1. As a result of my PD proposal we show that calretinin (Calr)-expressing neurons of the lateral thalamus ($Calr^+$LT neurons) convey the association of fast CS (tone) and US (foot shock) signals upstream from the LA in mice. This work was published in Nature Neuroscience in 2020 with my first authorship. We provided multiple lines of evidence to indicate that $Calr^+$LT cells do not simply relay single CS or US modalities, but compute associated and experience-dependent information (CS+US) before the LA:

- our activity-dependent anatomical and in vivo electrophysiological results indicated that $Calr^+$LT cells and their synaptic amygdala targets show larger activation to associated signaling than pure auditory or aversive processing.
- $Calr^+$LT cells collect signals from brain regions involved in the sensory processing of different modalities. The auditory and multisensory IC, the visual and multimodal SC, the somatosensory Pr5 and the nociceptive PAG cells are among the subcortical regions that form synaptic contacts with $Calr^+$LT neurons. Consequently, the $Calr^+$LT→AMG route can contribute to hippocampus-independent contextual memory formation and retrieval. Silencing this pathway not only prevented auditory-cued fear learning but also the establishment and recall of contextual fear memory
- the thalamus forms reciprocal connections with its corresponding cortical regions. $Calr^+$LT neurons do not project to the Au1, but preferentially target higher-order cortical regions like the AuV, the TeA and the insular cortex, which further suggests that these thalamic neurons are not directly involved in primary sensory processes.
- Thalamic-input-mediated inhibitory and disinhibitory mechanisms, which play important roles in sensory gating during fear learning, have also been identified in the amygdala complex. Our data show that the $Calr^+$LT cells, which can elicit the above-mentioned cortical disinhibition, also have control over the GABAergic cells in the amygdala.
- The direct $Calr^+$LT→AMG input can drive fast and robust evaluation and behavioral action in a dangerous situation, before any recognition. At the same time, sending collaterals to the AuV/TeA (which in turn provides strong cortical input to the LA), $Calr^+$LT neurons can provoke cortical processes that are slower but more precise and involve consciousness.
- $Calr^+$LT and cortical inputs can spatially converge on amygdala cells. Challenging the established idea of the coincident detection of separate CS and US inputs, we propose that CS+US-carrying $Calr^+$LT and cortical signals interact in the LA. Summation of these inputs can then promote memory formation by driving NMDA-dependent plasticity at the LT→LA input gated by inhibitory processes.
- this thalamic input can also interact with neuromodulatory pathways in the amygdala. Integrating all the above-noted cortical and subcortical signals, the amygdala can quickly select adaptive and adequate threat responses.
- our data suggest that the $Calr^+$LT→AMG population, transferring associated signals to the amygdala, cortical and basal ganglia networks, is in a unique position to develop and alter cue-related emotional behaviors.

Publication:

Associative and plastic thalamic signaling to the lateral amygdala controls fear behavior. Barsy B, Kocsis K, Magyar A, Babiczky Á, Szabó M, Veres JM, Hillier D, Ulbert

I, Yizhar O, Mátyás F. Nat Neurosci. 2020 May;23(5):625-637. doi: 10.1038/s41593-020-0620-z.

2. It has been shown that gamma oscillation in the amygdala provides a framework for learning (eg. Courtin et al., 2014; Kanta et al., 2019; Headley et al., 2021). Thus, in a follow-up study, we investigated the role of the Calr^{+}LT in the amygdalar network activity underlying learning processes. We found the US-evoked gamma oscillation in the amygdala wais shaped by it input from the Calr^{+}LT. DTR-mediated ablation of Calr^{+}LT cells diminished the US-evoked the amygdalar gamma rhythm while optogenetic stimulation of Calr^{+}LT population enhanced the same oscillation. In turn, mice with Calr^{+}LT ablation could not process fear learning, these animals showed generalized fear responses. Altogether, the Calr^{+}LT population can shape amygdalar function partially through its involvement in gamma oscillation genesis. I participated in this project with the behavioural experiments. Submitted publication is expected in 2024.

Earlier version of this project was summarized in an MSc thesis and in the evaluation of an UNKP project:

- Szabó Mónika: Különböző molekuláris hátterű thalamikus gátlások hatása a félelmi memórianyomok kialakulására, 2018. ELTE, Budapest.
- Kocsis Kinga. A talamikus bemenetek szerepe az amigdaláris oszcillációk kialakulásában (ÚNKP-19-3-III-PPKE-68).

3. In an other follow-up study of our publication, we started to examine the mechanism of innate fear-processing. First, we were focusing on the innate auditory signals. A fundamental form of communication in rats and mice is the ultrasonic vocalization (USV), especially in stressful situations. In rats, the alarm call is released in dangerous events on 22 kHz and activates the mesolimbic cholinergic system. However, in mice, the frequency of the alarm call and the neural mechanism of this communication is still unknown. Thus, we recorded USV of mice in stressful situation (restraint). The signals were acoustically analysed. We found 4 different clusters of calls, one of them was in range 100-150 kHz. This high frequency USV has not been identified in the literature, so we replayed it for mice and examined their behaviour. We found that the animals receiving high frequency USV showed significantly more freezing (22±2,3 t%) than the group receiving low frequency USV (10,9±1,6 t%). These results formed the basis of a BSc dissertation and presented at IBRO workshop, 2022. Since 2022, further electrophysiological and behavioral experiments have been carried onunder my supervision to investigate the Calr^{+}LT involvement is this USV signalling. Our preliminary results show that roughly 5-10% of Calr^{+}LT responded to high frequency USV calls. This proportion is similar to the one found in case of sound-evoked responses in our (Barsy, Kocsi et al., 2020) and others studies (eg. Grewe et al., 2017). Submitted publication is expected also in 2024.

Earlier version of this project was summarized in an BSc thesis and MITT_IBRO poster:

- Zsoldos R. Felnőttkori distressz vokalizációk és szerepük a fajon belüli kommunikációban. *BSc szakdolgozat*, ELTE, Budapest. 2022.
- **Analysis of ultrasonic vocalizations (USV) in mice.** R. Zsoldos, K. Kocsis, B. Barsy, F. Jártó, S. Zsebők, A. Magyar, S. Borbély, F. Mátyás. *Poster presentation* at

the International Neuroscience Meeting, Budapest – IBRO Workshop, 27-28th January, 2022.

4. In parallel of the above detailed investigation, we started to investigate the role of $Calr^{+}$LT neurons in affective visual fear-processing, which is the basic mechanism of context-dependent behaviour. In this experiment, based on the widely used Pavlovian conditioning, a visual stimulus (LED flashing) was associated with an aversive effect (footshock). 1 hour after the conditioning, animals were perfused, and c-Fos activation was examined mainly in the amygdalar areas. As a continuation of the experiment, on the day after conditioning, we performed a recall experiment for learning to know the result of the process. 1 hour after the experiment, the animals were perfused and c-
Fos activation was investigated. According to our results, learning processes similar to sound-associated learning take place in mice also in the case of visual stimuli: conditioned memory is formed. Our preliminary anatomical analyzes revealed similar activation pattern within the PIL-AMG system as we found in case of the auditory conditioning.
To further prove the involvement of this PIL-AMG pathway in visual-driven conditioning response, we found that the PIL-projecting superior collicular cells is able to provide visual information for $Calr^{+}$LT neurons. Due to this SC-PIL-AMG pathway, mice can process visual fear conditioning. This project is carring out by my PhD student, Judit Berczik, and by Anna Bakacsi (also a PhD student), under my supervision. Publication is also expected within a year.

5. At the beginning of my PD proposal I also participated in a project which demonstrated that the calretinin containing (CR+) neurons in the dorsal medial thalamus (DMT) constitute a key diencephalic node that mediates distinct levels of forebrain arousal. Cell-type-specific activation of DMT/CR+ cells could elicit active locomotion lasting for minutes, stereotyped microarousals or transient disruption of sleep rhythms depending on the parameters of the stimulation. State transitions could be induced in both slow-wave and REM sleep. The DMT/CR+ cells displayed elevated activity prior to arousal, received selective subcortical inputs and innervated several forebrain sites via highly branched axons. Together, these features enable DMT/CR+ cells to summate subcortical arousal information and effectively transfer it as a rapid, synchronous signal to several forebrain regions to modulate the level of arousal. I participated in this complex project with the anatomical and behavioural experiments. These results were published in Nature Neuroscience in 2018.

Publication:
A highly collateralized thalamic cell type with arousal-predicting activity serves as a key hub for graded state transitions in the forebrain. Mátyás F, Komlósi G, Babiczky Á, Kocsis K, Barthó P, Barsy B, Dávid C, Kanti V, Porrero C, Magyar A, Szűcs I, Clasca F, Acsády L.Nat Neurosci. 2018 Nov;21(11):1551-1562. doi: 10.1038/s41593-018-0251-9. Epub 2018 Oct 22.PMID: 30349105

6. Continuing this investigation, with my PhD student, Judith Berczik, we examined the age-dependent role of midline thalamic BDNF level in innate and learned fear behavior. It is well-documented that arousal–dependent cognitive functions like memory processes, sleep-wake

cycles and stress management are age-dependent. The thalamo-frontal network goes through age-related changes, which could be responsible for these functional alterations. Furthermore, DMT is the key source of the thalamic brain derived neurotrophic factor (BDNF) for the cortex, which is an important factor in cognitive functions including memory processes.
First, we showed that the BDNF levels in the DMT and PFC have age-dependent alterations measured with Western Blots. Within the DMT, the BDNF is almost exclusive expressed by the CR+ cells found using a Double fluorescent in situ hybridization method.
Then we performed a DMT-specific BDNF lesion using a $BDNF^{fl/fl}$ mouse strain and AAV-transferred Cre recombinant enzyme. Different age groups (1-3 months, 3-6 months, 6-8 months, 12+ months) of male and female mice were applied in the tests. After AAV injections into DMT, learned and innate fear reactions were examined. According to our results, the midline thalamus participates in learning processes in an age- and gender-dependent manner, via BDNF-signaling.

Earlier version of this project was presented at MITT IBRO Workshop and won the first prize in Neuroscience category at SE PhD Scientific Days, in 2022.

- **Age-dependent role of midline thalamus in learning.** J. Berczik, A. Magyar, B. Barsy, S. Borbély, A. Kurilla, B. Szeder, V. Vas, L. Buday, L. Szilák, F. Mátyás. *Poster presentation* at the International Neuroscience Meeting, Budapest – IBRO Workshop, 27-28th January, 2022.
- **Age-dependent role of midline thalamus in learning.** J. Berczik, A. Magyar, B. Barsy, S. Borbély, A. Kurilla, B. Szeder, V. Vas, L. Buday, L. Szilák, Á. Szepesi, B. Rózsa, M. Diana, F. Mátyás. *Poster presentation* at SE PhD Scientific Days, 2022.

A manuscript entitled "**Age- and sex-dependent effects of midline thalamic BDNF on behaviour"** (J. Berczik, A. Magyar, S. Borbély, T. Orbán, O. Kolacsek, Á. Szepesi, B. Rózsa, M. Diana, F. Mátyás and B. Barsy) with my shared corresponding authorship is under preparation.

Additional notes

I was on maternity leave between 2019-2021. Between 2021-2022, I resorted the one-year long COVID-delay which was demandable as legal right. The closed kindergartens (for my 2-year-old child), the home-schooling (with my 7-year-old child) and the restricted attendance at the TTK during the Pandemy rendered impossible for me making experiments. I restarted the experimental work in 2022.

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Gamma oscillations in the basolateral amygdala: localization, microcircuitry, and behavioral correlates. Headley DB, Kyriazi P, Feng F, Nair S, Pare D. J Neurosci. 2021 Jun 1;41(28):6087-101.

Closed-loop control of gamma oscillations in the amygdala demonstrates their role in spatial memory consolidation. Kanta V, Pare D, Headley DB. Nat Commun. 2019 Sep 3;10(1):3970.