Detailed scientific report of OTKA FK124434 (november 2017 – october 2022)

The role of midline thalamic circuitries in the learnt and innate behaviours

Brain disorders are not separate abnormalities but breakdown of normal, physiological routines. Thus, the understanding of normal molecular, cellular or network machineries of the brain can provide theories for what turned wrong to cause any brain diseases. Although decades of neuroscientists' effort to explain how the brain works and shifts to an abnormal states, we are still far from a clear idea explaining most of dysfunctions.

Mental disorders (including mood disorders) are among the most common abnormalities of the human population; 1 in every 8 people in the world live with a mental disorder (WHO). It is generally accepted that an important circuit element of cognitive function, the thalamocortical network formed by the medial thalamus and prefrontal cortex and their dysfunction are also linked with several mental deficits like anxiety, schizophrenia, depression and substance use. Thus, understanding the operation of this thalamocortical system can provide essential information for us about mental diseases. However, the medial thalamus is not a single structure; it is composed by at least two neurochemically and functionally distinct neuronal population: the calretinin-expressing and non-expressing ones. Thus, in our modified research plan (accepted during the second year) we aimed to dissect the role(s) of the medial thalamocortical pathways using anatomical, electrophysiological, optogenetic as well as behavioural tools.

1) CR+ dorsal medial thalamus is ideally suited to control arousal both in sleep and awake periods

In this investigation we demonstrated that the calretinin (CR)-containing neurons in the dorsal medial thalamus (DMT) constituted a key diencephalic node that mediated distinct levels of forebrain arousal. During sleep, DMT/CR+ cells displayed elevated activity before arousal, and their optogenetic stimulation evoked awakening effect in a dose-dependent manner. These awakening periods highly resembled stereotyped microarousals, sleep-throughs with transient disruption of sleep rhythms or fully wake up states, depending on the parameters of the stimulation. During active states, inhibition of the DMT/CR+ population disrupted the ongoing behaviour causing short immobile states. DMT/CR+ received selective subcortical inputs including the reticular activating system, orexinergic as well as cholinergic networks, and innervated several forebrain sites via highly branched axons. Together, these features enabled 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.

Presentation: Article

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.

Contribution: shared first and last author.

2) Parallel medial thalamocortical pathways differently contribute to the forebrain arousal

In this work, by integrating in vivo acute and chronic electrophysiological recordings, anatomical and optogenetic approaches in mice we demonstrated that DMT/CR+ and DMT/CR- populations had qualitatively and quantitatively distinct cortical and subcortical input/output organization and possess diverse cortical functions. In general, DMT/CR+ (paraventricular thalamic) neurons had global efferent connections, drove persistent cortical activation and majority of them showed arousal-predicting firing pattern. In contrast, DMT/CR- (mediodorsal thalamic and paratenial) cells targeted much fewer brain regions, provided focal excitations and their activity pattern only followed the behavioural arousal. Within the PFC, the DMT/CR+ and DMT/CR- cells activated qualitatively and quantitatively different PFC networks with laminar and subregional-specificity. In addition, the proportions of the DMT/CR+ and DMT/CR- activated principal cells and interneurons were different in the prefrontal cortex. Finally, we also found potential indirect interaction between the CR+ and CR- systems, via the thalamic reticular nucleus and the deep-layers of PFC. These findings indicated that, although the DMT/CR+ and DMT/CR-negative-PFC networks are anatomically and functionally different, they can form sequentially activated 'inter-loop' system. Building on each other, they can collectively mediate complex, arousal-dependent cognitive functions.

Presentation: poster (Manuscript submission is scheduled for 2023)

The anatomical and functional complexity of medial thalamus-prefrontal cortex circuit
Aletta Magyar, Sándor Borbély, Judit Berczik, Kinga Kocsis, Ofer Yizhar, Ferenc Mátyás.
FENS 2022 Paris.

Contribution: last author.

3) Distinct cortical control by NAc- and AMG-projecting DMT/CR+ cells

Previously we showed that single DMT/CR+ cell can project to multiple cortical and subcortical areas (Mátyás, Komlósi et al., 2018). On the order hand, the nucleus accumbens (NAc; DMT/CR+^{NAc})- and the amygdala-projecting (AMG) DMT/CR+ cells were showed to possess distinct connectivity patterns and functions. Thus, we also investigated how the NAc- and AMG-projecting DMT/CR+ cells shape the prefrontal cortical activity both at single cell and network levels. First, we quantified the DMT/CR+^{NAc} and DMT/CR+^{AMG} axonal projections in layer selective manner. DMT/CR+^{NAc} cells preferentially innervated the superficial layers, while the axonal arbours of DMT/CR+^{AMG} cells were denser in the deep layers. Using, multisite electrophysiological recordings we found that optogenetic stimulation of the DMT/CR+^{NAc} population, compared to the DMT/CR+^{AMG} trials, provided stronger modulation over the pyramidal cells albeit, proportionally these perturbation affected similar number of cortical cells. Furthermore, DMT/CR+^{NAc} cells had larger impact on the inhibitory cell population, too. In consequence, DMT/CR+^{NAc} population had stronger effect both on the local and global cortical oscillations. Altogether these findings suggest that the DMT/CR+^{NAc} population has a stronger arousal effect on the forebrain due its stronger feed-forward inhibitory mechanisms.

Presentation: None. (Manuscript submission is scheduled for 2023)

4) Molecular characteristics and laminar distribution of prefrontal neurons projecting to the mesolimbic system

Every neocortical region has laminar structure. Each layer has distinct genetic, input-out organization and function. To map the anatomical and functional organization of the medial thalamocortical interaction in a layer-specific manner, we needed to identify the laminar organization of the medial prefrontal cortex. In a related work, using the mesolimbic system – including the nucleus accumbens (NAc) and the ventral tegmental area (VTA) – combining classical retrograde and conditional viral tracing techniques with multiple fluorescent immunohistochemistry, we sought to deliver a precise, cell- and layer-specific anatomical description of the cortico-mesolimbic pathways in mice. We demonstrated that NAc- ($mPFCN_{Ac}$) and VTA-projecting mPFC ($mPFC_{VTA}$) populations show different laminar distribution (layers 2/3–5a and 5b–6, respectively) and express different molecular markers. Specifically, calbindin and Ntsr1 are specific to $mPFC_{NAc}$ neurons, while $mPFC_{VTA}$ neurons express high levels of Ctip2 and FoxP2, indicating that these populations are mostly separated at the cellular level. We directly tested this with double retrograde tracing and Canine adenovirus type 2-mediated viral labeling and found that there is indeed minimal overlap between the two populations. Furthermore, whole-brain analysis revealed that the projection pattern of these populations is also different throughout the brain. Taken together, we demonstrated that the NAc and the VTA are innervated by two, mostly non-overlapping mPFC populations with different laminar distribution and molecular profile. These results can contribute to the advancement in our understanding of mesocorticolimbic functions and its disorders in future studies.

We also suggest the general adoption of the presented laminar map to precisely define and separate mPFC subregions and layers in future studies. Therefore, we used this laminar map to localize cortical innervation and optogenetically-driven activation of distinct medial (CR+, CR-, NAC- and AMG-projecting) thalamic cell groups in the previously described works (paragraphs 2 and 3).

Presentation: Article

Molecular characteristics and laminar distribution of prefrontal neurons projecting to the mesolimbic system. Babiczky Á, Matyas F.
Elife. 2022 Sep 5;11:e78813. doi: 10.7554/eLife.78813.

Contribution: last author.

5) AgRP neurons control structure and function of the medial prefrontal cortex via dopaminergic and thalamic interaction

In a collaborative work, we have started to analyse how subcortical signal can contribute to the functional organization of the medial thalamocortical network. Hypothalamic agouti-related peptide and neuropeptide Y-expressing (AgRP) neurons have a critical role in both feeding and non-feeding behaviors of newborn, adolescent, and adult mice, suggesting their broad modulatory impact on brain functions. Here we show that constitutive impairment of AgRP neurons or their peripubertal chemogenetic inhibition resulted in both a numerical and functional reduction of neurons in the medial prefrontal cortex (mPFC) of mice. These changes were accompanied by alteration of oscillatory network activity in mPFC, impaired sensorimotor gating, and altered ambulatory behavior that could be reversed by the administration of clozapine, a non-selective dopamine receptor antagonist. The observed AgRP effects are transduced to mPFC in part via dopaminergic neurons in the ventral tegmental area and may also be conveyed by medial thalamic neurons. AgRP cells

preferentially innervate the medial thalamic cells projecting to the medial prefrontal cortex, which thalamic population is largely composed by the CR+ thalamic cells (Mátyás, Komlósi et al., 2018). In our future work, the functional consequence of the AgRP influence over the medial thalamocortical circuit will be investigated. Nevertheless, our results unmasked a previously unsuspected role for hypothalamic AgRP neurons in control of neuronal pathways that regulate higher-order brain functions during development and in adulthood.

Presentation: Article

AgRP neurons control structure and function of the medial prefrontal cortex. Stutz B, Waterson MJ, Šestan-Peša M, Dietrich MO, Škarica M, Sestan N, Racz B, Magyar A, Sotonyi P, Liu ZW, Gao XB, Matyas F, Stoiljkovic M, Horvath TL.
Mol Psychiatry. 2022 Jul 29. doi: 10.1038/s41380-022-01691-8.

Contribution: co-author

In addition to these studies, 3 other works (in paragraph 6-8) were completed which partially linked to the OTKA research, albeit, these were not present in either version of the proposal.

6) Associative and plastic thalamic signalling to the lateral amygdala controls fear behaviour.

In a widely used fear learning paradigm, we identified an other thalamic node, the so-called CR+ lateral thalamic (LT) regions to form associative signal prior to the amygdala during the auditory fear conditioning and memory recall. Decades of research support the idea that associations between a conditioned stimulus (CS) and an unconditioned stimulus (US) are encoded in the lateral amygdala (LA) during fear learning. However, direct proof for the sources of CS and US information is lacking. Definitive evidence of the LA as the primary site for cue association is also missing. Here, we show that calretinin (CR)-expressing neurons of the lateral thalamus (CR+LT neurons) convey the association of fast CS (tone) and US (foot shock) signals upstream from the LA in mice. CR+LT input shapes a short-latency sensory-evoked activation pattern of the amygdala via both feedforward excitation and inhibition. Optogenetic silencing of CR+LT input to the LA prevents auditory fear conditioning. Notably, fear conditioning drives plasticity in CR+LT neurons, which is required for appropriate cue and contextual fear memory retrieval. Collectively, our results demonstrate that CR+LT neurons provide integrated CS–US representations to the LA that support the formation of aversive memories.

Presentation: Article

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.

Contribution: last author.

7) Slow insertion of silicon probes improves the quality of acute neuronal recordings.

In this collaborative work, led by Richard Fiáth, we investigated how the speed of electrode insertion affects the silicone probe recordings

Neural probes designed for extracellular recording of brain electrical activity are traditionally implanted with an insertion speed between 1 μm/s and 1 mm/s into the brain tissue. Although the physical effects of insertion speed on the tissue are well studied, there is a lack of research investigating how the quality of the acquired electrophysiological signal depends on the speed of probe insertion. In this study, we used four different insertion speeds (0.002 mm/s, 0.02 mm/s, 0.1 mm/s, 1 mm/s) to implant high-density silicon probes into deep layers of the somatosensory cortex of ketamine/xylazine anesthetized rats. After implantation, various qualitative and quantitative properties of the recorded cortical activity were compared across different speeds in an acute manner. Our results demonstrate that after the slowest insertion both the signal-to-noise ratio and the number of separable single units were significantly higher compared with those measured after inserting probes at faster speeds. Furthermore, the amplitude of recorded spikes as well as the quality of single unit clusters showed similar speed-dependent differences. Post hoc quantification of the neuronal density around the probe track showed a significantly higher number of NeuN-labelled cells after the slowest insertion compared with the fastest insertion. Our findings suggest that advancing rigid probes slowly (~1 μm/s) into the brain tissue might result in less tissue damage, and thus in neuronal recordings of improved quality compared with measurements obtained after inserting probes with higher speeds.

Since these results, we implemented this in our electrophysiological recordings.

Presentation: Article

Slow insertion of silicon probes improves the quality of acute neuronal recordings. Fiáth R, Márton AL, Mátyás F, Pinke D, Márton G, Tóth K, Ulbert I.
Sci Rep. 2019 Jan 14;9(1):111. doi: 10.1038/s41598-018-36816-z.

Contribution: co-author.

8. Control of aversion by glycine-gated GluN1/GluN3A NMDA receptors in the adult medial habenula.

In this collaborative work, we showed that GluN1/GluN3A-containing NMDA receptors in the medial habenula is not involved in regulation of fear conditioning and fear memory recall, but control aversive states.

The unconventional N-methyl-D-aspartate (NMDA) receptor subunits GluN3A and GluN3B can, when associated with the other glycine-binding subunit GluN1, generate excitatory conductances purely activated by glycine. However, functional GluN1/GluN3 receptors have not been identified in native adult tissues. We discovered that GluN1/GluN3A receptors are operational in neurons of the mouse adult medial habenula (MHb), an epithalamic area controlling aversive physiological states. In the absence of glycinergic neuronal specializations in the MHb, glial cells tuned neuronal activity via GluN1/GluN3A receptors. Reducing GluN1/GluN3A receptor levels in the MHb prevented place-aversion conditioning. Our study extends the physiological and behavioral implications of glycine by demonstrating its control of negatively valued emotional associations via excitatory glycinergic NMDA receptors.

Control of aversion by glycine-gated GluN1/GluN3A NMDA receptors in the adult medial habenula. Otsu Y, Darcq E, Pietrajtis K, Mátyás F, Schwartz E, Bessaih T, Abi Gerges S, Rousseau CV, Grand T, Dieudonné S, Paoletti P, Acsády L, Agulhon C, Kieffer BL, Diana MA.
Science. 2019 Oct 11;366(6462):250-254. doi: 10.1126/science.aax1522.

Contribution: co-author.