Inhibitory Control and Integration in the Thalamus

PhD thesis booklet

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Introduction

According to the classical view, the thalamus serves as a relay station for ascending sensory information arriving from sensory organs that then travels on towards the cerebral cortex for processing. Thus, its only role was thought to be the faithful transmission of these sensory messages without failure or modification. Several thalamic nuclei indeed fullfil this role and receive their main excitatory „driver" input from sensory subcortical areas, and send their axons to layer 4 of a specific cortical region with well defined function. For a long time, these nuclei served as the model of the organization principle of thalamocortical connectivity, and the classical view of the thalamus was generalized from their properties. The majority of thalamic regions, however show an entirely different connection pattern with their output cortical regions and input subcortical areas, thus they do not fit in the classical framework. They can target large and widespread cortical regions or specifically innervate multiple areas, and the laminar distribution of the axons also shows great variability. Some thalamic nuclei also innervate subcortical targets. Moreover their input dose not neceserally convey environmental sensory information.

The intralaminar (IL) complex is a group of thalamic nuclei with divers functions. They were shown to play a role in higher cognitive processing, in the regulation of sleep-wake cycle and motor tasks. They have a vast frontal cortical projection and also extensively innervate the striatum. Besides the variability of their output their innervation also contributes to their functional diversity and raises the possibility of the IL being more than a relay station that faithfully transmits incoming information. It seems to act more like a hub, receiving subcortical modulatory inputs (serotonergic, noradrenergic, cholinergic) and several other types of messages from multiple sources (superior colliculus, lateral cerebellar nucleus), and having access both to cortical areas and, via the striatum, to the movement-organizing basal ganglial circuit.

This functional complexity is not only caused by the diversity of their excitatory inputs but also the inhibition targeting these nuclei. In the thalamus, the general source of inhibition is the GABAergic cells of the thalamic reticular nucleus (TRN) but in particular thalamic

nuclei, like the IL complex inhibitory inputs from extrathalamic sources (zona incerta, anterior pretectum and the substantia nigra pars reticulata) are also present.

The possibility of the existence of additional extrathalamic inhibitory pathways besides the presented ones was raised after a paper published by Zeilhofer and colleagues in 2005. They created a type 2 glycine transporter (GlyT2)::eGFP transgenic mouse line expressing eGFP in glycinergic neurons. In the midbrain of these animals a strong fluorescent signal could be observed emitted by a profuse network of axons and terminals.

A different aspect of thalamic information processing is the putative integrative role of particular thalamic nuclei. In these regions, besides the classical subcortical sensory drivers, other terminals that share the morphological and physiological properties of a driver (large terminals with multiple active zones, fast EPSPs with large amplitude) can also be observed. These terminals are known to have L5 cortical origin and innervate the exact same proximal dendritic domain of TC cells as inputs coming from subcortical sources. The distribution of driver terminals with different origin was mapped in the whole primate thalamus and turned out that in some thalamic regions these inputs were completely separated, while in others they colocalized, but areas entirely lacking driver terminals also existed. The colocalization of driver terminals with different origin in the same thalamic area raises the possibility of their convergence on a single thalamic cell and suggests that the output of such a neuron will be determined by two entirely different messages.

Objectives

The diversity of excitatory and inhibitory afferents in the thalamus, often in the same nuclei, as well as the heterogeneity of projection patterns and targeted cortical and subcortical areas, indicate that besides the well-described relay function, a great part of the thalamus may play different roles in shaping information processing, and may also have complex executive functions.

In GlyT2::eGFP transgenic animals, our group discovered a massive cloud of glycinegic fibers targeting the IL complex, and found the possibility of convergence of excitatory driver inputs with different origin in the somatosensory thalamus of rodents.

In my PhD work for this thesis I have focused on two different aspects of thalamic activity control. First, I examined the role of glycinergic inhibition arriving to the IL complex in the regulation of motion. Second, I studied the convergence of excitatory drivers with different origin on the same TC cell.

My goals were:

1. To describe the glycinergic projection to the IL at the network and cellular levels, and to characterize the *in vivo* activity of the glycinergic cells.
2. To describe the effects of selective activation of the glycinergic pathway on IL activity and on the behavior of the animal.
3. To define the afferents of the glycinergic cells and the effect on their activity.
4. To show the putative integrative role of TC cells by characterizing the morphology of drivers with different origin converging onto the same neuron.

Methods

Experimental animals

GlyT2::eGFP mouse line

The type 2 glycine transporter (GlyT2) is a protein localized in the neuronal membrane and is responsible for capturing glycine molecules in the extracellular space and accumulating them inside the cell (Liu et al., 1993). Once the glycine reaches the proper intracellular concentration, the vesicular inhibitory amino acid transporter (VIAAT) accumulates it in the synaptic vesicle, and thus it can function as an inhibitory neurotransmitter (McIntire et al., 1997; Sagné et al., 1997; Wojcik et al., 2006). GlyT2 is expressed in every glycinergic inhibitory neuron, making it their selective marker (Zafra et al., 1995).

In 2005, Zeilhofer and his colleagues created a reporter mouse strain in which enhanced green fluorescent protein (eGFP) was expressed under the control of the promoter of the GlyT2 gene, so glycinergic neurons showed green fluorescence (Zeilhofer et al., 2005). Performing the tracing experiments and the juxtacellular recordings using these animals allowed us to *post hoc* identify the labeled neurons or fibers and the recorded units respectively.

GlyT2::cre mouse line

For the generation of this line, homologous recombination in bacteria was used to introduce the cre coding sequence into the BAC-DNA (clone RP23-365E4). The modified BAC-DNA was injected to the pronuclei of fertilized C57BL/6 oocytes. GlyT2::cre mouse lines were maintained on a C57BL/6J background. The selectivity of cre expression was demonstrated by performing immunostaining against the cre-protein (mouse anti-cre 1:10,000. Millipore, followed by Cy3-conjugated donkey anti-mouse 1:500) in crossed GlyT2::cre and GlyT2::eGFP mice. In these experiments, we examined 198 cre-positive neurons, out of which 196 (99%) were also eGFP-positive. We used these animals to selectively label glycinergic neurons using the cre-loxP system in gain of function experiments.

GlyT2::eGFP/Rbp4::cre mouse line

In these animals all glycinergic neurons show green fluorescence, and in addition cre recombinase is expressed in the cerebral cortex in layer 5 pyramidal neurons under the control of the promoter of the retinol binding protein 4 (Rbp4) gene. This double transgenic strain on the one hand allowed us to identify glycinergic neurons (as it was described previously), and on the other hand to selectively label layer 5 pyramidal neurons in a cre-dependent manner.

Morphology

Surgery

Tracer and virus injections were performed under ketamine/xylazine anesthesia (ketamine, 83-111 mg/kg; xylazine, 3.3-4.3 mg/kg). The head of the animal was fixed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, California 91042, Model 900 Small Animal Stereotaxic Instrument). If needed, intramuscular injection of ketamine/xylazine was given to maintain anaesthesia.

Preparation of the tissue

Fixation of the tissue: all animals, both for anatomical analysis and following acute or freely moving electrophysiological recordings, were perfused using similar methods and solutions. After fixation the brain was sectioned. Slices were cut at 50-60 μm thickness.

Dehydration and embedding of the tissue: to prepare tissue for embedding, sections were treated with OsO_4 followed by sequential incubation in ethanol (50%, 70%, 90%, absolute) and acetonitrile as a final step at room temperature. After the dehydration procedure, sections were incubated in Durcupan.

Immunohistochemistry: For all the reactions TBS buffer was used as a solvent. Following each step, 3x10 minutes rinse with clean TBS was applied. First, the sections were treated with bovine serum albumin (BSA) to mask unspecific antibody binding sites, and then, without washing, primary antibody solution was applied followed by the secondary fluorescent or biotinylated antibody. After washing out the biotinylated antibody, sections were incubated in avidin-biotin horseradish peroxidase complex (ABC), then either in diaminobenzidine (DAB) for brown precipitation or nickel intensified diaminobenzidine (DAB-Ni) for black precipitation.

Preparation for electron microscopy: to prepare electron microscopic sections, one region of interest (ROI) was re-embedded from the light microscopic material. The ROI was further

sectioned to 60 nm ultrathin sections using an ultramicrotome. Sections were mounted on copper and nickel pioloform-coated grids.

In vivo physiology

Surgery

Altogether 20 adult male C57Bl/6J BAC GlyT2::eGFP and GlyT2::cre mice (20-30g) were used for the experiments. Surgery, acute recording experiments and implantations were done under ketamine/xylazine anaesthesia. Initially, mice received an intraperitoneal injection of ketamine (111 mg/kg) and xylazine (4.3 mg/kg). For the maintenance of the anaesthesia, intramuscular injection of ketamine/xylazine was given every 30-50 min during the experiments.

In vivo juxtacellular recording and labeling, local field potential (LFP) recording

Bipolar LFP electrodes (FHC, resistance ~1 MΩ) were inserted into the frontal cortex of mice (Bregma 1.7 mm; lateral -0.8 mm). The recorded signal was amplified, band-pass filtered from 0.16 Hz to 5 kHz and from 100 Hz to 5 kHz to record the fast multiunit activity (Supertech BioAmp, Supertech, Pécs, Hungary) and digitized at 20 kHz (micro 1401 mkii, CED, Cambridge, UK). Concentric bipolar stimulating electrodes were inserted into the IL (Bregma -1.5 mm; lateral 2 mm; ventral from cortical surface -3.2 mm tilted at 20 degrees, electrode separation 0.8mm). PRF single unit activity was recorded by glass microelectrodes (*in vivo* impedance of 20-40 MΩ) pulled from borosilicate glass capillaries (1.5 mm outer diameter, 0.75 or 0.86 inner diameter, Sutter Instrument Co., Novato, CA, USA or WPI Inc. Sarasota, Fl, USA) and filled with 0.5 M K^+- acetate and 2% neurobiotin (Vector Laboratories, Burlingame, CA, USA). Electrodes were lowered by a piezoelectric microdrive (Burleigh 6000 ULN or ISS 8200, EXFO, Quebec City, Quebec, Canada) into the PRF (Bregma -4.4 mm; lateral -0.8 to -1 mm; ventral from cortical surface -3.8 to -4.8 mm). Neuronal signals were amplified by a DC amplifier (Axoclamp 2B, Axon Instruments/Molecular Devices, Sunnyvale, CA, USA), further amplified and filtered

between 0.16 Hz and 5 kHz by a signal conditioner (LinearAmp, Supertech). Neuronal signals were recorded by Spike2 5.0 (CED). For the juxtacellular labeling of the recorded neurons positive current steps (0.5-8 nA) were applied at 2 Hz via the recording pipette filled with neurobiotin. The neuron fired only during these current steps. During the induced firing neurobiotin was taken up by the cell filling the soma and proximal dendrites. In some cases, distal dendritic regions and axons were also labeled.

Following perfusion, coronal sections (50 μm) were cut from the PRF and the neurons were visualized with Cy3 conjugated streptavidin (1:2,000, Jackson). GlyT2::eGFP positivity was determined by confocal microscopy. The neurons were then developed using ABC and DAB-Ni, and the sections containing the labeled neurons were dehydrated and embedded in Durcupan. The dendritic trees of the labeled neurons were reconstructed using Neurolucida 5.2 software (MBF Bioscience, Magdeburg, Germany).

Analysis

To find the action potential clusters of the PRF cells, we used a built-in script of the Spike2 7.0 (CED) software. We determined clusters by separating the two peaks at the minimum of the bimodal inter-spike interval histograms (ISI). To determine the phase of each action potential relative to the cortical population activity, we used a previously described method (Slézia et al., 2011). Briefly, the envelope of the cortical multiunit trace (MUA envelope) was low-pass filtered at 4 Hz using a zero phase-shift finite impulse response filter. We calculated the Hilbert-transform of the filtered- and z-scored MUA envelope, and the phase was determined by taking the angle of the complex ‘analytic signal’. The prime advantage of this method is that it estimates the phase of any quasi-rhythmic signal (cortical slow oscillation under ketamine-xylazine anesthesia) in a temporally refined manner, defining a time series that quantifies the “instantaneous phase” of the ongoing oscillation. The circular mean angle was calculated for each recorded neuron, and the inter-quartile range was determined as the ±25th percentile around the circular mean.

In-vivo optogenetics and LFP recordings

Two weeks after transfection of PRF cells with an AAV2-EF1a_DIO-hChR2(H134R)-EYFP virus (for control experiments: AAV2-EF1a DIO-EYFP WPRE hGH) GlyT2::cre animals were anaesthetized with ketamine-xylazine (111 and 5 mg/kg) and placed in a stereotaxic frame. The skin was removed over the frontal, parietal and occipital hemispheres and the skull was cleaned with H_2O_2. Alltogether either six or seven craniotomies were performed; five for the screw electrodes (two over frontal, two over parietal cortical areas and one over the cerebellum for reference and grounding); one or two for the optic fibers (Thorlabs, FG105UCA, Ø105 μm core, 0,22 NA) over the central lateral thalamic and parafascicular nucleus (Bregma -1.9 to 2.3 mm; lateral -0.8 mm; ventral from cortical surface -2.5 to -2.8 mm). The implant was wrapped in copper grid and fixed with Paladur dental cement. The animal was allowed to recover on a heating pad. Mice were rested in their home cage for at least 5 days before testing.

We used the following custom optical system for fiber optic light delivery in freely moving mice: the beam was generated by a 473 nm DPSS laser (LRS-0473-PFM-00050-03, Laserglow Technologies, Toronto, Canada) and was directed to a fiber optic patchcord (Thorlabs) via a fiber port (Thorlabs). The patchcord (1.6 m) was connected to the mouse and allowed to rotate passively. With adequate beam alignment, rotations induced power fluctuations of less than 5% at the fiber output. The power density measured at the output of the delivery fiber was 318 mW/mm^2 for the 200 μm-diameter optic fiber (behavioral experiments) and 1272.73 mW/mm^2 for the 100 μm-diameter fiber (electrophysiological experiments).

To monitor freely moving brain activity, we either used bipolar local field potential (LFP) recording electrodes implanted into the frontal cortex, or in a different set of experiments screw electrodes placed above the same cortical area. A screw electrode served as the ground and reference, and was placed over the cerebellum. Both the recording electrodes and the reference electrode were soldered to a 18 Position Dual Row Male Nano-Miniature Connector (A79014-001, Omnetics Connector Corporation, 7260 Commerce Circle East,

Minneapolis, MN - 55432). For LFP measurements, 30 second-long stimulations were used (3-5 stimulations per day with 5-10 minutes between them). To record the LFP signals we used a 128 channel amplifier (Amplipex Ltd., Hungary). For wavelet generation, we used homemade MatLab scripts.

Motility tests were performed in Paris by our collaborator Marco M Diana. For technical details see: (Giber et al., 2015).

Results

I – Novel source of inhibition in the thalamus

Besides the well known inhibitory input from the GABAergic neurons of the thalamic reticular nucleus (TRN), particulart thalamic nuclei recive inhibition from several other sources. These so-called extrathalamic inhibitory inputs, as well as the TRN input, are purely GABAergic. In a GlyT2::eGFP transgenic mouse line, however, we discovered a massive glycinergic inhibitory input innervating the intralaminar (IL) thalamic nuclei.

To identify the IL-projecting glycinergic neurons, we injected fluorogold as a retrograde tracer among the glycinergic fibers in the thalamus to different antero-posterior coordinates and we observed fluorogold-positive backlabeled neurons in the ipsilateral pontine reticular formation (PRF), consisting of the oral and caudal part of the pontine reticular nucleus (PnO and PnC respectively). 55.3% of the thalamus-projecting cells expressed glycine, while the rest were glycine negative (n = 765 cells in 6 animals).

Glycinergic neurons targeted the soma and proximal dendritic regions of thalamic neurons and electron-microscopic analysis and postembedding GABA staining revealed that the fibers ended in large terminals, coreleased GABA and glycine, and contacted the relay cells via multiple synaptic release sites. The importance and evolutionarily-conserved nature of the pathway is supported by the finding that similar glycinergic innervation of the IL is also present in humans.

To monitor the *in vivo* activity of the glycinergic cells juxtacellular recording was performed in anaesthetised animals. 8 out of 11 recorded and post hoc identified GlyT2 positive neurons fired rhythmic clusters of action potentials interspersed with silent periods, while 3 neurons showed no or very low firing activity. The mean firing rate (MFR) of the neurons as well as the intra-cluster frequency (within and across cells) were quite variable and changed between 2-23 Hz and 12-100 Hz (mean = 41.34 ± 11.59, median = 29Hz) respectively. We found that in the case of each cell, action potential clusters occured at particular phases of the cortical oscillation, and together they covered the whole Up-Down cycle.

We showed that the glycinergic input had a strong inhibitory effect on IL thalamic cells under urethane anesthesia. This inhibition induced rhythmic firing that was conveyed to the cortex and evoked oscillating activity in the thalamocortical system, putatively involving more and more areas. The effect was immediate and lasted only during the laser stimulation. We did not observe any long-lasting changes in the overall state of the cortical activity.

In freely moving experiments, we have shown that, by activating the glycinergic fibers, a prominent behavioral effect could be evoked during which all ongoing behavioural activity was suspended. By gradually decreasing the intensity of the illumination, the effect was also gradually decreased. Parallel to the behavioral changes, altered cortical activity could be detected. Both the behavioral effect and the cortical activity change were transient, lasted only during the stimulation, and no long term state transition was observed. These results, together with the IL thalamic unit recordings in anesthetized animals, imply that the effect of the inhibition is motor-related and conveyed to the cortex via the IL thalamus.

In order to identify the origin of the afferents of the PRF, we injected fluorogold as a retrograde tracer into the PRF (n = 4). We found backlabeled neurons in L5 frontal cortical motor regions (secondary motor and cingulate corticies, M2 and Cg respectively) spanning large anteroposterior areas. To map the projection pattern of these cortical regions in the brainstem, we injected a cre-dependent virus construct into the previously retrogradely backlabeled cortical areas in RBP4::cre/GlyT2::eGFP double transgenic animals, as described in detail in the methods section. We observed massive cortical innervation of several midbrain structures and brainstem nuclei including the PRF. This indicated that the

frontal motor cortical projection to the brainstem is not selective neither to a specific cell type nor to a particular region.

The effect of the anaesthetics is not steady during long recording sessions. This is represented by the changes in the cortical oscillatory pattern. When anaesthesia becomes lighter or more superficial, the cortical slow oscillation will be interspersed with spontaneous desynchronized periods. We juxtacellularly-recorded PRF neurons and monitored the spontaneous changes in cortical activity. We observed that during slow oscillatory periods, a recorded PRF cell fired the previously-described phase modulated rhythmic AP clusters, but when the slow oscillation was replaced by a desynchronized period, the firing pattern of the PRF cell immediately followed this transition and became tonic. When the cortical activity was restored, the AP clusters also reappeared.

To experimentally reproduce changes in cortical activity, we used 2 molar potassium chloride (KCl) to induce cortical spreading depression (CSD) in the frontal motor regions. During the application of KCl, PRF cells became non-rhythmic and decreased their firing rate. After wash–out of KCl, cortical activity slowly recovered and was followed by the partial recovery of PRF firing.

We tested the efficacy of the cortico-PRF pathway by stimulating the cortex and monitoring the response of the PRF cells in anaesthetized animals. First we applied electrical stimulation (1-2 μA) using a bipolar stimulating electrode on the surface of the cortex. We found that the glycinergic neurons (n = 4) faithfully followed electrical stimulation with short (10 ms) latency at 1 Hz. Increasing stimulation frequency caused the response probability to became more variable. In the RBP4::cre\GlyT2::eGFP strain, we labeled the L5 pyramidal neurons and made them express the ChR2 molecule so that we could use light to activate them selectively. We used 5-ms-long pulses on the surface of the brain with ~1300 mW/mm^2 intensity. The recorded glycinergic cells in the PRF, just like in the case of electrical stimulation, followed the pulses with short latency and decreasing reliability with increasing frequency.

II – Integration in the thalamus - convergence of driver inputs on a single TC neuron

Excitatory driver inputs in the thalamus can have both subcortical (classic first order relay nuclei) and cortical L5 origin (higher order nuclei). The relative distribution of these drivers was mapped in the whole primate thalamus and the possibility of their convergence on the same thalamic cell was raised. To investigate the role of convergence we used the first order (ventral posterior nucleus of the thalamus, VPM) and higher order (posterior thalamic nucleus, POm) somatosensory thalamic nuclei of rodents as a model system.

We labeled the subcortical drivers by type 2 vesicular glutamate transporter (vGlut2) immunostaining. vGlut2 is known to be selectively expressed in subcortical thalamus-projecting excitatory neurons. Cortical L5 terminals were visualized by anterograde tracing. PHAL (n = 5 mice and n = 6 rats) or BDA (n = 4 rats) was injected precisely to L5 of the S1 cortex. It is important to note that by this method we could not label all the thalamus-projecting L5 cells, and thus only a fraction of the total L5 terminal population was labelled.

We found that vGluT2-positive subcortical drivers displayed an inhomogenous distribution in the POm, both in rats and mice. Based on this inhomogenous pattern, we determined vGlutT2-rich and vGluT2-poor regions and examined which of these zones were targeted by L5 cortical drivers. We detected large cortical terminals in vGluT2-rich zones, as well as in regions having no or only a few subcortical terminals. Within the convergent zones, large cortical and subcortical terminals were in close proximity and measurements on electron-microscopic samples indicated that they both targeted thicker proximal dendritic regions compared to a random sample (Mann-Whitney U test, $p<2.2e-16$ in both cases).

Correlated light and electron-microscopic analysis also revealed that they could indeed establish synaptic connections with the same thalamic cell. Besides anatomical evidence, physiological data also support the existance of convergence. Whisker stimulation, together with optical activation of S1 L5 pyramidal neurons in transgeic mice resulted in a larger evoked response than the linear sum of whisker and cortical stimuli.

Conclusions

I - Novel source of inhibition in the thalamus

In our experiments, we have described a glycinergic-GABAergic pathway originating in the brainstem reticular formation and innervating the IL nuclei of the thalamus. The pathway with similar morphological properties was also identified in humans. Glycinergic-GABAergic fibers ended in large terminals with multiple release sites and formed extremely powerful non-depressing inhibitory connections with the proximal dendrites of TC cells in the IL complex. Glycinergic-GABAergic cells in the PRF were shown to fire rhythmic clusters of action potentials coupled to cortical activity. Spontaneous desynchronization, as well as pharmacologically-induced inactivation of the cortex, resulted in a reduced firing rate and disrupted AP clusters in glycinergic neurons, while they were effectively stimulated by the activation of the PRF-projecting cortical cells. In freely moving animals, the selective activation of the glycinergic-GABAergic fibers caused behavioural arrest, or in the case of smaller stimulus intensities, turning movements contralateral to the stimulus.

In the thalamus, two major sources of inhibition can be differentiated with fundamentally different properties. The GABAergic neurons of the thalamic reticular nucleus are situated as a shell around the thalamus, and their axons homogenously interlace among TC neurons. The fibers end in small terminals and form synaptic contacts with the postsynaptic cells via one or two active zones, without any spatial selectivity in the dendritic domain.

In contrast, extrathalamic inhibition originates from various cell groups (Zi, APT, SNr) located outside of the thalamus. These cell groups specifically target particular thalamic regions and form large terminals with multiple release sites selectively on thick proximal dendrites. As it was demonstrated in earlier studies, the IL complex is targeted by several extrathalamic inhibitory nuclei. By discovering the PRF-thalamic pathway, we have described a novel extrathalamic source of inhibition in the IL that shares the morphological and physiological properties of the previously identified ones.

The role of the reticular and extrathalamic system in shaping thalamocortical communication and behavior is different, due to their morphological and physiological dissimilarities. The TRN has the ability to exert global inhibition on large thalamocortical networks. However, its topographic point-to-point connectivity with distinct thalamic cell groups also allows the TRN to act locally in a spatially determined manner. In the temporal dimension, the TRN has a prolonged hyperpolarizing effect, which results in low threshold rebound Ca^{2+} bursts of the thalmic neurons. These bursts activate other TRN cells, and thus the cycle continues and grows, involving larger and larger networks.

The extrathalamic system, however, exerts highly specific spatial and precise temporal connections with the innervated thalamic cells. They do not only target selected nuclei, but also distinct dendritic domains. Their temporal accuracy relies both on the properties of the established synaptic contacts and on the firing rate of the cells.

The spatial-temporal specificity is nicely demonstrated through the feedforward gating of sensory information by the zona incerta. Trigeminal fibers targeting the posterior thalamic nucleus give collaterals to the GABAergic cells of the Zi. The activated incertal cells rapidly and effectively hyperpolarize POm neurons, and thus subcortical excitation cannot evoke action potentials. This effect is localized in POm cells only, and happens with high temporal precision.

The PRF-IL pathway described in this work also shows precise inhibition in the temporal domain. As our experiments demonstrated, high frequency activation of the glycinergic fibers *in vitro* resulted in non-depressing inhibitory postsynaptic currents (IPSCs) on the thalamic cells. As it was described, PRF neurons fired rhythmic AP clusters in anaesthetized animals with high intra-cluster frequency, suggesting that the non-depressing nature of the synapse has relevance *in vivo*.

II - Integration in the thalamus

In our experiments, we mapped the relative distribution of anterogradely-traced S1 cortical fibers and immunolabelled trigeminal terminals in the somatosensory thalamus of rodents (rats and mice). Colocalization of these terminals was observed in the higher order

somatosensory nucleus (posterior thalamic nucleus). Both type of terminals showed the morphological characteristics of a driver input: they established synaptic contacts via multiple release sites onto large caliber proximal dendrites (compared to our random sample). The convergence of drivers with different origin on the same TC cell was shown on correlated light and electron micrographs, and was confirmed by electrophysiological recordings.

With these results we showed a novel form of thalamic information processing, where two essentially different information streams conditionally determined the activity of the TC cell. POm neurons were only able to fire and transmit information to the cortex if the cortical driver signal (evoked by optical stimulation in Thy1::ChR2 transgenic animals) co-occured with the sensory driver signal evoked by whisker deflection.

Bibliography of the candidate's publications

List of Publications

Giber K[*], Diana MA[*], **M Plattner V[*]**, Dugué GP, Bokor H, Rousseau C V, Maglóczky Z, Havas L, Hangya B, Wildner H, Zeilhofer HU, Dieudonné S, Acsády L (2015) A subcortical inhibitory signal for behavioral arrest in the thalamus. Nat Neurosci 18:562–568.

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[*]equal contribution

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