INVESTIGATION OF CHRONIC PAIN AND COGNITIVE MECHANISMS IN MOUSE MODELS

PhD Thesis

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I. INTRODUCTION

Pain -as defined by the International Association for the Study of Pain- is an unpleasant sensory and emotional experience that results from actual or potential tissue damage. It can be characterized by **nociception** which can be examined in animal research and an affective component as the emotional appearance of pain which can be studied only in humans (1). Nociceptors specialize in the perception of mechanical, thermal, or chemical stimuli, which can be unimodal (thermal, mechanical) or polymodal (heat, mechanical, chemical stimuli) based on the sensitivity of the receptors. Based on duration, pain can be acute, which is a sudden short-lived condition, the most important defense mechanism of the body, and chronic pain which persists also after tissue healing (2,3). It can be caused by injury or inflammation that also affects the peripheral nerves or the spinal cord. **Neuropathic pain** is a long-term condition that results from nerve damage. Hypersensitivity caused by a non-painful stimulus is called **allodynia**, while the sensation of pain caused by a mildly painful stimulus is called **hyperalgesia** (2). Peripheral nociceptors consist of approx. 50-70% capsaicin-sensitive sensory nerves (4), which can be characterized by three functions: the first is a classic afferent function, when the stimulus causes the sensory nerve endings to transmit the stimulus to the central nervous system, thus developing nociception, the feeling of pain. The second, the local efferent function, in which sensory neuropeptides [calcitonin gene-related peptide (CGRP) and tachykinins (substance P (SP) and neurokinin A)] are released from the sensory nerve ending, causing vasodilation and plasma extravasation. The third, systemic efferent function, in which (in addition to pro-inflammatory neuropeptides) analgesic and anti-inflammatory neuropeptides (somatostatin and pituitary adenylate cyclase activating polypeptide) are also released from the sensory nerve endings (5,6).

Complex Regional Pain Syndrome (CRPS)

CRPS is a chronic pain condition that usually develops after limb trauma. It is characterized by limited movement of the limbs, sensory, autonomic, motor, skin, and bone changes, however, the main symptoms are pain and hyperalgesia (7–9). Psychological symptoms (anxiety, depression) and sleep disorder may also occur. Complex symptoms require integrated interdisciplinary treatment designed for the individual. The primary is to reduce pain, preserve or restore the function and improve the quality of life. Complete recovery is difficult to achieve even with early appropriate treatment (10). There are two main types: CRPS I is without nerve injury and CRPS II is with nerve injury (11). The pathophysiology of the disease is still unknown, but -according to research- autoantibody-mediated immune responses may play a

role, because some patients react to immunoglobulin treatment (10,12–14). Most patients with CRPS show improvement within a month with or without treatment (15), However 20% of patients develop persistent pain (16). Levels of some neuropeptides (SP, CGRP), tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) increased in CRPS samples (9,17). Sensory neuropeptide release induced by electrical stimulation or capsaicin injection stimulates the production of anti-inflammatory cytokines in rat skin such as tumor necrosis factor (TNF), interleukin-1 (IL-1) and IL-6, and nerve-derived growth factor (18). CRPS is a pain condition mediated by neuropathic mechanisms, in which microglia and astrocyte activation, neuroinflammation and central nervous system sensitization play a role (19,20). Thus, neuroplastic effects cause changes in both sensory and motor functions. Central neurophysiological processes, together with peripheral inflammatory mechanisms, induce neurogenic inflammation (21–23). Peripheral injury results in overexpression of inflammatory cytokines (24), neuropathic pain develops, and also in the release of vasoactive neuropeptides (CGRP, SP). Decreased activity of inhibitory interneurons can also be detected in the spinal cord dorsal horn (8). The potential role of neuroinflammation was investigated by transcriptomic analysis of dorsal root ganglia (DRG) samples in a rat chronic post-ischemic pain model. Key mediators, signaling pathways and potential new analgesic targets have been identified (25). Pathophysiological changes in DRGs can affect chronic pain, including the development of CRPS. DRG stimulation can be an effective method of analgesia (26). Further investigation of neuroinflammation mechanisms can contribute to a better understanding of the mechanisms of CRPS as well as to the identification of new therapeutic targets. Our research team has previously developed a CRPS passive transfer trauma mouse model in collaboration with the University of Liverpool and examined its significance in translational research. In our experiments, plantar skin-muscle incision was performed in mice to model tissue microinjury. Major symptoms of the disease such as pain, hyperalgesia and edema have been induced by daily administration of purified immunoglobulin G (IgG) fraction obtained from CRPS patients (27,28). The use of translational animal models allows these pathophysiological mechanisms to be mapped and can contribute to effective therapy. Molecular mechanisms and functions of signaling pathways involved in the development of pain can be identified, which can contribute to a promising new perspective in pharmaceutical research (29,30).

The importance of developing new types of analgesics

The current available analgesics (non-steroidal anti-inflammatory drugs (NSAIDs), opioids) are in many cases ineffective and cannot be used in the long term due to severe side effects for chronic, neuropathic pain conditions. (31). In CRPS, a number of drugs are used to reduce pain and inflammation as well as to improve functional status, such as NSAIDs, corticosteroids, antiepileptics, antidepressants, opioids, bisphosphonates, and calcium channel blockers (32). Prednisolone treatment improved the functional ability and quality of life of the affected limb, and in people who develop CRPS after traumatic injury, prevents the disease from entering the chronic phase (33,34). Implantation of a spinal cord stimulator using electrical impulses reduces pain in about 50% of the patients (35). Appropriate therapy for CRPS is based on drug combination and early physiotherapy (36). Plasmapheresis may be effective in some patients, suggesting a significant role for autoimmunity (10,12–14). CRPS is a real therapeutic challenge in pain relief, where new types of analgesics are needed (35).

Another promising new drug target might be the somatostatin 4 (SST4) receptor (37–40), which has analgesic, anti-inflammatory, and antidepressant effects. Several pharmaceutical companies began to deal with the development of small-molecule SST4 agonists (41,42). Potential drug candidates may be new analgesics that also have antidepressant effects (43). Orally administered new SST4 agonists exerted a strong anti-hyperalgesic effect in chronic neuropathy model (41). The SST_4 receptor may play a role in sensory and motor functions (44), in stress reactions, learning processes (45), mood regulation (46,47), cognitive performance, and neurodegenerative processes (48). During drug development, it is very important to understand the complex functions of the SST_4 receptor in the central nervous system.

Somatostatin and its receptors: focus on the SST_4 receptor

Somatostatin is a 14 or 28 amino acid peptide in the peripheral and central nervous systems and in many areas of the body (49–52). Broad-spectrum inhibitory neurotransmitter, which has complex effect in the central nervous system (45,53,54), and mediates auto-, para- or endocrine effects in the periphery (55,56). Somatostatin is expressed in regions of the brain that regulate pain and mood, mainly in the posterior-lateral prefrontal and cingulate cortex, and in the amygdala (57–59). Inhibits the release of many excitatory and inhibitory neurotransmitters (serotonin, acetylcholine, glutamate and glutamate and gamma-aminobutyric acid) (60). Our research group has previously proved the systemic anti-inflammatory and analgesic effect of somatostatin released from activated capsaicin-sensitive peptidergic sensory nerves in the periphery, called “sensocrine” function (38,61). Somatostatin also plays a role in many neurological disease such as Alzheimer’s, Huntington’s disease (62), and epilepsy (63).

Five distinct somatostatin receptor genes were cloned from a variety of species which is G protein-coupled receptor superfamily and have been hypothesized to be membrane glycoproteins, which contain seven α-helical transmembrane domains. Expression of *Sstr4* shows that it can be determined in brain areas related to behavior, cognition and memory, as in the hippocampus and striatum (64–67). The SST_4 receptor may be a promising target to inhibit neurogenic inflammation, neuropathic pain, and depression (11,43,68,69).

Relationship between aging and somatostatin

Aging significantly affects cognitive functions, memory, and learning. Slowing or preventing cognitive and memory impairments are important for drug development (54). Somatostatin mRNA levels decrease significantly with age in the striatum, frontal and parietal cortex of female Wistar rats (70). Somatostatin can play a potential protective role in the development of Alzheimer's disease and cognitive impairment (48). Somatostatin agonists improve memory function as well as long- and short-term memory in neurodegeneration mouse model (65,71). In addition to aging, sex differences and cognitive performance are also critical factors (72), in preclinical models. The number of somatostatin-regulated growth hormone-producing cells decreases with age (73–75).

Learning and memory

The process of **learning** means the acquisition of knowledge and skills, as a result of which, for example, our habits, attitudes and knowledge develop. **Memory** is information processing capability of the body in which the main brain areas are the amygdala, the hippocampus, the cerebellum and the prefrontal cortex (76). We differentiate three types: short and long term as well as working memory. Behavioral and cognitive tests in rodents (77–79) are suitable for mapping the aging process and neurodegenerative diseases of the brain (48). The most commonly used cognitive tests are the Morris water maze, the radial arm maze (RAM) and Y-maze, novel object recognition (NOR) and fear conditioning tests.

II. GENERAL AIMS

1. Our first goal was to explore the peripheral and central nervous system pathophysiological mechanisms after injection of plasma IgG fractions from patients into mice after small plantar incision in our recently developed and validated new CRPS translational mouse model.

1.1. Examination of functional changes (nociception, edema)

1.2. Determination of peripheral vascular and cellular inflammatory mechanisms (*in vivo* optical imaging and cytokine measurement)

1.3. Examination of the central nervous system neuroinflammation processes (glial cell activation)

1.4. Transcriptomic examination of dorsal root ganglia

1.5. Validation of identified pathophysiological processes by gene-deficient mice and pharmacological methods, determination of new pharmacotherapeutic options

2. In the focus of our research was a new analgesic target molecule, investigating the role of the somatostatin receptor 4 in mice detecting spontaneous locomotor activity and cognitive functions in learning and memory tests (spontaneous alternation, work and reference memory), as well as in exploration tests (novel object recognition), especially regarding to the age and sex differences.

III. METHODS AND RESULTS

Investigation of the role of neuroinflammation mechanisms in a passive transfer-trauma mouse model of CRPS

Experimental protocol

Our experiments were performed with C57BL/6J and microglia-specific *IL-1β* conditional KO and IL-1αβ KO female mice. After habituation and control measurements, plantar skin-muscle incision was performed on day 0 of the experiment, when a 0.5 cm long incision was made in the middle of the right hind paw of the mice (27,28). Our collaboration partner Dr. Andreas Goebel *(University of Liverpool, United Kingdom)* provided the purified IgG fraction from CRPS patients whose baseline pain intensity was 7/10 on an 11-point numerical rating scale (where 0 = no pain, 10 = maximum tolerable pain). Mice were treated intraperitoneally (i.p.) daily with CRPS or healthy IgG serum, and the control group received saline. In some experiments, IgG treatment was supplemented with prednisolone (4 mg/kg i.p.), IL-1 antagonist anakinra (10 mg/kg i.p.), soluble tumor necrosis factor etanercept (5 mg/kg i.p.) or Janus kinase inhibitor tofacitinib (30 mg/kg subcutaneous). We measured the mechanical pain threshold with a dynamic plantar aesthesiometer, the paw edema with a plethysmometer, and the MPO enzyme activity with *in vivo* optical imaging system. On the last day of the experiment (3, 6 or 14 days), the animals were anesthetized with ketamine-xylazine (100 mg/kg and 5 mg/kg i.p.). Plasma and paw samples were then stored at -80 °C until neuropeptide and cytokine concentrations were determined. Mice were perfused transcardially with 4% paraformaldehyde solution then the brain and spinal cord were isolated for immunohistochemical examination of astrocyte and microglia cell activation (28). The immunopositivity of astrocytes and microglia was examined by determining the cell density in L4–L6 spinal cord dorsal horn (laminae I–II) and deeper laminae, and in the periaqueductal gray (PAG) matter and somatosensory cortex (SSC) areas of the brain.

The mechanical pain threshold measured for transcriptomic analysis was performed on days 1, 3, 4, and 5. On the last day of the experiment, L4-L6 DRG samples were isolated and frozen in liquid nitrogen and then stored at -80 °C until ribonucleic acid (RNA) extraction. Transcriptomic analysis was performed after RNA sequencing.

CRPS IgG enhances mechanical hyperalgesia caused by paw injury in mice

The day after the plantar incision, a 45-50% decrease in mechanonociceptive threshold in the right paw developed in all three treatment groups then, in the following days, mild, non-

significant mechanical hyperalgesia persisted in saline and healthy IgG treated animals. CRPS IgG increased the development of mechanical hyperalgesia compared to healthy IgG-treated which further intensified in the late phase of the experiment. The day after operation, 30% volume increase occurred in the affected limb in all three treatment groups. The developed edema was significantly higher in the CRPS IgG-treated group until the end of the experiment while it was normalized within 1–2 days in the healthy IgG and saline groups.

The mechanical pain threshold measured for transcriptomic analysis of DRGs was also reduced by 45-50% with CRPS IgG treatment on day 5 after incision. Daily repeated administration of CRPS IgG significantly maintained the decrease of the mechanonociceptive threshold compared to the healthy IgG-treated group.

CRPS IgG enhances neutrophil myeloperoxidase activity in mice in the early phase

We measured of reactive oxygen species and reactive nitrogen species produced by neutrophil myeloperoxidase (MPO) in neutrophils and macrophages with L-012 chemiluminescent *in vivo* optical imaging. Increased MPO activity due to plantar incision was significantly increased in CRPS IgG-treated animals 2 days after injury. At this time point, there was no difference between the treated groups with fluorescence imaging showing plasma extravasation.

CRPS IgG does not affect the production of peripheral inflammatory mediators

Concentrations of SP and CGRP were measured by radioimmunoassay in hind paw homogenates, and concentrations of IL-6, TNF-α, MCP-1, and IL-1β were measured by cytometric bead array in the same samples. Similar to previous results (27), SP levels increased in the injured paw in the CRPS IgG group on day 6, while CGRP levels did not change significantly. In the case of injured paw, the level of inflammatory mediators was significantly increased compared to the intact side, however the difference gradually decreased over time. We detected no differences between the treatment groups. Thirteen days after incision, most mediators were undetectable at the time of maximum hyperalgesia There were no significant changes in plasma cytokines concentrations.

CRPS IgG enhances glial cell activation in pain-related central nervous system

The number of astrocytes and microglia cells was examined in the L4–L6 spinal cord dorsal horn (laminae I–II) and deeper laminae at 3, 6, and 13 days after hind paw incision. The glial fibrillary acidic protein (GFAP) immunopositivity on day 6, and ionized calcium-binding adaptor molecule 1 (Iba1) immunopositivity on day 13 were evaluated after paw incision. The number of astrocytes was increased in the early period (days 3 and 6), while the number of microglial cells was elevated throughout the whole experimental period (day 13) in the CRPS

IgG-treated group. In the model, we observed transient astrocyte and persistent microglial activation.

CRPS IgG results the neuroinflammation-related genes expression changes after paw injury in the dorsal root ganglia of mice

By the transcriptomic analysis, the heat map shows a group of 125 differentially expressed (DE) genes in the injured side DRG samples from mice treated with CRPS IgG compared to healthy IgG-treated mice. On the heat map, the treated samples were well separated according to the two groups. 48 genes were upregulated, 77 genes were downregulated in the CRPS IgG treated DRG samples. From these genes, we selected 12 up- and 12 down-regulated genes based on the p-value which may play a role in CRPS. We determined the cellular localization and potential functions of the proteins encoded by these genes. The *Ranked list enrichment*/KEGG database included cytokine-cytokine receptor interactions, and the GO database included cytokine activity, neuropeptide receptor and voltage-gated channel activity, and leukocyte chemotaxis, among many other processes. The DE genes list/GO terms include peptidase regulation and positive regulation of inflammatory response. In the transcriptomic study, TNF and Janus kinase signal transducer and transcriptional activator (JAK-STAT) signaling pathways were identified at the gene expression level in mice DRG samples, the CRPS IgG-treated group was compared to healthy IgG-treated. CRPS IgG induced neuroinflammatory changes in DRG, in which activation of TNF and JAK-STAT signaling played a key role.

Inhibition of TNF and Janus kinase (JAK) signaling reduces CRPS IgG-induced increase in mechanical hyperalgesia

The day after the plantar muscle incision, a 37-49% decrease in mechanical pain threshold was measured. Mechanical hyperalgesia was significantly higher in the CRPS IgG-treated group compared to healthy IgG-treated mice ($38.8 \pm 4\%$ vs. $15.3 \pm 7.3\%$ on day 3 of the experiment; $29.4 \pm 7.5\%$ vs. $5.5 \pm 4.9\%$ on day 7 of the experiment). Twice-a day administration of the JAK inhibitor tofacitinib (30 mg/kg *subcutaneous*) and daily administration of the soluble TNF receptor etanercept (5 mg/kg i.p.) significantly alleviated the CRPS IgG-induced mechanical hyperalgesia. The day after the incision 27-35% paw swelling developed in each group, which normalized by the end of the experiment. CRPS IgG treatment did not affect the size of edema, considering that the CRPS patient from whom the sample was derived did not have limb swelling either. CRPS IgG significantly increased GFAP immunoactivity in the spinal cord dorsal horn, in PAG, and SSC compared to healthy IgG-treated on day 7. Etanercept treatment reduced the number of GFAP-positive cells in the spinal cord dorsal horn. Etanercept and tofacitinib treatment also reduced cell activation in PAG and SSC. Iba1 protein expression was

increased in the spinal cord dorsal horn in CRPS-treated animals which etanercept treatment mildly reduced, however, tofacitinib significantly reduced. Etanercept and tofacitinib successfully relieved CRPS IgG-induced increased Iba1 immunopositivity in PAG.

Il-1 deletion and block of receptors prevent CRPS-related hyperalgesia and gliosis

The glucocorticoid **prednisolone** (4 mg/kg i.p./day) and the interleukin-1 receptor antagonist **anakinra** (10 mg/kg i.p./day) were examined on CRPS IgG-induced symptoms and inflammatory changes. Mechanical hyperalgesia develops in all treatment groups on the day after surgery. Glucocorticoid treatment transiently reduced CRPS IgG-induced mechanical hyperalgesia on days 2 and 3 but this effect was undetectable on day 7. Anakinra treatment prevented the development of CRPS IgG and almost completely reversed the activation of glial cells in the spinal cord dorsal horn on day 6 on the injured side. Predisolone treatment did not cause such changes. In addition, administration of late anakinra (from day 8) reduced the mechanical hyperalgesia induced by CRPS IgG and the associated increased microglia activation in the spinal cord dorsal horn on day 13. Of the peripheral mediators, anakinra reduced only MCP-1 levels in paw tissue on day 3.

CRPS IgG-treated *IL-1αβ* KO mice developed only mild mechanical hyperalgesia and paw swelling after incision. *IL-1β* flox (fl/fl) mice were generated, increased microglia IL-1β may affect CRPS-related increased mechanical hyperalgesia. Elimination of microglial IL-1β significantly reduced mechanical hyperalgesia and paw edema in the CRPS IgG-treated group, although this effect was smaller than in *IL-1αβ* KO mice. Iba1 activation was decreased in *IL-1αβ* KO mice, but did not change in microglia CRPS IgG-treated *IL-1β* conditional KO mice. Microglial *IL-1β* cells are important components of chronic neuroinflammation associated with persistent pain, and IL-1β-producing cells or IL-1α-mediated processes may also contribute to the symptoms of CRPS.

Investigation of the role of the SST_4 receptor in mouse models of learning and memory

Experimental protocol

Male and female *Sstr4* gene-deficient (39,43,80) and wild-type mice at different ages (3, 12, 17 months) were used for learning and memory tests. The original *Sstr4* heterozygous (*Sstr4*+/-) breeding pairs were provided by Prof. Dr. Pierce C. Empson and his research team (*Laboratory of Molecular Neuroscience, The Babraham Institute, Babraham Research Campus, United Kingdom*).

Methods

The Y-maze is the Y-shaped maze which is suitable for rodent spatial memory and route-learning capabilities (81,82).

The Radial arm maze (RAM) is an 8-arm maze suitable for testing working memory and long-term (reference) memory (83,84). Four sugar pellets (rewards) were placed in four defined arms. The test lasted until the animal found all the four pellets, or the time limit elapsed (85).

The Novel object recognition (NOR) test is widely used to examine exploratory behavior and memory processes (86). Spontaneous locomotor activity and anxiety were detected in mice in an Open field (OF) box (43,87,88). After placing the objects in the OF box, we measured the time spend with the exploratory of the familiar and the novel objects in mice; the ratio of which was determined as the recognition index (89).

Sstr4-deletion has no effect the spatial memory, which decreases with age

In the Y-maze, young male and female, WT and *Sstr4* KO mice visited significantly more arms than their older counterparts. In 17-month-old WT females, route-learning capabilities decreased compared to KO and male counterparts. SST_4 receptor deficiency did not affect the behavior of mice in any of the groups.

Sstr4 deficiency and aging impair the exploratory activity of female mice, which limits the evaluate of working memory

In the RAM test, young female *Sstr4* KO mice visited, repeated and missed significantly less arms than their WT counterparts. As the sex differences, young females repeated significantly more arms and visited more arms than males, although this parameter was not significant. Young male and female WT mice visited, repeated, and missed more arms and found more pellets than their older counterparts. Aging and SST_4 receptor deficiency also significantly improved the working memory in females, but not in male mice. The reference memory of older

male WT mice was better, while the working memory of younger was better than the same old female controls.

Aging decreases spontaneous locomotor activity and increases anxiety levels in both sexes and genotypes

In the OF test, 12- and 17-month-old mice moved significantly less and spent less time in the middle of the OF box for both sexes and genotypes. In the young WT group, females spent significantly more time in the middle of the OF box, than males of the same age.

Decreased exploratory behaviour in *Sstr4*-deficient young and old mice

In the NOR test, young male *Sstr4* KO mice spent significantly less time exploring familiar and novel objects than their WT counterparts. Young males were significantly more interested in exploring objects than females and their older counterparts. 12- and 17-month-old mice were less interested regardless of sex and genotype.

IV. SUMMARY OF THE NEW RESULTS

1. From CRPS patients, repeated systemic injection of IgG in mice after small plantar incisions induced a significant increase in mechanical hyperalgesia without signs of peripheral inflammation, and resulted in glial cell activation (neuroinflammation) in the dorsal horn of the spinal cord and in the pain-related PAG and SSC. We demonstrated primarily the role of microglia-derived IL-1β in neuroinflammation mechanisms.
2. In this novel translational passive transfer trauma CRPS mouse model, we identified neuroinflammation mechanisms and processes involved in inflammatory cytokine signaling in primary sensory neurons by hypothesis-free transcriptomic examination of dorsal root ganglia.
3. The glucocorticoid prednisolone was effective only in the early phase, however the IL-1 receptor antagonist anakinra, the soluble TNF receptor etanercept, and the JAK-STAT signaling inhibitor tofacitinib prevented CRPS IgG-induced increased hyperalgesia and glia activation throughout the experimental period.
4. We proved in our mouse experiments that spontaneous locomotor activity, exploratory behavior, and spatial memory function decreased with age. We found interesting sex differences depending on age: young females were more active than males in spontaneous motor activity, however, showed weaker exploratory behavior in the Novel object recognition test. The oldest females had worse the spatial memory function than males. In the case of the novel analgesic target molecule deficiency (SST_4 receptor), the spontaneous locomotor activity was reduced only in young female mice while exploratory behavior (new object recognition) was reduced in young males.

V. CONCLUSIONS

1. The novel CRPS passive transfer trauma translation mouse model was developed, characterized and validated by pharmacological tools useful for identifying pathophysiological processes in the disease. Hypothesis-free transcriptomic experiment of dorsal root ganglia was a suitable tool to determine the sensitizing mechanisms of pain at the level of primary sensory neurons.
2. In CRPS-related chronic pain, it is not peripheral inflammation but the neuroinflammation mechanisms (peripheral and central sensitization) in the sensory ganglia and central nervous system that play a crucial role by inflammatory cytokine signaling.
3. Inhibition of IL-1, TNF and JAK-STAT signaling pathways can provide new analgesic potential by expanding the applicability of commercial drugs (anakinra, etanercept, tofacitinib).
4. The new analgesic and antidepressant target -the SST4 receptor- did probably not affect the spontaneous locomotor activity and learning ability in either the age and sex differences, which indicated a favorable side effect profile of the SST_4 agonist drug candidates.

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VIII. PUBLICATION LIST

Articles related to the present thesis:

Helyes Zs, Tékus V, **Szentes N**, Pohóczky K, Botz B, Kiss T, Kemény Á, Környei Zs, Tóth K, Lénárt N, Ábrahám H, Pinteaux E, Francis S, Sensi S, Dénes Á, and Goebel A: Transfer of complex regional pain syndrome to mice via human autoantibodies is mediated by interleukin-1-induced mechanisms; *PNAS* (2019), 116(26)13067-13076.doi.org/10.1073/pnas.1820168116

Pohóczky K, Kun J, **Szentes N**, Aczel T, Urbán P, Gyenesei A, Bölcskei K, Szőke É, Sensi S, Dénes Á, Goebel A, Tékus V, Helyes Zs Discovery of novel targets in a CRPS mouse model by transcriptomics: TNF and JAK - STAT pathway; beküldött kézirat 2021

Szentes N, Tékus V, Mohos V, Borbély É, Helyes Zs: Exploratory and locomotor activity, learning and memory functions in somatostatin receptor subtype 4 gene-deficient mice in relation to aging and sex; *GeroScience* (2019) 41:631–64. doi: 10.1007/s11357-019-00059-1.

Articles not related to the thesis:

Ádám István Horváth, **Nikolett Szentes**, Valéria Tékus, Maja Payrits, Éva Szőke et al.: Proof-of concept for the analgesic effect and thermoregulatory safety of orally administered multi-target compound SZV 1287 in mice: a novel drug candidate for neuropathic pain; *Submitted: Biomedicines, 2021*

Ádám Horváth, Valéria Tékus, Noémi Bencze, **Nikolett Szentes** et al.: Analgesic effects of the novel semicarbazide-sensitive amine oxidaseinhibitor SZV 1287 in mouse pain models with neuropathicmechanisms: Involvement of transient receptor potential vanilloid 1and ankyrin 1 receptors; *Pharmacological Research*, Volume 131, May 2018, Pages 231-243

György Schneider, **Nikolett Szentes**, Marianna Horváth, Ágnes Dorn et al.: Kinetics of Targeted Phage Rescue in a Mouse Model of Systemic *Escherichia coli* K1; *BioMed Research International*, Volume 2018, Article ID 7569645, 8 pages

Eszter Pakai, Valeria Tekus, Csaba Zsiboras, Zoltan Rumbus1, Emoke Olah, Patrik Keringer, Nora Khidhi, Robert Matics, Laszlo Deres, Katalin Ordog, **Nikolett Szentes** et al.: The neurokinin-1 receptor contributes tothe early Phase of lipopolysaccharide-induced Fever via stimulation of Peripheral cyclooxygenase-2 Protein expression in Mice; *Frontiers in Immunology*, 05 February 2018

Horváth Ádám, Borbély Éva, Bölcskei Kata, **Szentes Nikolett**, Kiss Tamás et al.: Regulatory role of capsaicin-sensitive peptidergic sensory nerves in the proteoglycan-induced autoimmune arthritis model of the mouse; *Journal of neuroinflammation* 2018 Dec 3;15(1):335.

Awards achieved as a PhD student

Szentes Nikolett, Tékus Valéria, Mohos Violetta, Helyes Zsuzsanna: A szomatosztatin 4 receptor szerepének vizsgálata tanulás és memória egérmodelljeiben; A Magyar Élettani Társaság, A Magyar Kísérletes és Klinikai Farmakológiai Társaság és a Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság közös Vándorgyűlése (Debrecen) 13-16.06.2017 - Poster special award

Nikolett Szentes, Valéria Tékus, Violetta Mohos, Zsuzsanna Helyes: Investigation of learning, memory, locomotor activity and anxiety in somatostatin receptor 4 deficient mice, interactions with aging and sex; 11th FENS Forum of Neuroscience Konferencia (Berlin) 07-11.07.2018 - Poster certificate

Szentes Nikolett, Tékus Valéria, Pohóczky Krisztina et al.: Neuroinflammációs mechanizmusok szerepe a centrális szenzitizációban Komplex Regionális Fájdalom Szindróma passzív transzfer-trauma egérmodelljében; FAMÉ 2019 - Magyar Kísérletes és Klinikai Farmakológiai Társaság, Magyar Anatómus Társaság, Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság, Magyar Élettani Társaság Közös Vándorgyűlése (Budapest) 05-08.06.2019 - First place prize for poster presentation

Nikolett Szentes, Krisztina Pohóczky, Valéria Tékus et al.: Neuroinflammatory mechanisms of central sensitization are mediated by TNF- and IL1-driven pathways in a passive transfer-trauma mouse model of Complex Regional Pain Syndrome (CRPS); European Pain Federation (Valencia) 04-07.09.2019. - First place poster award of young researchers in pain syndromes poster section

Szentes Nikolett, Tékus Valéria, Pohóczky Krisztina et al.: Neuroinflammációs mechanizmusok szerepe a centrális szenzitizációban Komplex Regionális Fájdalom Szindróma (CRPS) passzív transzfer-trauma egérmodelljében; MOFT - Magyarországi Fájdalom Társaság 2019. évi Konferenciája (Szeged) 08-09.11.2019- First place prize for poster presentation, in the form of oral presented

Zsuzsanna Helyes, Valéria Tékus, **Nikolett Szentes**, Krisztina Pohóczky et al.: Transfer of complex regional pain syndrome to mice via human autoantibodies is mediated by interleukin-1-induced mechanisms; Magyar Kísérletes és Klinikai Farmakológiai Társaság Ifjúsági Pályázat 2019, 03.04.2020 - First place prize

Cumulative impact factor of publications related to the thesis: 9,412
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Number of independent citations (MTMT): 30
Number of citations (Google Scholar): 71