wistar rats, prevalently during QW. Notably, brief light stimulation effectively halted ongoing spontaneous ASs in GAERS rats (i.e. both SWDs and immobility), immediately altering thalamic multi-unit activity from rhythmic to irregular firing, irrespective of the SWD phase at which it was delivered. These findings support the view that the excitability of cortico-thalamic-cortical network is highly behavioural state-dependent, with increased susceptibility to the induction of ASs during QW, thus questioning the necessity of low-threshold burst firing of TC neurons in the generation of these seizures. Moreover, they highlight the dual control of ASs by TC neurons, underscoring their potential as therapeutic targets for AS modulation.

1. Introduction

Absence seizures (ASs), a common feature of many idiopathic generalized epilepsies, are characterized by bilateral 2.5–4 Hz spike-and-wave discharges (SWDs) in the EEG, and a loss of consciousness (Crunelli et al., 2020). They are known to start from localized cortical networks, defined as the cortical initiation network (CIN) of ASs (Crunelli et al., 2020), before spreading to the rest of the neocortex, thalamus and other subcortical regions (Bai et al., 2010; Holmes et al., 2004; Lörincz et al., 2007; Meeren et al., 2002; Polack et al., 2009; Studer et al., 2019; Tangwiriyasakul et al., 2018). The functional integrity of the cortico-thalamo-cortical (CTC) network and their inputs to and from the basal ganglia (Crunelli et al., 2020; Riban et al., 2004) to the expression and maintenance of ASs is well documented. In particular, the thalamic contribution to ASs has been highlighted by various evidence. Thus, lesions and pharmacological block of various thalamic nuclei, including the thalamic relay and reticular nuclei, were shown to prevent ASs in a rat model (Avanzini et al., (1992); (de Curtis and Avanzini, 1994; Vergnes and Marescaux, 1992), and low-frequency electrical stimulation of intralaminar thalamic nuclei has been known to elicit ASs in normal animals (Jasper and Droogleever-Fortuyn, 1946; Morison and Dempsey, 1941). Notably, recent data suggests that these intralaminar nuclei are responsible of generating the “spike” component, while the wave component is generated by neocortical microcircuits (Terlau et al., 2020).

In rat ASs models, the CIN is located in the primary somatosensory

* Corresponding authors.
E-mail addresses: crunelli@cardiff.ac.uk (V. Crunelli), magor.lorincz@med.u-szeged.hu (M.L. Lőrincz).
[1] Equal contribution.
[2] Senior authors.

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cortex (Meeren et al., 2002; Polack et al., 2009), and thus the ventrobasal thalamic nuclei (VB), the somatotopic thalamic region of the somatosensory cortex, is known to play a key role in the modulation of these non-convulsive seizures. The involvement of T-type Ca^{2+} channels-mediated burst firing of VB thalamocortical (TC) neurons in the control of ASs was hypothesized by Sorokin (Sorokin et al., 2017). By delivering optogenetic *hyperpolarizing* pulses that triggered bursts in VB TC neurons *in vitro*, they suggested that this type of burst firing is sufficient for the initiation of SWDs in WAG/Rij rats, a genetic rat model of ASs (Sorokin et al., 2017). However, direct excitation of hyperpolarized or depolarized TC neurons *in vivo* was not tested. This issue, together with recent findings showing that T-channels-dependent burst firing of TC neurons is not necessary for AS generation and maintenance *in vivo* (McCafferty et al., 2018), invalidate the requirement of burst firing in the bidirectional control of these seizures by the thalamus as previously suggested (Sorokin et al., 2017).

Using a depolarizing opsin (channelrhodopsin 2, ChR2), here we investigated the ability of direct *depolarization* of TC neurons in the VB of freely behaving GAERS (a well-established AS model) (Depaulis et al., 2016) to elicit ASs during interictal periods, and tested whether these seizures had the same vigilance-state dependence and sensitivity to the anti-absence drug ethosuximide (ETX) as spontaneous ASs (Depaulis et al., 2016; Lüttjohann et al., 2011). We also studied whether light stimulation (LS) of these ChR2-transfected thalamic neurons was able to evoke ASs in the inbred non-epileptic control (NEC) rats, that do not have spontaneous ASs, and in those outbred Wistar rats that do not have spontaneous ASs (Depaulis et al., 2016). Moreover, to test the bidirectional control of ASs by TC neurons, we investigated the ability of LS to block spontaneous ASs in GAERS rats and the phase-dependence of this effect. We found that excitation of ChR2-transfected TC neurons in the VB reliably elicited ASs in GAERS rats during quiet wakefulness but not during slow wave sleep (SWS), and that were blocked by ETX. LS of TC neurons in Wistar rats occasionally led to short ASs during QW and no AS could be evoked in NEC rats during any behavioural stage. Furthermore, excitation of ChR2-transfected TC neurons in GAERS during an ongoing spontaneous AS immediately halted the seizure independent from the SWD phase at which it was delivered. These findings suggest that i) direct excitation of TC neurons provides effective bidirectional control of generalized AS networks, i.e., can initiate or stop these seizures, and ii) the behavioral state-dependence of AS induction may have important implications for understanding AS generation, maintenance, and termination.

2. Materials and methods

Adult male GAERS (n = 10), NEC (n = 5) and Wistar (n = 5) rats bred at Cardiff and Szeged University were provided with normal diet and water *ad libitum* and kept under a light:dark cycle of 12:12 hours with light on at 7:00 am. All experimental procedures were performed in accordance with the UK Animals (Scientific Procedures) Act (1986), the European Community Council Directive (2010/63/EU), and local ethics committee and expert group guidelines (Lidster et al., 2015). All efforts were made to minimize animal suffering and the number of animals used.

GAERS, Wistar and NEC rats (250–300 g) were anaesthetized with isoflurane (2–5 %), positioned in a stereotaxic frame and their body temperature was maintained at 37°C with a heating pad. They were bilaterally injected (1 µl/hemisphere, 1.14e9 GC/µl) with pAAV-CaMKIIα-hChR2(H134R)-mCherry (Addgene plasmid 26975) (Fig. 1A) into the VB thalamus (AP: –3.14 mm, ML: ± 2.80 mm, DV: −5.75 mm) (Paxinos and Watson, 2013) (Fig. 1B), and implanted with bilateral home-made microdrives carrying independently moveable tetrodes and fiber optics targeted to the same region (AP: –2.50 to –4.30 mm, ML: ± 1.4–3.9 mm), as previously described (Taylor et al., 2014). Four gold-plated epidural screws (Svenska Dentorama AB, Sweden) were also implanted in pairs, over frontal (AP: + 2.0 mm, ML: ± 2.0 mm) and (AP: – 2.2 mm, ML: ± 5.5 mm) parietal cortices to record the EEG. Two screws were implanted over the cerebellum and served as ground electrodes. Fiber optics were implanted at −5 to −5.25 mm DV, and kept into optimal position in the days leading up to the recording. Then, they were moved alongside tetrodes, as necessary during the experiments to locate and stimulate $ChR2^{+}$ cells in the VB thalamus. Animals were allowed to recover for at least five days after surgery.

To habituate the rats, they were connected to the recording apparatus and placed in a Plexiglas box within a Faraday cage for 1–2 hours for a few days before the experiments. On the days of the experiments, rats were placed in the box for a habituation period of 30 minutes prior to initiating the simultaneous electrophysiological and video recording that occurred between 10.00 am and 4.00 pm, with the order of recordings being randomized in different sessions for each rat. Thalamic multi-unit activity (MUA) and EEG were recorded using a Neuralynx Digital Lynx SX. AW, QW and SWS were classified by an experienced researcher and defined as periods of motor movement, immobility with or without active sniffing grooming and head movements, and large amplitude slow EEG waves, respectively, with post-hoc analysis of videos being used to confirm visual observations made during the experiment. Notably, the position of the fiber optic and tetrodes were kept constant to test the same population of TC neurons for their ability to evoke ASs in different vigilance states. Blue light stimulation (LS) with laser pulses of 473 nm was delivered to freely moving animals during seizure-free periods of AW, QW and SWS, or upon detection of a SWD with a closed-loop system based on a real-time coastline and in-house

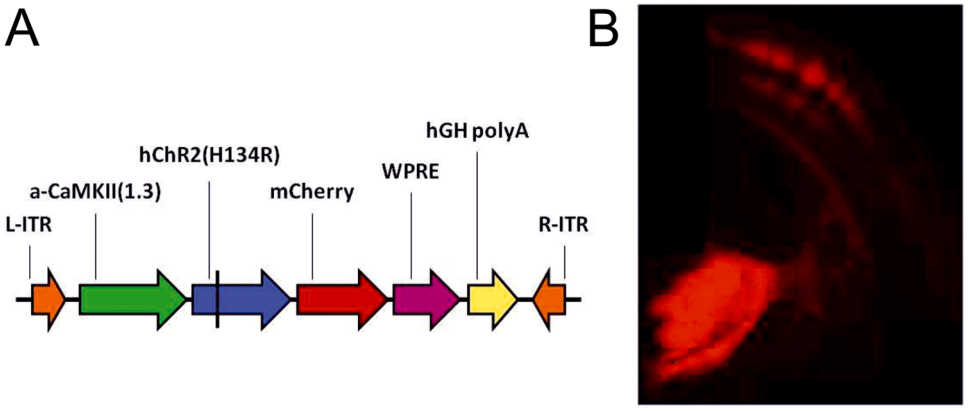

Fig. 1. Channelrhodopsin-2 (ChR2) transfection of TC neurons in the VB. (A) Schematic of the pAAV-CaMKIIα-hChR2(H134R)-mCherry plasmid bilaterally injected into the VB thalamus. (B) Thalamocortical section displaying ChR2-mCherry expression throughout the VB and projections traversing the NRT to the neocortex, with clearly defined barrel walls visible.

developed algorithm using the signal of one of the frontal EEG leads. Note that our algorithm was unable to distinguish between SWDs and the pre-ictal rhythmic 5–9 Hz oscillations (Nikalexi et al., 2025; Pinault et al., 2001). The laser power (5–40 mW) and the duration of the LS (5 ms - 3 s) were varied systematically during the experiments and stimulation consisted of either single pulses or multiple pulses at 7 Hz. In order to deliver the optogenetic stimulation at different times during a SWD, we added a randomly varying delay to the closed loop stimulation, so that it occurred at different times during the 150 ms duration of a SWD. The timing of stimulation to the SWD phase was then assessed post-hoc during the analysis of the data.

SWDs were detected semi-automatically using the SeizureDetect script in Spike2 v7.03 (Cambridge Electronic Design, UK), and were further verified through visual inspection of EEG traces. The presence of immobility in video recordings, together with the presence of SWDs in the EEG, was used to confirm the occurrence of an ASs, as previously described (Venzi et al., 2016; McCafferty et al., 2018; Iacone et al., 2021). Importantly, the behavioral component was not used merely to identify motionlessness, which may also occur during quiet wakefulness, but to detect behavioral arrest that is time-locked to SWDs - typically involving abrupt cessation of ongoing activity such as grooming, exploration, or rearing. In some cases, subtle postural changes or frozen posture could also be observed, allowing clearer differentiation from other behavioral states. This multimodal approach strengthens the identification of SWDs that are behaviorally relevant and consistent with absence seizures. The percentage of induction and blockade of seizures refers to the proportion of stimulations that successfully induced or blocked a seizure, calculated as the number of positively detected seizures divided by the total number of stimulations, expressed as a percentage. Power spectra were computed using MATLAB (R2019a, MathWorks, USA). Saline or a low dose of ethosuximide (ETX) (100 mg/kg) (Sigma, UK) were injected intraperitoneally, when required.

The extent, location, and selectivity of viral transfection, as well as the position of tetrodes and fiber optics, were assessed by collecting brains at the end of the experiments, washing them in PBS, and fixing them in 4 % PFA for 24 hours. Coronal thalamocortical slices (50 μm) that retained the integral connections between thalamus and cortex were then cut and processed for histology. Data from animals with poor transfection ($n = 1$) or misplaced fiber optics and tetrodes ($n = 1$) were excluded from the final analysis. Quantitative data are reported in the text and figures as mean ± s.e.m. (unless stated otherwise), Willcoxon rank-sum, Student's t-tests or one-way Anova were used for statistical analysis as appropriate, and statistical significance was considered for $p < 0.05$.

3. Results

The histological analysis revealed that the VB neurons transfected with the ChR2- and mCherry-containing viral vector (Fig. 1A) were strongly expressing mCherry. There was no staining in the nRT and the transfected VB neurons were identified as TC neurons based on immunostaining of ChR2-mCherry (see Fig. 8 in (David et al., 2013)) and the presence of ChR2-mCherry stained fibers projecting through the nRT up to the primary somatosensory cortex within clearly defined barrel walls (Fig. 1B).

3.1. Induction of ASs

In QW, single-pulse LS of VB TC neurons during interictal periods in GAERS resulted in the reliable generation of ASs, i.e. SWDs in the EEG that were invariably accompanied by behavioural arrest and cessation of ongoing behaviors (Fig. 2A), with an incidence of 73.65 ± 5.1 % ($n = 600$ trials in 6 GAERS, 442 successes) (Fig. 2E). These LS-elicited ASs had behavioural and electrographic properties very similar to those of spontaneous ASs. In particular, the power spectra and autocorrelograms of LS-evoked SWDs were highly comparable to those of spontaneous SWDs (Fig. 2A, B, C). At the cellular level, the thalamic MUA activity of LS-induced SWDs showed the stereotypical switch from the interictal irregular firing to the ictal rhythmic firing of spontaneous SWDs (Fig. 2B, D). Moreover, the duration of LS-elicited SWDs (17.9 ± 2.9 s) was similar to that of spontaneous SWDS (18.5 ± 1.9 s) ($p > 0.05$) as was their frequency (LS-evoked: 7.5 ± 0.3 Hz; spontaneous: 7.4 ± 0.2 Hz; $p > 0.05$) ($n = 500$ LS-evoked SWDs in 6 different stimulation sites, $n = 500$ spontaneous SWDs, in 3 GAERS) (Fig. 3C). Notably, the effectiveness of the single-pulse LS-elicited induction of ASs was not increased by delivering 7 LS pulses at 7 Hz (not shown).

ASs evoked by LS had the same pharmacological profile of spontaneous ones since they were partially blocked by a low dose of ETX (100 mg/kg/ip) (pre-ETX: 79.3 ± 5.7 %, 238/300 trials succesful; ETX: 49.4 ± 6.1 %, 148/300 trials succesful; $n = 3$ GAERS; $p < 0.01$) (Fig. 2E, left plot). However, the duration of the SWDs that were evoked by LS in the presence of ETX was markedly smaller than those elicited before the injection of this anti-absence medication, even when the LS intensity was increased to 40 mW (55.6 ± 13.28 %, 167/300 trials successful) (Fig. 2E, right plot). All together, these findings demonstrate that direct and selective excitation of TC neurons during QW induces ASs in GAERS with similar characteristics to spontaneously occurring ASs.

3.2. Behavioural state-dependent induction of ASs

The ability to induce ASs during interictal periods was heavily influenced by the state of arousal, with higher rates of induction during QW (73.65 ± 5.1 %, $n = 600$ trials in 6 GAERS, 442 successes) compared to AW (25.64 ± 3.7 %, $n = 600$ trials in 6 GAERS, 154 successes) (Figs. 2A and 3A, respectively). The use of shorter (5–25 ms) or longer (200 ms) LS pulses did not significantly affect the vigilance-state dependence of AS induction during QW and AW (Fig. 3C, D). In contrast, LS of TC neurons did not induce ASs during SWS, regardless of the light pulse duration and intensity (incidence: 0.2 ± 0.1 %, $n = 600$ trials in 6 GAERS, 1 success) (Fig. 3B, C, D). Furthermore, increasing the intensity of LS from 5 to 40 mW did not enhance AS induction during SWS (not shown). Additionally, no SWDs or behavioral arrest was induced by either single or repetitive pulses of LS delivered to VB TC neurons in NEC rats during any behavioral state (0 successes in 500 trials from 5 NEC rats), indicating the natural resistance of this non-epileptic control rat strain to both spontaneous and LS-induced ASs.

It is well known that some Wistar rats (10–15 %), as well as other outbred rat strains, such as Sprague-Dawley and Long Evans (Buzsáki et al., 1990; Polack and Charpier, 2006; Shaw, 2004) show spontaneous ASs, though shorter in duration and with generally lower SWD power than well-established mouse and rat AS models. Thus, it was important to test whether optogenetic stimulation would elicit ASs in "normal" Wistar rats, i.e. Wistar rats that do not have spontaneous ASs. We found that in these "normal" Wistar rats that did not exhibit spontaneous seizures, ASs could be reliably induced by LS of VB TC neurons during QW (but not during AW and SWS) (Fig. 3E, F) (AW: 13 %, 52 successes, QW: 66 %, 264 successes, SWS: 4 %, 16 successes in 400 trials from 4 rats). These seizures had the same frequency (7.77 ± 0.06 Hz) (Fig. 3G) as the spontaneous ones in GAERS but they were much shorter in duration (4.95 ± 0.1 Hz) (Fig. 3F).

3.3. Block of ASs

Having established that direct LS-dependent depolarization of TC neurons delivered during interictal periods could elicit ASs, we next investigated the ability of the same LS to block ASs. Whereas sham stimulation (0 mW) was ineffective, we observed that a single, short (5 ms) pulse of LS of VB TC neurons could block the vast majority (74.88 %) of SWDs (331/442 SWDs blocked in 4 GAERS) and associated behavioral arrest and the rat started to move immediately after the stimulation, i.e. the ASs had been blocked (Fig. 4A, C). Increasing the LS

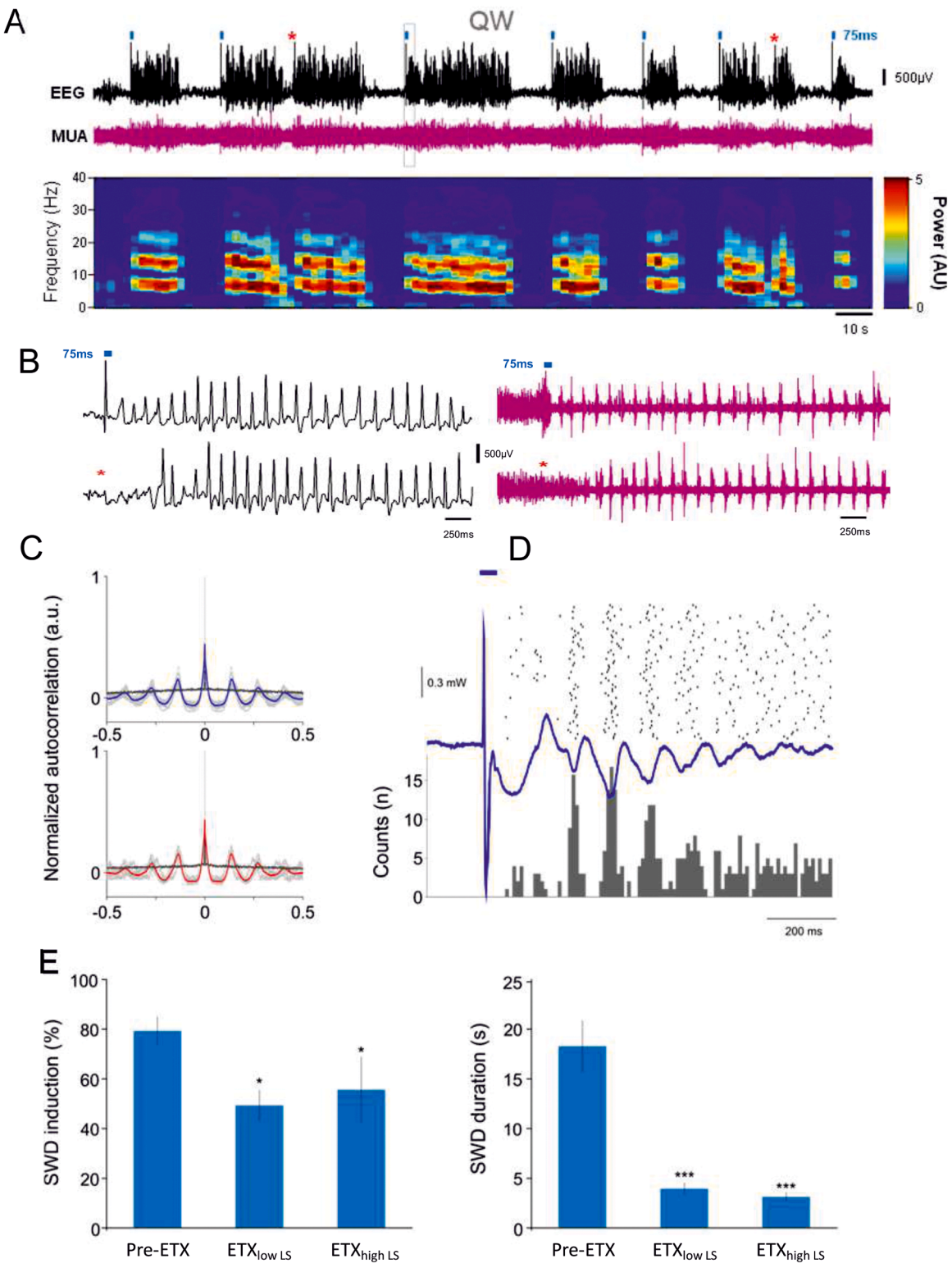

Fig. 2. Selective light stimulation (LS) of VB TC neurons elicits ETX-sensitive SWDs in GAERS. (A) Simultaneous EEG (top, black trace) and thalamic multiunit activity (MUA) (bottom, red trace) during LS (75 ms pulse, blue bar) of VB TC neurons during quiet wakefulness (QW) in GAERS. Two spontaneous SWDs, and corresponding thalamic MUA, are marked by red asterics. The corresponding EEG spectrogram (below) show similar high power in the 7–8 Hz and first harmonic at 14–16 Hz for both LS-induced and spontaneous SWDs. (B) The initial portion of the SWD elicited by the third LS (black box in A) and the preceding spontaneous SWD are illustrated at a faster timebase than in (A). They show striking similarities in both the EEG and the switch from irregular to rhythmic firing at 7 Hz of the thalamic MUA between the LS-evoked SWD (top traces) and the spontaneous SWD (bottom traces). Note also the strong similarity of the MUA autocorrelations shown in (C) (bottom: spontaneous SWD, top: evoked SWDs; gray: 10 individual sweeps, black and blue: averages, black: average interictal). (D) Peristimulus time histogram and spike rasters (centered on the LS time, blue bar on top) during SWDs. (E) Bar graphs showing the effect of ETX (100 mg/kg/ip) on the percentage of induction (left plot) and the duration (right plot) of LS-elicited SWDs. Note the similar effect of ETX when using both low (10 mW) and high (40 mW) LS intensity, ($ETX_{low\ LS}$) and ($ETX_{high\ LS}$), respectively.

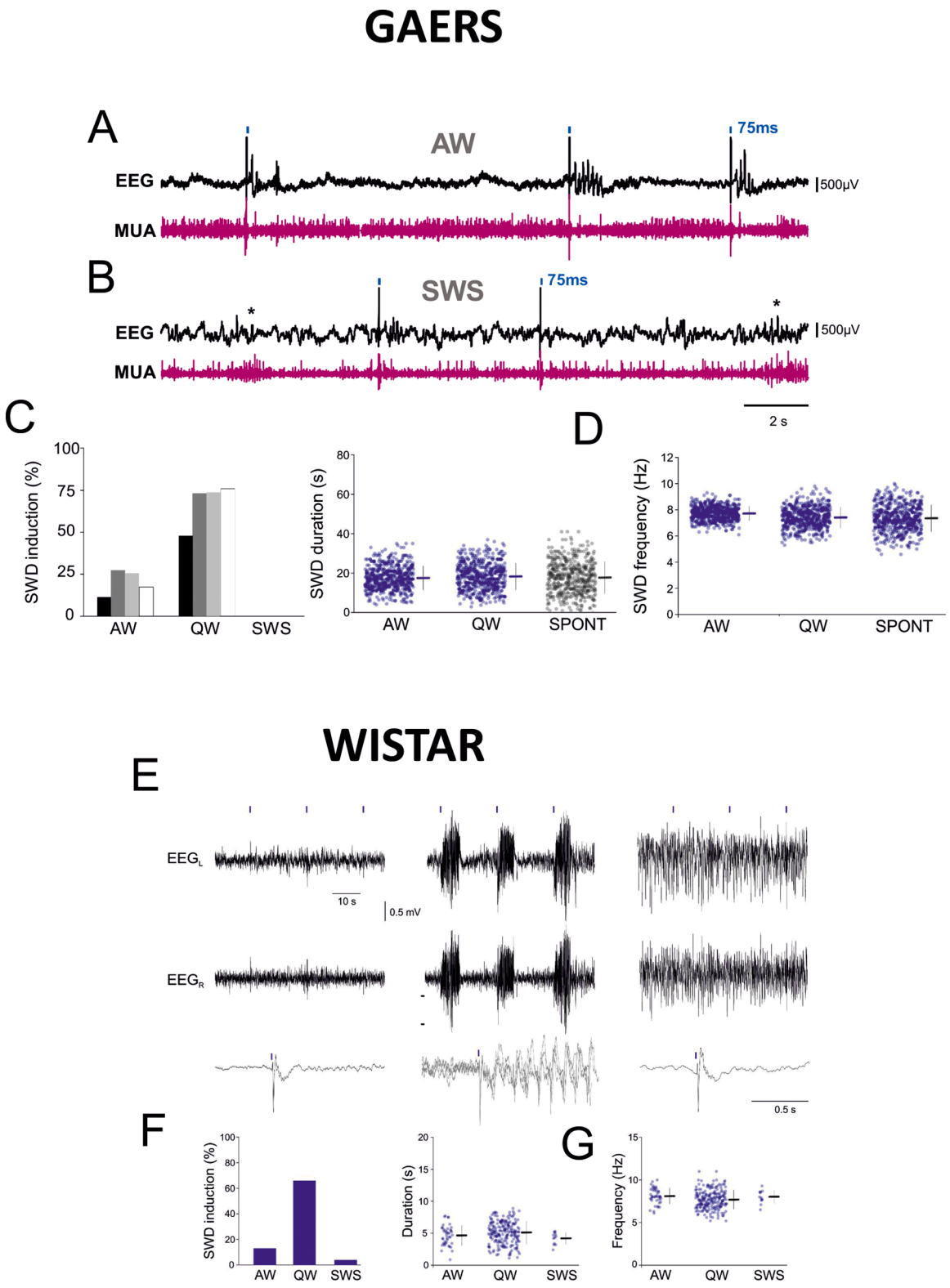

Fig. 3. Behavioural-state dependence of SWD induction in GAERS and Wistar rats. (A) Example EEG (top, black trace) and thalamic MUA (bottom, red trace) show that LS during active wakefulness (AW) does not reliably generate SWDs in GAERS. (B) Example EEG and thalamic MUA showing that LS during slow wave sleep (SWS) fails to elicit SWDs in GAERS. Two spontaneous sleep spindles are marked by asterisks. (C) Bar graphs showing the percentage of SWDs induced during QW, AW and SWS by different lenghts of LS in GAERS. (D) Scatter plots comparing the duration (left) and frequency (right) of SWDs induced by LS during QW and AW with those of spontaneous SWDs in GAERS. (E) Left and right EEG (top and middle traces) during 3 consecutive LS (10 ms pulse, blue bar) delivered to VB TC neurons show brief SWDs during QW, but not during AW and SWS, in normal Wistar rats. The bottom traces are averaged EEG responses (for AW and SWS) and 4 overlayed sweeps for QW (aligned on the LS time. (F) Bar graphs showing the percentage of SWDs induced during QW, AW and SWS in Wistar rats. (G) Scatter plots comparing the duration (left) and frequency (right) of the SWDs induced by LS during QW, AW and SWS in Wistar rats.

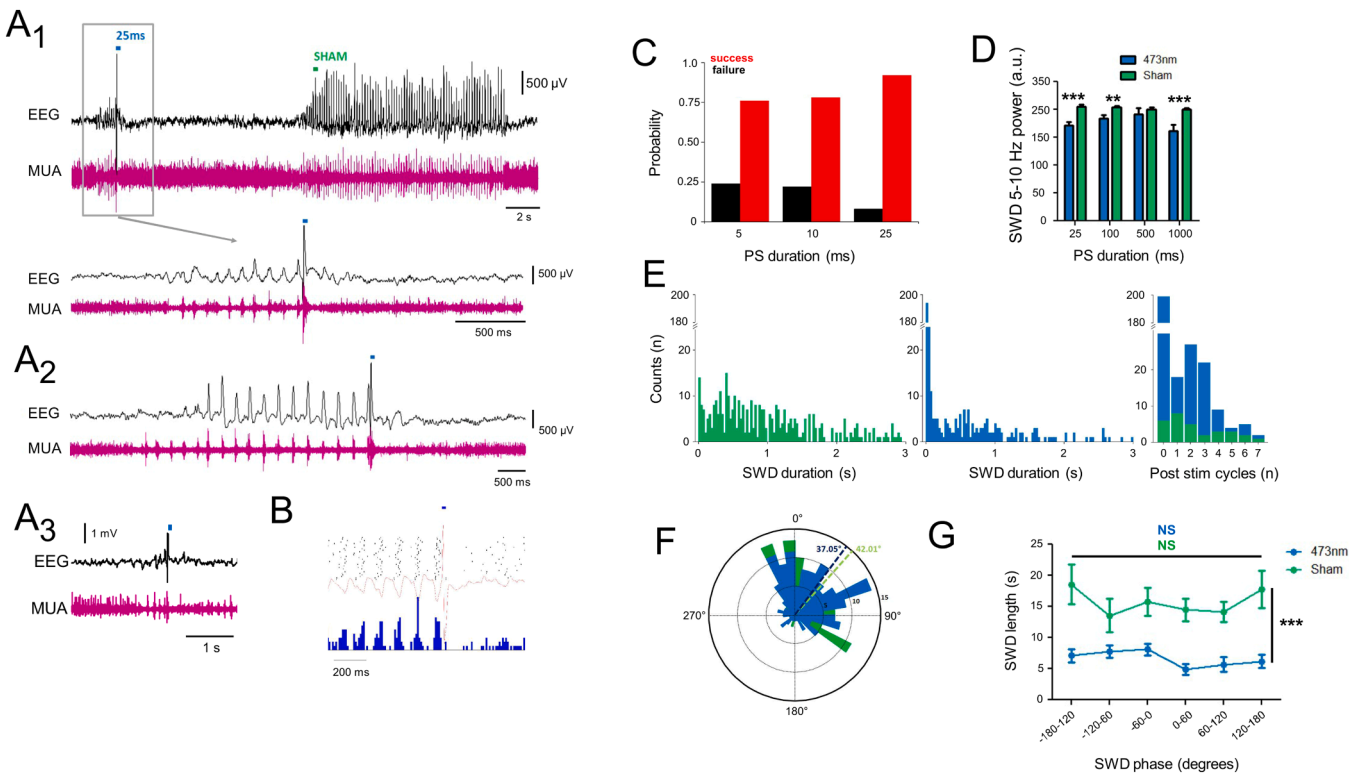

Fig. 4. Selective LS of VB TC neurons blocks ongoing SWDs in GAERS. (A1) Representative EEG (black) and thalamic MUA (red) traces demonstrating that selective LS of VB TC neurons (single 25 ms light pulse, 20 mW), but not sham stimulation (0 mW), terminates naturally occurring SWDs. The boxed segment is enlarged below. (A2) LS during a more established seizure, characterized by larger-amplitude SWDs, immediately terminates the seizure and re-establishes irregular firing in the MUA recording. (A3) LS is highly effective in blocking SWDs at their onset. (B) Peristimulus time histogram and rasters of EEG SWDs centered on the LS (blue bar). The red line represents the averaged EEG. (C) Probability of SWD termination by LS of VB TC neurons as a function of stimulus duration. (D) Comparison of 5–10 Hz EEG power following LS versus sham stimulation, showing significantly reduced power after LS. (E) Distribution of SWD durations (0–3 s) for sham stimulation (left) and LS (middle). Data for the first second post-stimulation (first 7 SWD cycles) are overlaid in the left plot. (F) Rose plot depicting stimulation onset phases for LS (blue) and sham (green) trials, with mean vectors represented by dashed lines. (G) Phase-specific effects of sham (green) and LS (blue) on SWD duration. In (F) and (G), zero indicates the peak of the SWD spike.

pulse duration to 25 ms increased the probability of an ASs block to 92 % ($p > 0.05$, one-way Anova, Fig. 4C).

To assess whether the efficacy of seizure interruption by LS depended on the timing of stimulation relative to seizure progression, we compared early (within 500 ms of onset) and late (>500 ms) LS applications. LS applied early in the SWD resulted in seizure termination in 54.91 % of trials (67/122) (Fig. 4A3), while LS applied during the developed phase terminated 82.5 % of SWDs (264/320) (Fig. 4A2). This difference was not statistically significant ($p > 0.079$, Fisher's exact test), suggesting that thalamic stimulation is capable of disrupting SWDs both at their onset and during their later, more developed phases. Notably, at the cellular level the block of SWDs was invariably accompanied by a change in the thalamic MUA from rhythmic to irregular firing (Fig. 4B). Moreover, 55 % and 68 % of ASs were blocked immediately and by the third SWD cycle, respectively, following the LS (Fig. 4F) (see also Figure 3A1-A3). Even in ASs that were not blocked by PS, the SWD power was smaller than that of seizures subjected to sham stimulation Fig. 4D. The efficacy of blocking seizures was not dependent on the phase of the SWD cycle at which the LS was administered (Fig. 4F, G).

4. Discussion

The main findings of this study are that: i) brief, selective and direct depolarization of TC neurons can block spontaneously occurring ASs in freely moving GAERS; ii) direct LS-evoked depolarization during interictal periods can elicit ASs similar to spontaneous ones; iii) ASs are more easily evoked by LS delivered during QW than AW and never during SWS; iv) brief LS of VB TC neurons during QW could generate ASs in freely moving Wistar rats though with a very low probability; and v) single or repetitive direct excitation of TC neurons fails to elicit ASs in freely moving NEC rats. Our LS of ChR2-transfected TC neurons led to their direct depolarization of these neurons in freely moving GAERS. The resulting tonic firing generated by a single brief optogenetic pulse (that leads to direct depolarization of TC neurons) is shown here to be highly effective in eliciting or halting ASs depending only on whether it is delivered interictally or ictally, respectively: that is, depolarization of TC neurons during QW can switch the CTC network from an apparently normal function to an AS pathophysiological activity and vice versa. Interestingly, whereas we show that selective stimulation of TC neurons abolishes ASs is a SWD-phase independent manner, sensory stimuli that do not block SWDs are more effective in reducing the firing of both cortical and thalamic neurons when delivered during the SWD spike than during the wave (Williams et al., 2020).

While the role of the somatosensory CTC network in generating SWDs in genetic rat AS models is well known (Crunelli et al., 2020), the dual function of VB TC neurons in generating and terminating SWDs is novel. In particular, the ability of direct stimulation of TC neurons to elicit ASs indicate that excitation of these thalamic neurons can force the trajectory of the generalized CTC network activity to re-enter the paroxysmal state of an AS *before* the CIN would bring about the next spontaneous seizure. Essentially, the interval between two spontaneous consecutive seizures is longer than the refractory period of ASs. This suggests that whatever process stops an AS is quickly reset, enabling the start of a new seizure. Furthermore, the fact that TC neuron LS can block ASs regardless of the phase of the SWD cycle indicates the relative

fragility of the overall paroxysmal oscillators. This duality, whereby the same stimulation may either elicit or terminate seizures depending on the current thalamocortical network state, is consistent with principles from dynamical systems theory, in which small perturbations near critical points can push the system toward or away from seizure-like attractor states (Breakspear, 2017; Lytton, 2008). In other words, an AS may end due to intrinsic properties of individual neurons or networks within the CTC circuitry disrupting the paroxysmal cycle, or due to external synaptic input affecting the entire network. The evidence from children with ASs suggests that there may be a vulnerable state at the beginning of some seizures, which results in more significant physiological changes and altered levels of consciousness (Guo et al., 2016). Indeed, the cortical 5–9 Hz oscillations, that were originally described in GAERS rats by Pinault et al. (2001), were directly shown recently to represent a more vulnerable state to cortical and intralaminar thalamic stimulation than EEG periods that lacked these oscillations (Nikalexi et al., 2025).

Rhythmic, synchronized thalamic firing is important for the generation of ASs and controlled by cortically driven, feed-forward thalamic inhibition (McCafferty et al., 2018). The results of the present study clearly show that the most effective way of evoking an AS is the specific stimulation of VB TC neurons during QW, a state in which both cortical and thalamic neurons are prone to low frequency oscillations in normal rodents (Molnár et al., 2021; Reinhold et al., 2023). Notably, the prevalence of SWDs induced by electrical stimulation of the CIN in WAG/Rij rats during QW (Lüttjohann et al., 2011) is smaller (~20 %) than the one we observed (~75 %), probably due to differences in the type of stimulation (electrical *vs* optogenetic, respectively) and the lack of neuron-type selective activation in the former study. In contrast, optogenetic stimulation of TC neurons during AW yielded less effective results, in line with the lack of low frequency oscillations during this state (McGinley et al., 2015). Moreover, LS of TC neurons during SWS never resulted in the generation of an ASs in agreement with the rare occurrence of spontaneous ASs in WAG/Rij rats during SWS (Smyk et al., 2019). This result is surprising, given the membrane potential changes dependent on brain state and the biophysical properties of TC neurons. Specifically, the membrane potential of TC neurons is most hyperpolarized during SWS than AW and QW (Hirsch et al., 1983; Urbain et al., 2019): therefore, a depolarizing input, as the one provided by LS of ChR2-transfected TC neurons most likely generates a T-type Ca^{2+} channel-mediated burst firing. On the other hand, when the same LS is applied during AW the less hyperpolarized membrane potential of TC neurons will likely result in the generation of tonic firing in these neurons. Conversely, hyperpolarizing TC neurons with NpHR during wakefulness will likely generate T-type Ca^{2+} channel-mediated burst firing at the end of the LS, which has been shown to be able to trigger ASs (Sorokin et al., 2017). Thus, TC neuron burst firing may be sufficient to drive SWDs. But, is it necessary?

The contribution of thalamic T-type Ca^{2+} channels to ASs generation has long been debated and is still controversial. On one hand, *in vitro* recordings suggested that the T-type Ca^{2+} channel mediated burst firing of TC neurons plays a pristine role in generating the SWDs of ASs (Bal et al., 1995), but pharmacological blockade of T-type Ca^{2+} channels in TC neurons in freely moving rats fails to suppress spontaneous ASs (McCafferty et al., 2018), indicating that T-type Ca^{2+} channels are not necessary for the expression of SWDs. The behavioural vigilance state-dependent expression of SWDs by selective activation of TC neurons shown in this study also support the view that the burst firing by these thalamic neurons is not necessary for SWD generation. If this were the case, SWDs would also be generated during SWS, where given the hyperpolarized membrane potential of TC neurons a depolarizing stimulation (such as the one provided by LS of ChR2-transfected TC neuron) would most likely trigger burst firing in TC neurons: yet no ASs are generated during this behavioral state. In addition, recent findings have reported a significant coordination between global EEG microstate dynamics and general local cortical assembly activity during periods of QW, but not during sleep (Boyce et al., 2023). This coordination may explain why LS of TC neurons during QW is more effective in triggering synchronized oscillations, including SWDs. Taken together, these results provide additional evidence against the necessity of low threshold spikes of TC neurons for the generation of ASs.

CRediT authorship contribution statement

Morais Tatiana P.: Writing – review & editing, Investigation. **Nagy Olivér:** Investigation, Data curation. **Taylor Hannah F.:** Writing – review & editing, Investigation, Data curation. **David Francois:** Writing – review & editing, Data curation. **Mátyás Ferenc:** Investigation, Data curation. **Lorincz Magor:** Writing – original draft, Supervision, Funding acquisition, Data curation, Conceptualization. **Crunelli Vincenzo:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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