Basic Medical Sciences Doctoral School

Specific network motifs of the medial entorhinal cortical microcircuitry

Ph.D. thesis

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1. General introduction

1.1. The entorhinal cortex

Our brain filters, processes, stores and retrieves information which helps us navigate in space, form memories of the past and predict future events. All these events are conducted by specific neuronal micro- and macro-circuits.

It is generally accepted that the medio-temporal cortex is crucial for memory formation and spatial navigation. The quite heterogeneous information converging from other cortical areas are first arriving to the entorhinal cortex (EC), which serves as a "gate" to the hippocampal formation. Moreover, the hippocampus-processed information enters first again the EC from where it is distributed to other, specific cortical areas. The EC not only relays the information to and from the hippocampal formation, but also pre- and post-processes it.

1,1,1, Anatomical organization of the rodent and human entorhinal cortex

The rodent EC derives its name from the fact that it is partially enclosed by the rhinal sulcus. The entorhinal cortex is surrounded by several cortical areas. Based on cytoarchitectonic criteria, the entorhinal cortex can be divided into a lateral and a medial area. The medial entorhinal cortex (MEC) contains six layers, and it is implicated in physiological processes underlying navigation, learning, and memory and is often the site for early insults during

pathophysiological conditions such as epilepsy and Alzheimer disease (Beed et al., 2010; Canto et al., 2008; Witter & Amaral, 2004).

The human entorhinal cortex is relatively larger and more complex compared to non-human primates and other mammals. This increased size and complexity is thought to be associated with the enhanced cognitive capabilities of humans. In humans, the entorhinal cortex is located on the ventromedial surface of the temporal lobe and can be divided into eight different subdivisions. Topographically, the human entorhinal cortex is really similar to those of non-human primates. The laminar organization of the human and other non-human models is similar as well; there are no major differences except the layerVc, which is visible in non-human primates (Amaral & Insausti, 1990; Insausti, 2013; Insausti et al., 1987, 1995; Witter & Amaral, 2021).

1.1.2. Connectivity of the entorhinal cortex

The MEC, and the entire EC itself, serves as a major gateway between the hippocampal formation and the neocortex in humans, in non-human primates and other mammals as well.

From the classical perspective, there are two major projections connecting the MEC with the hippocampus: the tri-synaptic pathway, which connects the layerII of the MEC with the DG and the CA3, ending in CA1 of the hippocampus; and the monosynaptic pathway bridging the layerIII of the MEC with the CA1 of the hippocampus (Steward & Scoville, 1976; Witter et al., 2000). The inputs from MEC are integrated in CA1 and return to MEC

layerV-VI directly or indirectly via subiculum (Naber et al., 2001; Witter et al., 2000), or to other cortical areas (Cenquizca & Swanson, 2007; Fanselow & Dong, 2010).

1.1.3. Role of the medial entorhinal cortex

The MEC is an important brain region involved in spatial navigation and memory formation in humans, primates and other mammals (Grande et al., 2022; Maass et al., 2015). It is characterized by its unique cellular composition called grid cells. These cells exhibit spatially periodic firing patterns and are crucial for encoding the position within an environment and are thought to contribute to the formation of a particular hexagonal grid of firing fields. Grid cells have been found in all principal cell layers of the MEC, with a higher proportion existing superficially, particularly in layerII (Sargolini et al., 2006).

The MEC, as the brain's GPS, provides spatial information for the hippocampus, playing a crucial role in the formation of episodic memory and spatial representation (Knierim et al., 2014). In addition to the abovementioned grid cells, we find in the MEC several other spatially modulated cell types, such as border cells, which respond to environmental boundaries, and head direction cells, which give information about the human's or animal's orientation in space (Bjerknes et al., 2014; Taube, 2007).

1.2. GABAergic cells in the medial entorhinal cortex

Principal cells in the MEC receive strong inhibitory inputs, both via spontaneously released GABA (Greenhill et al., 2014; Woodhall et al., 2005) and via action potential-driven GABA release (Gloveli, Schmitz, & Heinemann, 1997; Gloveli, Schmitz, Empson, et al., 1997; Jones & Bühl, 1993). Action potential-driven GABA release can both mediate feed-forward inhibition (Finch et al., 1986; Gloveli, Schmitz, & Heinemann, 1997; Gloveli, Schmitz, Empson, et al., 1997; Jones & Bühl, 1993), or provide feedback inhibition (Armstrong et al., 2016; Beed et al., 2010; Couey et al., 2013; Kumar et al., 2007; Varga et al., 2010). These inhibitory interneurons represent a very heterogeneous population, and classically can be divided into three non-overlapping classes expressing parvalbumin (PV+), somatostatin (SOM+), or the serotonin receptor type 3a (5HT3R+) (Tukker et al., 2022). Another major GABAergic cell type briefly described in this dissertation is the NPY+ and neuronal nitric oxide synthase (nNOS+) expressing neurogliaform cell (NGF cell).

1.3. Inhibition in brain circuits

The firing and function of the principal cells can be stabilized by GABAergic interneurons discussed in section 1.2, at least in two different ways: feed-forward and feedback inhibition.

Feed-forward inhibition can reduce the firing responses of principal neurons by competing with excitation or decreasing output spiking. It is involved in different forms of plasticity (Basu et al., 2013) and modulates temporal association memory (Kitamura et al.,

2017; Roux & Buzsáki, 2015). The other form of inhibition, feedback or recurrent inhibition, occurs when a postsynaptic interneuron is activated by an excitatory cell in a specific circuit, and the inhibitory cell itself prevents further activation of the excitatory neuron by giving feedback to it (Miles, 1990).

It is important to mention that these principles do not describe the heterogeneity of inhibition in its ensemble. Feedback and feed-forward inhibition are the two most common forms of inhibition in the brain, and understanding the types of connections is crucial for the current thesis.

1.4. Acute brain slice electrophysiology combined with optogenetics

Electrophysiology, since its emergence, has been a method largely used to describe electrical changes throughout the phospholipid membrane bilayer (Hodgkin et al., 1952). In the brain, in vitro acute slice electrophysiology is used as a powerful experimental tool to investigate structural and functional characteristics of synaptic connectivity in neuronal circuits (Eguchi et al., 2020; Li & McIlwain, 1957; Llinás & Sugimori, 1980; Yamamoto & McIlwain, 1966).

To investigate the circuits and connections within the brain, paired and multiple channel recordings can be used in slice electrophysiology. However, this remains a challenging method, and the success rate of the connectivity between the recorded cells remains very low (Szabadics et al., 2007; Vandrey et al., 2022; Winterer et al.,

2017). To overcome these challenges and low success rates, we can use optogenetics, which is still considered a novel tool to investigate the connectivity and function of the brain.

In optogenetics, we use specific opsins, genetically coded light-sensitive proteins, which are injected with the help of viral vectors into genetically modified mice. These proteins will therefore be expressed in the desired, specific cell types. Afterwards, we can manipulate these specific neurons with the help of LED illumination at different wavelengths.

Optogenetics can be used as a toolbox to investigate local and projection-specific connectivity, establish polysynaptic or monosynaptic connections, characterize synapses, describe the functionalities of different cell types in different brain regions, modulate cell signaling, etc. (Deisseroth, 2015; Lee et al., 2020; Pastrana, 2011).

2. Aims

The function and connectivity of the EC largely differs from other cortical areas in multiple aspects. However, to understand its computational power, many generalized microcircuit motifs are incorporated into the current models of information processing within this brain area. Are these models indeed suitable for EC? In other words, are there EC specific wiring of the neuronal machine?

In my thesis I focused on these specific EC motifs. The specific aims were to:

1. Describe the function of layerI GABAergic interneurons, mainly the role of neurogliaform cells in the local entorhinal cortical microcircuitry.

2. Test the connectivity between neurogliaform cells and layerII principal cells, including stellate and pyramidal cells.

3. Describe the connectivity of somatostatin-expressing GABAergic cells in the EC.

4. Compare the postsynaptic effects of previously mentioned somatostatin-expressing GABAergic cells with the postsynaptic effects exerted by parvalbumin-expressing interneurons.

3. Materials and methods

3.1. Experimental animals

The experiments were approved by the Ethics Committee on Animal Research of Pécs, Hungary (license #: BA02/2000-1-2015 and BA02/2000-21/2021). The animals used in our experiments were male and female 4-14-week-old transgenic mice and additionally, 6-8-week-old Wistar rats were employed for anatomical and in vitro electrophysiological paired recording experiments. The animals were housed in 12 h light/12 h dark cycle.

3.2. Viral injection

For viral injection, the animals were deeply anesthetized (isoflurane, 4% initial dose for induction then 1% during the surgery). A small craniotomy was drilled in the skull above the MEC (coordinates were 3.75 mm lateral from the midline and 0.2 mm anterior to the transverse sinus (Heys & Dombeck, 2018)). To

selectively express ChR2 adeno-associated virus vector coding ChR2-mCherry was injected at postnatal day P25–30 into the MEC. Mice were sacrificed 2-3 weeks postinjection for slice preparation.

3.3. Acute brain slice preparation for in vitro electrophysiology

Optogenetic and in vitro slice electrophysiological experiments were performed in acute horizontal brain slices. For some of our recordings performed in the somatosensory cortex and in the hippocampus, we used acute coronal brain sections. Under deep isoflurane anesthesia, mice were decapitated, and 300 μm horizontal or coronal slices were cut and were used in in vitro electrophysiological experiments.

3.4. In vitro electrophysiological experiments and analysis

Patch pipettes were pulled from borosilicate glass capillaries with filament with a resistance of 3–5 MΩ. Slices were visualized using an upright microscope equipped with differential interference contrast (DIC) optics and fluorescence excitation source. Whole-cell experiments were performed at 32-34 °C in ACSF bubbled with 95% O_2 and 5% CO_2. The pipette recording solution contained "CsCl containing – high chloride – intracell", "Low Cl containing intracell" and "Very Low Cl containing intracell" containing 0.2% biocytin.

3.5. In vivo electrophysiological recordings

All surgery were done under deep isoflurane anesthesia. Head bar implantation, acclimation, and craniotomy was done as described earlier (Varga et al., 2012). A linear silicon probe with 32 recording sides (A1x32-Poly2, Neuronexus) was placed into the CA1 region of dorsal hippocampus. The MEC was targeted with a 2 or 3 shank silicon probe. Each shank contained 8 recording sides with 50 μm spacing (Buzsaki32 design, Neuronexus). For optogenetic manipulation optical fibers were attached to the probe. Each recording lasted 30–90 min.

3.6. Immunohistochemistry, confocal imaging, and Neurolucida-tracing

Anatomical, morphological, and immunohistochemical studies were done on 60- or 70-μm-thin sections. Confocal images were taken using a Nikon Eclipse Ti2-E fluorescence confocal microscope with 10×, 20×, and 60× objectives and a Zeiss LSM710 microscope. The three-dimensional reconstructions of axonal and dendritic arborization were performed using the Neurolucida software with a 60× 1.4 NA objective. Cell densities were defined as cells/mm^2 and counted using the ImageJ software.

3.7. Statistical analysis

Statistics were performed using GraphPad Prism. Normality's of samples were tested using the D'Agostino–Pearson test or the Kolmogorov–Smirnov test (for small representations).

Samples were compared with unpaired T-test and non-normally distributed ones using the Mann–Whitney test. Kruskal–Wallis's test followed by post-hoc Dunn's pairwise comparison tests for non-normally distributed data and ANOVA test with Tukey multiple comparison test for normally distributed data were used for three or more group comparison. In the case of some of our nonparametrically distributed in vivo data, Wilcoxon signed-rank test was applied. Data were presented as mean ± S.E.M. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications in the field. The animal and the cell numbers are presented as N and n.

4. Results

4.1. Axons of layerII pyramidal cells mainly target layerII

The layerII principal cells axonal targets have been reported to be located in layerII and to some extent in deeper layers (Sürmeli et al., 2015). High connection probability from layerII pyramidal cells to stellate cells has been reported (Winterer et al., 2017). To test this, we selectively expressed ChR2 in pyramidal cells using Calb-Cre mice and examined the effect of short (5 ms) light pulses on layerII stellate cells. We found that large amplitude excitatory postsynaptic potentials (EPSPs) (delay time: 2.0 ± 0.2 ms, n = 6, average amplitude of EPSPs: 10.6 ± 2.9 mV, at 5 mW light intensity) could be elicited in the stellate cells, which frequently

resulted in action potentials (AP). We also tested whether layerII fast spiking parvalbumin-expressing interneurons are also innervated by axons of layerII pyramidal cells. We found strong excitation (delay: 2.2 ± 0.7 ms, average amplitude of EPSPs: 6.8 ± 2.1 mV at 0.5 mW light intensity) on layerII interneurons (n = 5, 2/2 tested for parvalbumin), which reliably elicited APs when paired-pulse light-excitation was applied.

4.2. LayerI neurogliaform cells receive excitatory monosynaptic inputs from layerII pyramidal cells

ChR2 expressing projections are not restricted solely to layerII but can also be found in layerI. Therefore, we then investigated the effect of light pulses on layerI located interneurons. The majority of interneurons are neurogliaform cells, which express GABAARα1 (Armstrong et al., 2012) and Reelin. In our colocalization experiments, we found that 53 ± 12% (totally 735 Reelin and/or GABAARα1 + cells, N = 2) of GABAARα1 expressing cells in layerI also express Reelin. Their dendrites and axons are mostly restricted to layerI (Craig & Mcbain, 2015). Light excitation of ChR2+ axons elicited EPSPs in neurogliaform cells (delay: 1.9 ± 0.1 ms, amplitude: 6.0 ± 0.7 mV, n = 34, light intensity: 5 mW). TTX and 4-AP wash-in experiments verified that the recorded EPSPs are monosynaptic.

Calbindin is expressed not only in layerII pyramidal cells but also in some GABAergic interneurons (Canto et al., 2008), potentially resulting in a mixed GABAergic and glutamatergic effect of light-activation of calbindin+ ChR2-expressing cells in Calb-Cre

animals. We, therefore, tested whether neurogliaform cells receive inputs from calbindin+ interneurons as well. For this, we used a high-chloride intracellular solution, kept the membrane potentials at −60 mV, and washed the sections in NBQX and CNQX (both in 10 μM concentration). The AMPA receptor blockers have completely removed the postsynaptic effect on the neurogliaform cells (before drug wash, the average amplitude of the EPSPs was 6.8 ± 1.5 mV, n = 3, light intensity: 5 mW), therefore we assume that there is no GABAA receptor-mediated input to the neurogliaform cells from calbindin+ interneurons within the entorhinal cortex.

4.3. Neurogliaform and non-neurogliaform cells react differently to layerII excitatory inputs

Neurogliaform cells are considered late-spiking cells (Kawaguchi, 1995), which means that when somatic membrane depolarization is applied to them, they elicit their first action potentials only after several hundred ms during the depolarization step. The neurogliaform cells in the MEC show similar late-spiking intrinsic membrane properties (rheobase: −36.6 ± 0.9 mV, first AP after 278.5 ± 48.0 ms, resting membrane potential of NGF cells: −65.1 ± 0.5 mV, n = 34; time constant: 7.5 ± 0.4 ms). There is only limited data, however, about the EPSP-generated firing properties of this cell population (Chittajallu et al., 2020). Therefore, we then tested how neurogliaform cells and other non-late spiking interneurons react to trains of EPSPs generated by layerII pyramidal cells. LayerII interneurons (n = 5) reliably elicited action potentials during the train

of stimulation (5 EPSPs at 17 Hz, 10.7 ± 2.7 mV, light intensity 5 mW). In the layerI neurogliaform cells, the same intensity of light elicited smaller amplitude EPSPs (average amplitude of EPSPs: 6.2 ± 1.1 mV, n = 17, light intensity 5 mW) and could not elicit action potentials. However, when the membrane potentials were slightly depolarized from resting (from −64.5 ± 0.6 mV to −52.8 ± 0.9 mV), action potentials have been initiated. The occasional action potentials occurred during the first and/or second light pulses, and no action potentials could be elicited during the remaining 3 light pulses.

4.4. The distribution of neurogliaform cells does not correlate with dendritic clustering of layerII pyramidal cells

The connection probability between two cells has been shown to largely depend on the distance between the neurons (Holmgren et al., 2003). Following this assumption, we have attempted to record monosynaptic connectivity between layerII principal cells and closely located neurogliaform cells (n = 15 stellate and n = 24 pyramidal cells recorded simultaneously with neurogliaform cells in layerI). Since no connection has been found between the recorded cell pairs, we have analyzed their axo-somato-dendritic morphology both in rats and mice. The layerII pyramidal cells instead of having apical dendrites running straight up to layerI perpendicular to the layer, as layerII–III pyramidal cells in the neocortex (M.L. Feldman, 1984) and layerIII pyramidal cells in entorhinal cortex have (Canto & Witter, 2012; Craig & Mcbain, 2015), MEC layerII calbindin+ pyramidal cells send their most prominent

"apical" dendrites laterally. The layerII stellate cells in contrast, have both laterally and medially running dendrites and mostly no apical dendrites running straight up to the layerI. These features of the layerII principal cells set different connectivity rules compared with the known connection probability rule of thumbs in other cortical areas. Then, we checked whether neurogliaform cells are actually localized in areas of layerI where dendrites of the layerII pyramidal cells are less abundant. For this, we labeled tangentially sectioned layerI of the entorhinal cortex area for markers of layerII pyramidal and neurogliaform cells. Patchy structures are recognizable in layerI as well; however, instead of the hexagonal arrangement of the patches, we have found amorph WFS1+ areas intermingled with putative layerIII-V pyramidal cells apical dendrites. We have estimated the density of neurogliaform cells in the patch and interpatch areas. Our semiquantitative measurement of alpha-actinin (α-actinin) immunoreactive putative neurogliaform cells in rats (Price et al., 2005; Ratzliff & Soltesz, 2001) revealed that neurogliaform cells are relatively homogenously distributed within the patch and interpatch areas (within patches: 169 ± 24 cell/mm^2 outside patches: 122 ± 5 cell/mm^2, p = 0.1288 Welch corrected t-test).

4.5. In the MEC, SOM+ interneurons show strong layer-specific target selectivity, meanwhile PV+ interneurons innervate all principal cells equally

We induced ChR2 expression either in SOM+ or in PV+ interneurons in the MEC. We investigated the effect of SOM+

interneuron innervation on multiple layers of the MEC. For this, we recorded principal cells from different layers of the MEC in the SOM-ChR2 mice, in order to examine their light-evoked postsynaptic responses. Dense SOM immunoreactive axonal cloud and SOM-Cre-ChR2 axons (but no PV+ boutons) can be found in layerI. However, SOM+ interneurons elicited only moderate postsynaptic responses in layerII principal cells located closely near layerI. Both reelin+ stellate ($n = 33$, $N = 18$) and WFS1/calbindin+ pyramidal cells ($n = 9$, $N = 7$) responded only moderately (layerII stellate: 2.2 ± 0.38 mV, layerII pyramidal: 0.66 ± 0.16 mV) to light-evoked SOM+ interneuron activation. The recorded cells showed dense dendritic arborizations surrounded by robust ChR2-mCherry expression from SOM+ axons; thus, the moderate postsynaptic effect was not due to truncated dendritic trees.

In contrast to layerII, the pyramidal cells of layerIII ($n = 15$, $N = 12$) and layerV ($n = 8$, $N = 6$) responded with a magnitude higher amplitude to the whole-field illumination of the same duration and intensity (layerIII: 13.58 ± 2.02 mV, layerV: 16.6 ± 2.09 mV). Comparing the amplitudes of the light-induced postsynaptic potentials (PSPs) in the stellate cells with the PSPs in pyramidal cells of layerII, as well as comparing the PSPs in layerIII vs layerV pyramidal cells showed no differences; however, every other comparison (layerII cells vs layerIII or layerV cells) showed notable differences ($p < 0.0001$, variance analysis with Tukey's multiple comparison test). Therefore, we concluded that SOM+ preferentially innervates deep layers (layerIII–V) instead of superficial layerII principal cells.

Next, we sought to map the cell type and layer specificity of PV+ GABAergic inhibitory motifs in the MEC. We applied short (3 ms), whole-field light illuminations (Pfeffer et al., 2013) on the PV-ChR2-expressing slices and recorded the postsynaptic effects in principal cells in different layers. Stellate ($n = 14$, $N = 5$) and pyramidal cells ($n = 4$, $N = 3$) responded with a fast, monosynaptic (2.17 ± 0.56 and 1.9 ± 0.6 ms delay time, respectively, $p = 0.58$) potential change. However, we found equally large postsynaptic potential changes in layerIII ($n = 11$, $N = 8$) and also in layerV ($n = 8$, $N = 3$) pyramidal cells as well. These inhibitory events were comparable with the effect on layerII principal cells (layerII pyramidal: 13 ± 1.08 mV, layerII stellate: 16.5 ± 1.79 mV and layerIII: 18.45 ± 1.49 mV, layerV: 19.25 ± 1.75; $p = 0.23$, one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test). Therefore, we concluded that, in contrast to previous immunohistochemical predictions (Boccara et al., 2010), deep-layer (layerIII–V) pyramidal cells receive strong PV+ innervation; thus PV+ GABAergic cells have an overall strong GABAergic inhibition on all principal cell types in layerII–V of the MEC.

The differences in SOM+ interneuron innervation on principal cells of the deep vs superficial layers in the MEC may reflect a more general cortical/hippocampal microcircuit organization. To examine this, we investigated the monosynaptic inhibitory effects of all types of SOM+ interneurons in the somatosensory cortex and in the CA1 region. We induced ChR2 expression in SOM+ cells in the somatosensory cortex and in the dorsal hippocampus CA1 within the

same SOM-Cre mouse line (SOM-Cre) and with the same AAV-ChR2 injection. The postsynaptic effects of short, whole-field light illuminations on the ChR2-expressing slices were recorded in pyramidal cells in different layers of the somatosensory cortex and the dorsal hippocampus CA1 region. We found that the overall SOM+ innervation in the recorded regions does not differentiate between superficial or deeper located pyramidal cells. Thus the preferential innervation of the deep-layer pyramidal cells in the MEC by SOM+ GABAergic cells may suggest that they have specific roles in this brain area.

4.6. SOM+ interneurons inhibit pyramidal cells longer than PV+ interneurons

Subsequently, we tested whether the PV+ and SOM+ local inhibitory interneurons have different effects on the firing activity of pyramidal cells in the MEC. First, we recorded the multi-unit activity of the MEC in head-fixed awake transgenic (PV-Cre-ChR2 and SOM-Cre-ChR2) mice. When 10 ms light pulses were applied to the MEC expressing ChR2 Cre-dependently, putative PV+ or SOM+ interneurons increased their firing rate (PV-ChR2: from 8.2 ± 1 to 50 ± 6 Hz, $n = 122$, $N = 6$ SOM-ChR2: from 3.5 ± 1 to 27.3 ± 7 Hz, $n = 36$, $N = 4$), and putative layerIII–V pyramidal cells were inhibited. The duration of inhibition in PV-ChR2 animals was shorter than in SOM-ChR2 animals (50% recovery from inhibition: PV-Cre-ChR2: 37 ± 1 ms $N = 6$, $n = 136$, SOM-Cre-ChR2: 89 ± 7 ms $N = 4$, $n = 51$, $p < 0.0001$, Wilcoxon rank-sum test). The duration of inhibition in PV-Cre-ChR2 animals was comparable with what was reported in

previous in vivo studies on the MEC (Buetfering et al., 2014). However, the prolonged inhibition in SOM-Cre-ChR2 animals suggested an atypical underlying inhibitory mechanism.

4.7. The neuromodulator SOM influences the firing probability of layerIII–V pyramidal cells

SOM as a neuromodulator has been shown to evoke hyperpolarization in CA1 pyramidal cells (Baraban & Tallent, 2004) and has been hypothesized to be released from synaptic boutons of SOM-expressing interneurons (Katona et al., 2014).

Elongated inhibitory action of SOM+ cells compared to PV+ cells has been reported, for example, in the prefrontal cortex (Kvitsiani et al., 2013), but the underlying mechanisms have not been revealed. To understand the mechanisms causing this unexpected difference between PV+ and SOM+ inhibitory effect on layerIII–V pyramidal cells, we determined first whether the kinetics of the postsynaptic currents evoked by PV+ and SOM+ interneurons were similar. Dendritic filtering of PSPs can cause slower kinetics (Hoffman et al., 1997). In our experiments, however, the rise times (SOM: 5.96 ± 1.22 ms ($n = 11$, $N = 2$), PV: 5.77 ± 0.99 ms ($n = 11$, $N = 3$), $p = 0.91$) and decay times (SOM: 179 ± 34.5 ms, PV: 199.8 ± 21.6 ms, $p = 0.17$, Mann–Whitney test) of the two groups did not differ. This finding is in agreement with that of the comparison of the basket cell and Martinotti cell inhibitory PSP kinetics (Szabadics et al., 2007). Moreover, applying the GABAA receptor blocker

GABAZINE completely eliminated the postsynaptic effect of both PV+ and SOM+ interneurons.

Afterwards, we compared the duration of the light-induced inhibition in SOM-ChR2 and PV-ChR2 MEC layerIII–V pyramidal cells in vitro. We held the recorded cell's membrane potential at the level where low-frequency firing occurred and excited the SOM-ChR2+ or PV-ChR2 + cells/axons with light pulses. The firing of principal cells recovered much earlier after the excitation of PV+ interneurons than in the SOM-ChR2 animals (PV 220 ± 17 ms, $n = 16$, $N = 2$; SOM 340 ± 32 ms, $n = 10$, $N = 3$; $p = 0.0023$). These in vitro electrophysiological results were comparable with our in vivo findings suggesting that SOM+ cells provide a prolonged inhibition to layerIII-V pyramidal cells. Taken all these together, our findings suggest that neither GABAB nor the dendritic filtering is involved in this mechanism. Furthermore, we found that the neuromodulator SOM may be responsible for this prolonged inhibition, but these results are beyond the scope of the current dissertation and are not shown.

5. Discussion and conclusions

We identified two unique motifs in the entorhinal cortex employing novel techniques and methodologies. Using selective genetic markers of layerII pyramidal cells of the entorhinal cortex and optogenetics combined with slice electrophysiology, our results show that layerI neurogliaform GABAergic cells receive monosynaptic excitatory inputs from the layerII calbindin-positive pyramidal cells,

and they perform feedback inhibition, which seems to be a specific entorhinal cortical motif.

Moreover, our results revealed that GABAergic inhibition elicited by SOM+ local interneurons show strong layer preference in MEC. The activities of layerIII–V pyramidal cells, which have important implications for handling memory, are heavily influenced by SOM+ inhibition. LayerII principal cells, however, receive only moderate level of inhibition from local SOM+ GABAergic cells. LayerI neurogliaform cells also receive strong inhibition from these SOM-expressing neurons. This sparse inhibition may have a permissive role in the dendritic encoding of converging spatial information in principal cells in layerII of MEC.

Taken all together, our results briefly are the following:

1. Neurogliaform cells receive strong, monosynaptic inputs from layerII pyramidal cells.

2. The activity of NGF cells is largely set by the synapses arising from both inhibitory and excitatory neurons.

3. NGF cells provide strong feedback inhibition in the local entorhinal cortical microcircuit.

4. Specifically in the EC the dendritic inhibition provided by the somatostatin-positive cells is weaker on layerII principal cells than on deep layer principal cells.

5. The inhibition provided by SOM+ neurons is significantly prolonged in comparison to the inhibition given by PV+ interneurons.

As a summary, we can say that our work shed light on two specific entorhinal cortical motifs and highlighted that to understand the complex processes of learning, memory formation, and spatial navigation, we need to understand the local neuronal networks.

6. List of publications

This dissertation is based on the following articles:

Szocs, S., Henn-Mike, N., Agocs-Laboda, A., Szabo-Meleg, E., & Varga, C. (2022). Neurogliaform cells mediate feedback inhibition in the medial entorhinal cortex. Frontiers in Neuroanatomy, 16(August), 1–11. https://doi.org/10.3389/fnana.2022.779390 (IF = 2.90)

Kecskés, M., Henn-Mike, N., Agócs-Laboda, Á., **Szőcs, S.**, Petykó, Z., & Varga, C. (2020). Somatostatin expressing GABAergic interneurons in the medial entorhinal cortex preferentially inhibit layerIII-V pyramidal cells. Communications Biology, 3(1), 1–13. https://doi.org/10.1038/s42003-020-01496-x (IF = 6.26)

Other publications not related to the dissertation:

Makkai, G., Abraham, I. M., Barabas, K., Godo, S., Ernszt, D., Kovacs, T., Kovacs, G., **Szocs, S.**, & Janosi, T. Z. (2023). Maximum likelihood-based estimation of diffusion coefficient is quick and reliable method for analyzing estradiol actions on surface receptor movements. Frontiers in Neuroinformatics, 17(March), 1–10. https://doi.org/10.3389/fninf.2023.1005936 (IF = 2.50)

Kövesdi, E., Udvarácz, I., Kecskés, A., **Szőcs, S.**, Farkas, S., Faludi, P., Jánosi, T. Z., Ábrahám, I. M., & Kovács, G. (2023).

17B-Estradiol Does Not Have a Direct Effect on the Function of Striatal Cholinergic Interneurons in Adult Mice in Vitro. Frontiers in Endocrinology, 13(January), 1–14. https://doi.org/10.3389/fendo.2022.993552 (IF = 6.05)

Luo, W., Egger, M., Domonkos, A., Que, L., Lukacsovich, D., Cruz-Ochoa, N. A., **Szőcs, S.**, Seng, C., Arszovszki, A., Sipos, E., Amrein, I., Winterer, J., Lukacsovich, T., Szabadics, J., Wolfer, D. P., Varga, C., & Földy, C. (2021). Recurrent rewiring of the adult hippocampal mossy fiber system by a single transcriptional regulator, Id2. Proceedings of the National Academy of Sciences of the United States of America, 118(40), e2108239118. https://doi.org/10.1073/pnas.2108239118 (IF = 12.77)

7. Presentations

Conference presentations and posters related to this thesis:

Szilárd, Szőcs; Nóra, Henn-Mike; Ágnes, Agócs-Laboda; Zoltán, Petykó; Csaba, Varga: Feedback inhibition in the entorhinal cortex mediated by neurogliaform cells; International Neuroscience Meeting, Budapest, 2022: IBRO Workshop: 27-28 January 2022. Budapest, Hungary: Abstract book (2022) 277 p. p. 126

Szilárd, Szőcs; Nóra, Henn-Mike; Ágnes, Agócs-Laboda; Csaba, Varga: Feedback inhibition in the entorhinal cortex mediated by neurogliaform cells; Society for Neuroscience, San Diego, CA, USA (2022)

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