Brain State Dependent Activity in the Lateral Geniculate Nucleus

Benedek Molnár

Summary of the PhD Thesis

Supervisor: Magor L. Lőrincz PhD
Associate Professor

Doctoral School of Biology
University of Szeged
Department of Physiology, Anatomy and Neuroscience

2019

Összefoglaló

Az agyiállapot-függő talamokortikális (TC) sejtaktivitás fontos szerepet játszik a szenzoros kódolásban, illetve agyi oszcillációkban és kognícióban. A corpus geniculatum laterale (CGL) közvetíti a vizuális információt az agykéregbe, ám a CGL állapotfüggő spontán aktivitása, ill. a vizuális kiváltott válaszai egyelőre nem egyértelműek. Éber, fejbefogott egerek CGL-neuronjaiban történő pupillometriai, extracelluláris és intracelluláris mérések kombinációjából kiderül, hogy a TC-sejtek és a valószínűsíthetően lokális interneuronok fordított viszonyban vannak egymással az éberség szintjétől függően. Míg a TC-neuronok aktivitása pozitívan korrelált az éberség szintjével, addig az lokális interneuronok negatívan korreláltak. Ezen kívül a talamikus sejtek vizuális kiváltott válaszainak az orientációs finomhangolása megváltozik az agyi állapotok hullámzásával. Az intracelluláris elvezetések alapján kimutattuk, hogy CGL TC-sejtjeinek membránpotenciálja szoros korrelációt mutatnak a pupillaátmérő változásaival. A kortikotalamikus visszacsatolás inaktiválásakor, amit a muscimol alkalmazásával értünk el a dura mater felületén, szignifikánsan csökken a korreláció az agyi állapotok és a talamikus sejtek aktivitása között. További kísérleteink alapján a laterális hypothalamusból (LH) a nucleus raphe dorsalisba (NRD) érkező GABAerg axonok fotostimulációja növeli az NRD sejtjeinek a tüzelését, feltételezhetően diszinhibíción keresztül.

Introduction

Brain states can fluctuate on various timescales and can profoundly influence neural and behavioral responses. Detailed observation of rapid state fluctuations can significantly account for variability and allow for a more accurate exploration of the neural mechanisms of behavior at all levels, from sensory coding to decision making and motor responses. This is most obvious when comparing spontaneous neuronal activity or responses to sensory stimuli between states of sleep and wakefulness, but recently, the prominent influence of spontaneous variations within the waking state on both cortical neuronal responses and perceptual abilities has been documented in both humans and rodents.

Anatomically, the thalamus is a nuclear complex located in the diencephalon and comprising of four parts (the hypothalamus, the epithalamus, the ventral thalamus, and the dorsal thalamus). Functionally, the thalamus is a relay center subserving both sensory and motor mechanisms. Multiple cortical areas receive afferents from a single thalamic nucleus and send back information to different thalamic nuclei. The corticofugal projection provides positive feedback to the "correct" input, while at the same time suppressing irrelevant information. Sensory pathways (except the olfactory pathway) are relayed by specific thalamic nuclei that all project to specific corresponding cortical areas, and in turn, receive exclusive and specific inputs from cortical areas (layer 6) to which it projects. Some thalamic nuclei do not receive sensory inputs, but instead are part of either higher-order associative corticothalamic circuitry, the midline and

intralaminar thalamus and the extra-thalamic GABAergic reticular shell. These are implicated in sensory information (secondary) integration, arousal and attention and the genesis of sleep spindles, respectively. The lateral geniculate nucleus (LGN) is the visual relay nucleus of the thalamus, which is known to primarily taking input from the retinal ganglion cells (retinothalamic pathway) and after local processing, it projects visual information to layer 4 of the primary visual cortex (V1) (visual thalamocortical pathway). Analysis of LGN-dependent fMRI activity in non-V1 extrastriate cortex suggests that the LGN also projects to regions further downstream in the visual pathway. While the LGN receives substantial input from the retinal ganglion cells, it receives far greater innervation from higher-order regions, such as modulatory feedback from layer 6 of V1 and the thalamic reticular nucleus (TRN). It also receives varying degrees of modulatory activity from the raphe nuclei (serotonergic), pedunculopontine and laterodorsal tegmental nuclei (cholinergic), and locus coeruleus (noradrenergic).

The membrane potential of LGN cells has long known to be correlated with different brain states. During non-rapid eye movement (NREM) sleep the membrane potential is effectively hyperpolarized, and shows oscillatory activity with typical bursts, while the membrane potential becomes depolarized by 8–12 mV when the brain state is shifted to rapid eye movement (REM) sleep or to quiet wakefulness. Strikingly, while the major excitatory glutamatergic input to TC neurons in the LGN is originating from the retina, and cortical modulation was thought be conveyed through local GABAergic interneurons, studies have shown that cortical axons make twice the number of synapses on relay

cells than on interneurons. Furthermore, activation of corticothalamic fibers results in a slow depolarization of thalamic relay cells through reduction of a potassium current through the activation of metabotropic glutamate receptors (slow EPSP). This slow depolarization blocks rebound burst firing and promotes single spike activity, thereby promoting a state of thalamic activity that is associated with enhanced sensory transmission and arousal.

The relay is disengaged not by silencing relay cells but, rather, by forcing these cells to burst rhythmically and independently of driver input. Slow waves and spindles occur largely during slow-wave sleep, while gamma waves are present throughout brain states, but are most prominent in the alert and attentive animal. Thalamocortical (TC) cells show membrane potential bistability that accounts for oscillatory activity. Low voltage-activated T-type $Ca2^{+}$ channels are important components of the large array of voltage-dependent membrane channels used by neurons to express different network dynamics. The primary contribution of the low threshold, transient Ca^{2+} current to the subthreshold electrical activity of central neurons has long been considered to be the low-threshold Ca^{2+} potential (LTCP).

In cortical areas state dependent neuronal activity is determined by both thalamic and neuromodulatory inputs. Intrinsic and network generators exist in both the neocortex and the thalamus, which are capable of locally eliciting oscillations at alpha (8–13 Hz), theta (4–7 Hz), spindle (7–14 Hz), slow (<1 Hz) and delta (0.5–4 Hz) frequency. Classically, slow wave sleep is characterized

by a high density of cortical field potential power at 0.5–4 Hz, and reduced muscle tone, while waking is associated with a relative suppression of low frequency activity (usually termed “activation” or “desynchronization”), increased muscle tone, and behaviorally relevant eye movements. Recently, intracellular membrane potential (V_m) and local field potential (LFP) recordings from cortical neurons collected simultaneously with pupil diameter in head-restrained, spontaneously locomoting or whisking mice revealed a marked relationship between pupil size, low frequency (2–10 Hz) fluctuations in membrane potential/LFP, and exploratory behaviors.

The hypothalamus, more specifically the lateral hypothalamus (LH), is known for its key function as a homeostatic regulator, and integrator of environmental stimuli and internal signals. Although an indirect connection to the LGN, empirical evidence has shown that a subset of GABAergic lateral hypothalamus (LH_{GABA}) cells sends monosynaptic connections to (also GABAergic) TRN cells, and that these cells exerted a strong $GABA_A$-mediated inhibitory action on TRN cells during spontaneous NREM sleep-to-wake transitions.

Although DRN is most often implicated in mood regulation, it is fair to assume that it also has a role in sleep regulation as well, since the main pathophysiological mechanisms involving DRN, such as depression, is frequently accompanied by sleep disturbances. Also, various waking brain states beside sleep-wake cycles can be suspected to be influenced by the DRN, as its major neuromodulator serotonin rapidly influences sensory, motor, and cognitive functions.

Materials and Methods

Our experiments were conducted on 17 adult male or female C57BL/6 mice which were awake, drug-free and head-restrained throughout the recording sessions. Prior to surgical preparation, the animals were anaesthetized with Isoflurane (dose: 1 L/min 1-1.5% isoflurane and 99-98.5% O_2) until surgical plane anesthesia was achieved, i.e., negative paw withdrawal reflex. During surgical preparation the skull of the animal was exposed by removing the skin overlying the cranium and a stainless-steel head post cemented over the frontal suture with dental pattern resin. Craniotomy positions were marked with a permanent marker pen at the following stereotaxic coordinates. 5 days after the surgical preparation the mice were handled gently each day in order to reduce excessive stress or anxiety during the recording sessions. On the day of the recording, craniotomy was performed at the previously marked stereotaxic positions under isoflurane anesthesia (dose: 1 L/min 1-1.5% isoflurane and 99-98.5% O_2) and mineral oil was applied on the dural surface to prevent dehydration. Finally, mice were transferred to a recording setup where their head posts were fixed to a custom clamping apparatus and recording sessions started at least 30 minutes following awakening.

Single-unit and local field potential (LFP) recordings were performed from the LGN and V1 structures with either borosilicate glass micropipettes filled with 0.5 M NaCl solution containing with 1.5% w/v Biocytin or Silicon probes (single shank, 32-channel). In the LGN, high-

impedance sharp electrodes (10-80 MΩ) were used, while recordings in V1 were performed using lower resistance electrodes (3-5 MΩ). Intracellular recordings, using the current-clamp technique, were performed with standard wall glass microelectrodes filled with 1 M potassium acetate (impedance 30-50 MΩ). Recorded neurons were included in the data set only if their resting membrane potential during the active period was more hyperpolarized than -45 mV and had overshooting action potentials. The biological signals were pre-amplified with Axon HS-9A headstages and amplified with an Axoclamp 900A amplifier (gain: 50-100x) and filtered (0.1 Hz -200 Hz for LFP, 0.3-6 kHz for units, DC-6 kHz for intracellular recordings). The amplified signals were then digitized with a CED Power3 1401 AD converter at 30 kHz sampling rate and Spike2 software was used for data acquisition. To confirm the morphology and location of the recorded neurons as TC neurons in the LGN, we also performed juxtacellular labeling of LGN cells (n=7) by applying pulses of anodal current (1-3 nA, 500 ms, 50% duty cycle) for 2-5 minutes, which allowed Biocytin from the micropipette solution to enter the cells. At the end of the recording sessions, mice were overanesthetized, their brain removed and transferred to 4% paraformaldehyde (PFA) solution in 0.1 M phosphate buffer (PB) for overnight storage at 4°C. Next day, the brain was washed in standard PB solution and 50 µm slices containing the LGN were cut with a VT1000S vibratome. During the histological processing, the slices were cryoprotected with 10% and 20% sucrose solution, cells were opened with a freeze-thaw method and TBS-Tween20 to be able to conjugate the Biocytin in labelled

cells with Cy3-Streptavidin as secondary antibody. After 2 hours of incubation with Cy3-Streptavidin, the slices were mounted on glass microscope slides and covered with cover slips. A BX60 fluorescent microscope and a Surveyor software were used to visualize the labeled neurons.

For in vivo optogenetic activation of LH axons in the DRN, Vgat-IRES99 Cre mice were injected with 150 nl AAV1-CAGGS-FLEX-CHR2-td tomato-SV40 bilaterally into the LH. Following 2 weeks of postinfection DRN neurons were recorded in awake head restrained mice using a multi-site Silicone electrode (Neuronexus, 32 channel, linear) coupled to an optical fiber.

For inactivation of the V1, we microinjected muscimol, a $GABA_A$ receptor agonist (200 nL, 1 mM), through a glass pipette (15 μm tip diameter) at coordinates 3.3 mm posterior; 2.3 mm lateral to Bregma at a depth of 0.5 mm from brain surface. The correlation of the LGN neuron baseline activity and pupil diameter was quantified and compared with control. Saline injections (200 nl) into the V1 did not affect the correlation of LGN neuron baseline activity and pupil diameter.

Pupillometry was conducted with an infrared camera operating at 50 fps focused on the ipsilateral eye of the animal illuminated with an infrared LED (850 nm). For the visual evoked responses, we placed a PC screen at a ~20 cm distance to the contralateral eye of the mouse displaying moving gratings of 8 different orientations in pseudorandom order.

Data analysis was performed offline with custom-written MATLAB routines and ImageJ plug-ins. Recordings during stages of natural sleep (as confirmed by cortical LFP analysis) have been excluded from data analysis. Pupillometry was carried out offline on recoded AVI files using a custom ImageJ plug-in. For comparing the FRs of thalamic neurons with respect to the pupil diameter, the upper and lower terciles of the pupil diameter distributions were used. LGN neurons were identified as TC or interneurons based on either their morphology, action potential duration (<0.35 ms for LGN interneurons, >0.35 ms for TC neurons), and action potential height ratio. A burst in thalamic neurons was defined as a cluster of spikes consisting of minimum of two action potentials, a maximum interspike interval of 10 ms, and had to be separated from other bursts by more than 100 ms. Brain states were detected from the V1 LFP signal using a semi-automated level threshold method in Spike2. Visual evoked responses were determined by comparing the firing rate during three periods of time: (1) 1 s before the appearance of the grating image, (2) 1 s during still image presentation and (3) 1 s of drifting grating. We used Wilcoxon rank-sum test to determine two p-values (P1 and P2). P1 is from comparing the firing rates of (1) and (2), and P2 is from comparing (1) and (3).

Results

The spontaneous activity of LGN is correlated with arousal

Extracellular and intracellular recordings of morphologically identified LGN neurons with simultaneous LFP in the V1 and pupillometry in awake head-restrained mice revealed the brain state-dependent activity of thalamic neurons in the LGN. The pupil diameter of awake mice spontaneously fluctuated during the recordings, with states of quiet wakefulness (QW) associated with constricted pupil and periods of active wakefulness accompanied (AW) by pupil dilation. States of QW were characterized by large-amplitude slow V1 LFP fluctuations, and small pupil diameter, while periods of active wakefulness (AW) by small-amplitude fast fluctuations in the LFP recorded in V1 with prominent pupil dilation. State transitions were accompanied by prominent changes in the activity of LGN neurons as well. Consistently enough, QW to AW transitions led to an increase in firing, and AW to QW transitions led to a decrease in firing in the majority of LGN neurons.

The increased firing rate in the majority of LGN cells was associated with cortical desynchronization in V1 and pupil dilation, while the marked drop in neuronal activity when pupil diameter decreased indicated relaxed wakefulness with synchronized activity in the neocortex and the replacement of high frequency brain waves with slower oscillations. Strikingly, a subset of LGN neurons characterized by high baseline FR also showed state transition-related changes of baseline activity. In this

neuronal subset, QW to AW transitions led to a decrease in firing rate, but AW to QW transitions resulted in an increase in firing, leading to the suspicion of them being local interneurons. To further drill down in the mechanism of state dependent activity of LGN neurons, we quantified the correlation between neuronal firing rates (FR) and pupillometry, and found three functionally distinct groups of cells: non-modulated, positively correlated (regarded as TC neurons), negatively correlated (regarded as putative interneurons) cells. A smaller percentage of neurons had a baseline activity that did not correlate with the pupil diameter, while the majority did. In most of the state-modulated neurons, FRs showed a statistically significant positive correlation with pupil diameter. Perhaps the most astounding finding was that we recorded a subset of neurons showing negative correlation to pupil diameter. The duration of the action potentials in these neurons was significantly narrower than in TC neurons, and the waveform of their action potential was more biphasic.

Corresponding publication: Molnár et al., 2021, Cerebral Cortex Communications.

The membrane potential of LGN TC neurons is correlated with brain states

To elucidate the intracellular mechanisms underlying the state-dependent fluctuation of thalamic neurons, we performed intracellular recordings of LGN TC neurons of awake mice while simultaneously monitoring the pupil diameter and recorded the LFP and multi-unit activity (MUA) in V1. In all the neurons recorded, we found an

apparent correlation between the membrane potential and pupil diameter, such that periods of pupil constriction were associated with low baseline FRs (6.98±3.19 Hz), hyperpolarized membrane potentials (−63.0±2.3 mV) and burst firing in two of the neurons recorded. Importantly, these bursts were recorded at a membrane potential inconsistent with LTS and more akin to HT bursts. Periods of pupil dilation, on the other hand, were associated with high frequency (19.96±10.98 Hz) tonic action potential output and less hyperpolarized membrane potentials.

Corresponding publication: Molnár et al., 2021, Cerebral Cortex Communications.

Visually evoked responses of LGN TC cells

Next, we asked whether the visual responses elicited in TC cells are state dependent. 31 of 98 recorded LGN neurons (32%) were evaluated, as they elicited significant visual responses defined as statistically significant increase in firing rate compared to baseline activity. Analysis of the visual responses and calculating the orientation selectivity index (OSI) values revealed a slight alteration of orientation selectivity in a brain state dependent manner. Strikingly, we observed a counterintuitive change in orientation selectivity, namely in the states when the normalized pupil size was greater than 0.5 (regarded as AW), more orientations evoked significant responses in TC cells (i.e. the OSI value decreased) than in the state when normalized pupil size was smaller than 0.5 (regarded as QW) [Unpublished data].

Corticothalamic modulation is hindered by visual cortex inactivation

We reasoned that corticothalamic projections are, at least in part, responsible for regulating LGN activity, thus we monitored the effects of inactivating the cortical feedback from V1 to the LGN with muscimol microinjections and its effect on the arousal-dependent activity of LGN neurons. In the control condition, the FR and pupil diameter changes are relatively simultaneous but following V1 inactivation periods of dilated pupil are not always followed by an increase in FR and FR changes do not always coincide with a change in pupil diameter. The FR of individual neurons before and after V1 inactivation varied considerably but did not reach statistical significance as a group. When comparing the correlation of thalamic single units and pupil diameter before and following V1 inactivation, we found a significant decrease suggesting that corticothalamic input from V1 is at least partly responsible for the state-dependent activity in LGN TC neurons.

Corresponding publication: Molnár et al., 2021, Cerebral Cortex Communications.

Lateral hypothalamus axons inhibit GABAergic DRN neurons

To test for functional connections from LH_{GABA} projections to DRN neurons, we recorded extracellular single unit activity from DRN neurons of awake, head-restrained VGAT-cre mice infected with AAV1-CAGGS-336 FLEX-CHR2-tdTOM-SV40 in the LH while photo-

stimulating ChR2-expressing LH_{GABA} axons in the DRN. Comparison of the activity of DRN neurons recorded in the presence and absence of LH_{GABA} axonal photostimulation confirmed the suppressive effect in a subset of DRN neurons (2/12, 17%), while the activity of the remaining neurons was increased (10/12, 83%). The overall activity of DRN neurons was slowly (~200 ms), but persistently (~1 sec) increased.

Corresponding publication: Gazea et al., 2021, The Journal of Neuroscience.

Co-author Statement

I, the undersigned, hereby declare that I have not nor will I use the results of the below publication for my doctoral dissertation, excluding the following parts: surgical preparation of mice for *in vivo* photostimulation of LH axons in the LGN and the *in vivo* photostimulation itself in awake mice. These results are my contribution to the below publication:

- Mary Gazea, Szabina Furdan, Péter Sere, Lukas Oesch, **Benedek Molnár**, Giuseppe Di Giovanni, Lief E. Fenno, Charu Ramakrishnan, Joanna Mattis, Karl Deisseroth, Susan M. Dymecki, Antoine R. Adamantidis, Magor L. Lőrincz (2021) Reciprocal lateral hypothalamic and raphé GABAergic projections promote wakefulness. *The Journal of Neuroscience* **41**(22) 4840-9; doi: https://doi.org/10.1523/JNEUROSCI.2850-20.2021

.....

Benedek Molnár
PhD candidate, co-author

.....

Magor L. Lőrincz, PhD
Corresponding author