MAGNIFICENT SEVEN 2

WARSAW — NENCKI INSTITUTE — 20 MAI 2019

11:30 – 12:00 (Exhibition hall)
Coffee and snacks

12:00 – 13:45 (Conference room 1st floor)

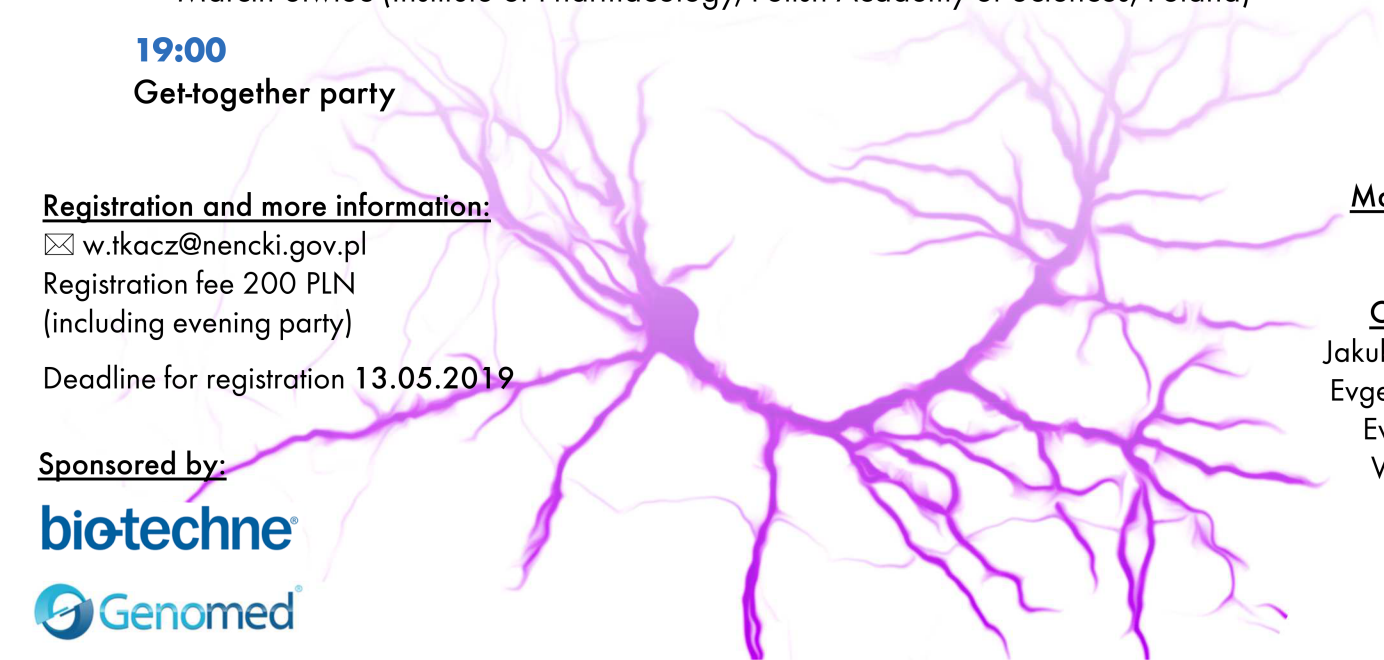
- The physicochemical properties play a role in the residence time of serotonin 7 (5-HT_7) receptor ligands
  Marcello Leopoldo (University of Bari Aldo Moro, Italy)
- The role of the serotonin 5-HT_7 receptor in regulation of the enteric nervous system and gastrointestinal functions under physiological and pathological conditions
  Daria Guseva (University of Hohenheim, Germany)
- Ameloration of Tau pathology by targeting 5-HT_7 receptor
  Evgeni Ponimaskin (Hannover Medical School, Germany)

14:00 – 15:00
Lunch

15:00 – 17:45 (Conference room 1st floor)

- Fluorinated indole-imidazoles: Selective, orally bioavailable 5-HT_7 receptor low-basicity agonists, molecular probes and potential neuropathic painkillers
  Adam Hogendorf (Institute of Pharmacology, Polish Academy of Sciences, Poland)
- Signaling between serotonin receptors and the extracellular matrix as a key to understanding pathogenesis of stress related disorders
  Monika Bijata (Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland)
- Modulation of the synaptic transmission in the basal amygdala by 5-HT_7 receptors
  Magdalena Kusek (Institute of Pharmacology, Polish Academy of Sciences, Poland)
- The functional role of 5-HT_7 receptors in the mouse dentate gyrus
  Marcin Siwiec (Institute of Pharmacology, Polish Academy of Sciences, Poland)

19:00
Get-together party



Registration and more information:
✉ w.tkacz@nencki.gov.pl
Registration fee 200 PLN
(including evening party)

Deadline for registration 13.05.2019

Sponsored by:
biotechne
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Main organizer:
Monika Bijata

Co-organizers:
Jakub Włodarczyk
Evgeni Ponimaskin
Ewa Bączyńska
Wioletta Tkacz

Marcello Leopoldo (University of Bari Aldo Moro, Italy)

The physicochemical properties play a role in the residence time of serotonin 7 (5-HT$_7$) receptor ligands

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The serotonin receptor 7 (5-HT$_7$R) is a G protein-coupled receptors (GPCR) involved in many physiological processes and in neurological and neurodevelopmental disorders such as Fragile X syndrome and Rett syndrome, which are characterized by abnormal neuronal connectivity and consequent intellectual disabilities. To unveil the molecular pathway linking 5-HT$_7$R to these diseases, the use of adequate pharmacological tools, such as the brain penetrant and selective 5-HT$_7$R receptor agonist LP-211, has been important. Usually, the pharmacological properties of pharmacological tool are defined by the affinity for the target receptor (Ki) and by the agonist (EC50) or antagonist (IC50) potency. In recent years, there is interest also to investigate how a given ligand kinetically interacts with the target receptor, by measuring the residence time, because it has been proposed that persistent ligand binding can be exploited in drug discovery to achieve more selective response profiles. Previous studies on LP-211 and related analogs have suggested that the residence time of these class of 5-HT$_7$R ligands might be structure-dependent. By assessing the residence time of a series of 5-HT$_7$R ligands characterized by different lipophilic properties (ClogP), we have found that it is not the overall lipophilicity of the molecule that determines its dissociation rate, but rather the lipophilicity at a specific position of the scaffold. Future studies will investigate if 5-HT$_7$R agonists characterized by different residence time display different effects on neuronal morphology in animal models of neurodevelopmental diseases.

Daria Guseva (University of Hohenheim, Germany)

The role of the serotonin 5-HT$_7$ receptor in regulation of the enteric nervous system and gastrointestinal functions under physiological and pathological conditions

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The enteric nervous system (ENS) is a complex network in the wall of the gastrointestinal (GI) tract, which consists of neurons and glia and controls a wide range of GI functions. It has increasingly become evident that intestinal inflammation is associated with structural and functional changes of the ENS, which can persist long after recovery, and may contribute to altered gut function in post-inflammatory irritable bowel syndrome. Serotonin (5-hydroxytryptamine, 5-HT) represents an important neurotransmitter in the ENS, which targets epithelial cells, smooth muscles and enteric neurons. Its largest pool is secreted by intestinal enterochromaffin cells (EC), and serotonergic neurons in the myenteric and submucosal plexuses. When released, 5-HT stimulates local ENS reflexes regulating secretion, absorption, local blood flow, and propulsive motility. We have previously shown that 5-HT regulates neuronal outgrowth and plasticity via 5-HT$_7$ receptor (5-HT$_7$R)-mediated activation of RhoA and Cdc42 GTPases in CNS neurons. The aim of the present study was to investigate the expression profile and the role of 5-HT$_7$R in enteric neurons morphology and plasticity under physiological and inflammatory conditions. To analyze the role of the 5-HT$_7$R in enteric neurons outgrowth and synaptogenesis, we used primary culture of isolated intestinal ganglia from adult 5-HT$_7$R knockout mice and their wild-type littermates (Hannover Medical School). Those preparations contain both myenteric as well submucosal neurons that survive up to 20 days. Using immunofluorescence, confocal microscopy, and morphometric measurements, we performed measurements of neurite outgrowth at day in vitro 2 (DIV2), and the number of synapses at DIV12. Quantitative analysis revealed a significant increase in the length of neurites and the number of synaptophysin-positive puncta in cultures treated with 5-HT$_7$R agonist 5-CT vs. control preparation. This morphogenic effect was fully inhibited by the specific 5-HT$_7$R antagonist SB-269970. For the analysis of the 5-HT$_7$R-mediated enteric neuron plasticity, we used longitudinal muscle myenteric plexus (LMMP) preparations from Wnt-1 Cre/GCaMP5 tdT-flox mice on C57BL/6 background (referred Wnt-GCaMP, University of Vermont) which were processed for Ca2+ imaging with stimulation of the 5-HT$_7$R by application of LP211. Results of this experiment are variable with increased activity being observed in some cells while decreased activity was noted in others. Additional studies will be necessary to determine if specific cell types are activated or inactivated. To assess the morphogenic role of the 5-HT$_7$R in enteric neurons in vivo under inflammatory conditions, we performed morphological analysis of axon density in intestines of IL10 knockout mice (Hannover Medical School) with chemically induced colitis. These mice were intra-peritonealy injected with 5-CT, SB-269970 or 5-CT and SB-269970, for 35 day followed by immunofluorescence analysis of intestinal cryosections using confocal microscopy. Quantitative analysis revealed that chronic stimulation of the endogenous 5-HT$_7$R, both under physiological and inflammatory conditions, resulted in an increase of the density of axons in the mouse colon. In accordance, inhibition of the receptor resulted in the reduction of the density of axons. To evaluate the changes in density of axons in the human intestine under inflammatory conditions, cryosections of colon from patients with Crohn's disease and controls (tumor patients) (Hannover Medical School) were subjected to immunofluorescence analysis following confocal microscopy and the quantification. We have identified a 3-fold decrease in the density of axons in the colon of patients with Crohn's disease in the mucosal, submucosal and muscle layers. We suppose that 5-HT$_7$R-mediated regulation of enteric neurons morphology can have a favorable impact in patients with IBD via restoration of the neuronal network in the intestinal wall. Taken together, we have demonstrated that 5-HT$_7$R-mediated signaling induces outgrowth, synaptogenesis and plasticity of intestinal neurons, and might play an important role in the recovery and normalization of the ENS function after intestinal inflammation.

Evgeni Ponimaskin (Hannover Medical School, Germany)

Amelioration of Tau pathology by targeting 5-HT$_7$ receptor

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Tauopathies comprise a heterogeneous family of neurodegenerative diseases characterized by pathological accumulation of hyperphosphorylated Tau protein. Pathological changes in serotonergic signaling have been associated with tauopathy etiology, but the underlying mechanisms remains poorly understood. Here, we studied the role of the 5-HT$_7$ serotonin receptor (5-HT$_7$R), in a model of tauopathy induced by overexpressing the human Tau[R406W] mutant associated with inherited forms of frontotemporal dementia. We showed that the constitutive 5-HT$_7$R activity is required for Tau hyperphosphorylation and tangle formation through G-protein-independent, CDK5-dependent mechanism. We also showed that 5-HT$_7$R physically interacted with CDK5. At the systemic level, 5-HT$_7$R-mediated CDK5 activation induces tangle formation leading to neuronal death, reduced LTP, and impaired memory in mice. Specific blockade of constitutive 5-HT$_7$R activity in neurons that overexpressed Tau[R406W] prevented Tau hyperphosphorylation, aggregation, and neurotoxicity. Moreover, 5-HT$_7$R knockdown in prefrontal cortex fully abrogated Tau[R406W]-induced LTP deficits and memory impairments. Thus, 5-HT$_7$R/CDK5 signaling emerged as a new, promising target for tauopathy treatments.

Adam Hogendorf (Institute of Pharmacology, Polish Academy of Sciences, Poland)

Fluorinated indole-imidazoles: Selective, orally bioavailable 5-HT$_7$ receptor low-basicity agonists, molecular probes and potential neuropathic painkillers

Hogendorf AS[1], Hogendorf A[1], Kurczab R[1], Satała G[1], Lenda T[1], Walczak M[2], Latacz G[2], Handzlik J[2], Kieć-Kononowicz K[2], Nikiforuk A[1], Krawczyk M[1], Mika J[1], Popiołek-Barczyk K[1], Ciechanowska A[1], Morriset-Lopez S[3], El Khamlichi C[3], Ponimaskin E[4], Bijata M[4,5], Tokarski K[1], Siwiec M[1], Roth B[6], Levy FO[7], Andressen KW[7], Andresen H[7], Kubicki M[8], Pilarski B[7], Bojarski AJ[1]

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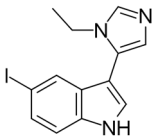
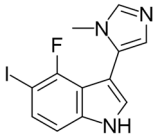


AGH-107
K_i = 6 nM



AGH-194
K_i = 2 nM

3-(1-alkyl-1H-imidazol-5-yl)-1H-indoles, invented in the Department of Medicinal Chemistry Institute of Pharmacology PAS, are the first-in-class non-basic full agonists of an aminergic receptor. Probably due to the very distinctive mode of action (the unprotonated form is considered active), they exhibit unprecedented selectivity profiles. Moreover, they show high efficacy, high metabolic stability, water solubility, oral bioavailability and excellent pharmacokinetics[1-3]. Lead compound AGH-194 has been shown to produce strong procognitive effects in rodent models and antihyperalgesia in mouse model of neuropathic pain. Development of truly selective, functionally potent and brain penetrable agonists is crucial to the understanding of any receptor function and pathology in the CNS. Thus, new, low-basicity 5-HT$_7$ agonists are highly appropriate molecular probes to explain the role of 5-HT$_7$ receptor in the aetiology of depression, anxiety, sleep disorders, memory impairment as well as cognitive processes.

1. Hogendorf, A. S.; et al. Sci. Rep. 2017, 7 (1), 1-15
2. Latacz, G.; et. al. MedChemComm 2018, 9, 1882-1890.
3. Hogendorf, A. S. et al. Eur. J. Med. Chem. 2019, 170, 261-275.

Monika Bijata (Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland)

Signaling between serotonin receptors and the extracellular matrix as a key to understanding pathogenesis of stress related disorders

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The rewiring of synaptic circuitry pertinent to memory formation in the brain has often been associated with morphological changes in dendritic spines and extracellular matrix (ECM) remodeling. Here, we linked these processes by uncovering the signaling pathway involving the serotonin 5-HT$_7$ receptors (5-HT$_7$R,) the matrix metalloproteinase-9 (MMP-9) and the small GTPase Cdc42. We find that 5-HT$_7$R stimulation increases local MMP-9 activity triggering dendritic spines remodeling. The underlying molecular machinery involves 5-HT$_7$R-mediated activation of MMP-9, which leads to cleavage of its substrates followed by Cdc42 activation. Our results thus reveal causal interactions in a previously unknown molecular mechanism regulating neuronal plasticity. The direct evidence for the possible behavioral role of described 5-HT$_7$R/MMP-9 pathway was an intriguing issue. We were wondering if 5-HT$_7$R/MMP-9/Cdc42 signaling pathway also exists in brain of adult animals and how does it affect animal behavior. Our results demonstrate that application of 5-HT$_7$R agonist prompts a significant increase of MMP-9 activity, whereas application of 5-HT$_7$R antagonist leads to MMP-9 decrease in hippocampus. Changes in MMP-9 activity reflected also behavior in animals: increased immobility time in tail suspension test (depressive behavior) after application of 5-HT$_7$R agonist and decreased immobility time in tail suspension test application after application of 5-HT$_7$R antagonist. These new data provide the first indication for the behavioral importance of 5-HT$_7$R/MMP-9 signaling.

Magdalena Kusek (Institute of Pharmacology, Polish Academy of Sciences, Poland)

Modulation of the synaptic transmission in the basal amygdala by 5-HT_7 receptors

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Institute of Pharmacology of the Polish Academy of Sciences, Department of Physiology, 12 Smetna St., Kraków, Poland

Introduction. The amygdala mediates unconscious reactions and is responsible for emotional memory formation and attachment of subjective emotional valence to various stimuli. Limbic structures of the brain, including the amygdala, express of 5-HT_7 receptors in a high density, which suggests their contribution to emotional processing. Many studies investigated the role of this receptor in brain regions involved in the pathophysiology of affective disorders. However, the function of the 5-HT_7 receptor in the amygdala remains poorly understood. Aim. The present experiments were aimed at determining the impact of 5-HT_7 receptor activation on membrane properties and synaptic transmission in anatomically and electrophysiologically identified pyramidal-like basal amygdala (BA) neurons. Methods. Whole-cell patch clamp recordings in both current and voltage clamp mode were performed to investigate ionic mechanisms following 5-HT_7 receptor activation. After decapitation, brains were removed and placed in an ice-cold artificial cerebrospinal fluid (aCSF) bubbled with carbogen. Brains were cut into coronal slices using vibrating microtome. Slices containing a part of the amygdala were incubated at 30°C in aCSF for minimum 3 h. For whole-cell recording, a slice was transferred to the recording chamber mounted on an upright microscope and superfused with aCSF. Cells were identified by a shape of cell body, membrane resistance and a response to depolarizing current pulses. After confirming the characteristics of the neuron spontaneous postsynaptic currents were recorded in the voltage-clamp mode. Spontaneous excitatory and miniature postsynaptic currents (sEPSCs and mEPSCs) were recorded at a holding potential of -70 mV. Spontaneous and miniature inhibitory postsynaptic currents (sIPSCs and mIPSCs) were recorded at a holding potential of 0 mV with pipette filled with cesium gluconate-containing solution. The measured parameters of the currents were their frequency and amplitude. To selectively activate 5-HT_7 receptor, 5-CT, an agonist of 5-HT_{1A}/5-HT_7 receptors, was applied for 15 min in the presence of 2μM WAY100635, a selective 5-HT_{1A} receptor antagonist. Conclusions. Activation of 5-HT_7 receptors decreased the mean frequency of sEPSCs without changing sEPSCs amplitude. The mean frequency and amplitude of sIPSCs were enhanced after 5-HT_7 receptor activation. 5-CT administration induced a hyperpolaryzation and an increase of the membrane resistance in a majority of recorded cells. The frequency and amplitude of miniature excitatory and inhibitory postsynaptic currents were not changed after 5-CT administration. The effects of 5-CT were blocked in the presence of 5-HT_7 receptor antagonist SB 269970. The application of 5-CT had no effect in slices prepared from 5-HT_7 knockout mice. These data suggest that the observed decrease in sEPSCs and an increase in sIPSCs frequency and amplitude may result from activation of 5-HT_7 receptors located on GABAergic interneurons that in turn innervate BA projection neurons.
Supported by grant 2016/21/B/NZ4/03618 financed by the National Science Center, Poland, and by statutory funds from Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

Marcin Siwiec (Institute of Pharmacology, Polish Academy of Sciences, Poland)

The functional role of 5-HT_7 receptors in the mouse dentate gyrus

Institute of Pharmacology of the Polish Academy of Sciences, Department of Physiology, 12 Smetna St., Kraków, Poland

Many years have passed since the discovery of the 5-HT_7 receptor - the youngest member of the serotonin receptor family. Numerous studies have investigated its physiological functions and roles in various pathophysiological mechanisms. Yet we still lack answers to fundamental questions. Which neuron subpopulations express it in different brain regions? What are the effects of its activation at the single cell and circuit level? Our group studies 5-HT_7 receptor function in brain regions associated with affective processing, such as the amygdala, frontal cortex and hippocampus. The hippocampal dentate gyrus (DG) is rich in 5-HT_7 receptor-expressing neurons. Recent studies have unveiled its vital role in the pathophysiology of affective disorders and responsiveness to antidepressant treatment. To date, there have been almost no studies documenting 5-HT_7 receptor function in the DG, which is an oversight given the role of this receptor in the pathophysiology of affective disorders. We aimed to characterize 5-HT_7 receptor-expressing neurons in the ventral DG using transgenic mice expressing enhanced green fluorescent protein (EGFP) under the control of the Htr7 promoter. We found that EGFP expression was mostly confined to parvalbumin-positive and somatostatin-positive GABAergic interneurons and not in granule- or mossy cells. Whole-cell patch clamp recordings from dentate granule cells showed that activation of 5-HT_7 receptors increased spontaneous inhibitory transmission, consistent with interneuronal receptor expression. Patched EGFP-expressing cells had electrophysiological characteristics of basket or somatostatin interneurons and visualization of their morphology following biocytin labelling confirmed their phenotype. Our findings suggest that activation of 5-HT_7 receptors promotes inhibitory control of dentate gyrus output by increasing GABAergic interneuron activity. Reliable inhibition of the dentate gyrus granule cell network is critical to healthy hippocampal function. Therefore further investigation of the functional consequences of 5-HT_7 receptor signalling in the dentate gyrus could give us new insights into the pathophysiological mechanisms related to dentate gyrus disinhibition.
Funding for this study was provided by the Polish National Science Centre Grant 2016/23/N/NZ4/03224