PhD Thesis

Role of central transient receptor potential ankyrin 1 ion channel in mouse models of posttraumatic stress disorder

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1. INTRODUCTION AND BACKGROUND

1.1 Transient receptor potential ankyrin 1 (TRPA1) ion channel: structure, agonists and its role in the peripheral and central nervous system

TRPA1 is a non-selective cation channel composed of four subunits with 6 transmembrane α-helical segments per subunit (S1-S6) (Meents et al. 2019). The hydrophilic loop between the leaning of the S5 and S6 segments forms the permeable pore of the channel for cations (*e.g*., Ca^{2+}, Na^{+}), allowing their influx from the extracellular space into the cytoplasm (Talavera et al. 2020). Cysteine amino acid side chains located in the linker domain region of the protein near to the N-terminal cytoplasmic part of the protein, between the ankyrin repeat and the first transmembrane segment (S1), are responsible for binding electrophilic agents that can be considered as agonists (Logashina et al 2019). Thus, the activation of TRPA1 receptors and the consequent opening of the ion channel can be induced by various exogenous and endogenous compounds possessing electrophilic structure (allyl isothiocyanate, lipid peroxidation products) on the one hand, while another group of agonists includes compounds with non-electrophilic structure and typically lipophilic properties (menthol, arachidonic acid) (Logashina et al. 2019, Talavera et al. 2020).

TRPA1 receptors are found at the nerve endings of the dorsal root as well as the polymodal nociceptive afferents of the trigeminal ganglia (Talavera et al. 2020), thus contributing to the pain signal, mechanical allodynia, inflammatory and immune responses associated with certain disorders (*e.g.,* neuropathic pain) (Koivisto et al. 2012; Julius 2013; Marone et al. 2018). TRPA1 receptors expressed on trigeminal nociceptive terminals may also play a role in the perception of certain volatile irritant/aversive odorants (Wang et al. 2018). Our research group investigated the central nervous system expression of *Trpa1* mRNA in mice using RNAscope *in situ* hybridization (ISH) techniques, with the highest copy number detected in urocortin 1 (UCN1)-containing neurons of the centrally projecting Edinger-Westphal nucleus (EWcp) (Kormos et al. 2022), furthermore these results were confirmed by PCR studies performed on *post-mortem* human brain samples (Kormos et al. 2022). The functional activity of the TRPA1 ion channel in the EWcp urocortinergic cells was established by our research group by electrophysiological studies (Al-Omari et al. 2023), as well as we showed that *Trpa1* mRNA expression is reduced in EWcp/UCN1 neurons in the mouse model of chronic variable mild stress (CVMS) and in EWcp samples originating from suicidal individuals, raising the possibility of a role for TRPA1 receptors in the process of stress adaptation (Kormos et al. 2022).

1.2 The role of EWcp and UCN1 in stress adaptation

According to the stress theory, based on the work of János Selye and Walter Cannon, physiological and psychological stressors induce neuroendocrine changes in the body, the main purpose of which is to develop adaptive responses to stress. The first steps in this process are the sympatho-adrenomedullary catecholamine mobilisation and activation of the hypothalamic-pituitary-adrenocortical (HPA) axis (Adams and Hempelmann 1991). However, besides these processes, several other neuronal structures can contribute to the regulation of stress adaptation responses, including the EWcp expressing multiple neuropeptides (cholecystokinin, substance P, UCN1, cocaine- and amphetamine-regulated transcripts), whose UCN1-containing fibres project in neurons of the paraventricular nucleus of the hypothalamus (PVN) and regulate HPA axis function (Zuniga and Ryabinin 2020).

The role of CRH in the regulation of the HPA axis has long been known. Later discovered members of the CRH family are the urocortins (UCN1, 2 and 3), which also contribute to the stress adaptation (Vaughan et al. 1995; Hsu and Hsueh 2001; Gaszner et al. 2004). UCN1 is synthesized in the highest amounts in the urocortinergic cells of EWcp in the brain, sending fibres to certain nuclei of the hypothalamus (PVN, supraoptic nucleus, lateral hypothalamic nucleus) and various limbic (prefrontal cortex (PFC)), bed nucleus of the stria terminalis (BNST), amygdala) and brainstem (dorsal raphe nucleus) centers, thus regulating, among others, stress adaptation, thermoregulation, food intake and mood (Fekete and Zorrilla 2007; Zuniga and Ryabinin 2020). The effects of CRH family members are mediated by CRH receptors (CRHR), of which subtypes 1 (CRHR1) and 2 (CRHR2) were identified (Hsu and Hsueh 2001; Bale et al. 2002). UCN1 is characterized by an approximately 40-fold higher binding affinity for the CRHR2 subtype compared to CRH (Bale et al. 2002; Im et al. 2015), suggesting that UCN1 is the most important endogenous ligand for CRHR2 (Vaughan et al. 1995; Bale et al. 2002). According to the literature, the two subtypes of CRHR mediate opposite effects: while CRHR1 activation induce anxiogenic action, CRHR2 mediate primarily anxiolytic effects (Bale, Lee and Vale 2002; Gaszner et al. 2004). These suggest that the main CRHR2-dependent responses mediated by EWcp/UCN1 neurons may contribute to the recovery of normal homeostasis and the termination of the stress response in the late phase of stress adaptation (Adams and Hempelmann 1991; Hsu and Hsueh 2001; Gaszner, et al. 2004). In our previous experiments, in the mouse model of CVMS and in EWcp samples deriving from suicidal individuals, the reduced *Trpa1* mRNA expression detected on EWcp/UCN1 neurons suggests that TRPA1 receptors expressed on UCN1 neurons may contribute to the role of

urocortinergic cells in stress adaptation and thus may be involved in the pathomechanism of certain stress-related maladaptive disorders.

1.3 Posttraumatic stress disorder (PTSD)

PTSD is a stress adaptation disorder that develops because of intense emotional/physical trauma (*e.g.*, accident, childhood abuse, sexual abuse, war-related trauma). The disorder is characterised by episodic recurrent severe anxiety and psychomotor agitation (hyperarousal) triggered by a key factor reminiscent of the traumatic event, the recurrent experience of memories of the trauma in waking state (spontaneous or intrusive flashbacks) or in the form of nightmares, and associated avoidance behaviours (Auxéméry 2018).

Neuroinflammation, one of the most extensively studied neurobiological mechanism involved in the pathogenesis of PTSD, may be induced by excessive or chronic stress (Hori and Kim 2019). The effect of stress can increase the production of proinflammatory cytokines (interleukin 1 (IL-1); interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α)) by immune cells indirectly through sympathetic nervous system activation and consequent catecholamine mobilisation due to the increased CRH production by the PVN (Tan et al. 2007). On the other hand, upon stress exposure, the glial cells of the CNS (microglia, astroglia) can directly produce the mentioned cytokines, thus contributing to the development of neuroinflammation (Hori and Kim 2019). It is also important that elevated levels of IL-1, IL-6 and TNF-α play a role in maintaining the stress response by increasing CRH secretion (Hori and Kim 2019). Oxidative stress is another important neurobiological factor involved in the pathogenesis of the disease . Glial cells activated during the proinflammatory state may contribute to this process, in addition reactive free radicals of microglial and astrocyte origin can themselves induce glial cell activation, thus enhancing the neuroinflammation process (Miller et al. 2015; Oroian et al. 2021). The most important brain areas involved in the development of PTSD are the medial prefrontal cortex (mPFC), the basolateral (BLA) and central (CeA) nuclei of the amygdala, and the hippocampus as well as BNST among the limbic areas (Miller et al. 2018; Kamiya and Abe 2020).

Manualised psychotherapeutic procedures are the first choice in the treatment of the disorder, with the main aim of processing traumatic memories and associated emotions, reassessing trauma-related meanings, reducing avoidance behaviours and restoring adaptive functions (Goodnight et al. 2019; Perczel-Forintos and Lisincki 2020; Schrader and Ross 2021). However, literature data suggest that the long-term efficacy of pharmacotherapy available for the treatment of PTSD, which primarily involve the use of selective serotonin reuptake

inhibitors, falls significantly short of psychotherapeutic approaches, and the maintenance of sustained remission requires ongoing drug therapy, which is associated with an increased risk of side effects (Lee et al. 2016; Schrader and Ross 2021). In addition, the drugs used to treat the disease improve the patients' condition only by blunting the symptoms (depressed mood, anxiety), while the neurobiological mechanisms underlying the disease (*e.g.*, inadequate extinction of the conditioned fear response) are not significantly affected (Lee et al. 2016). Based on the above detailed information, it would be worthwhile in the future to develop drugs with greater efficacy and a more favourable side-effect profile in the treatment of the disease.

1.4 Structure, connections and physiological significance of the olfactory tract

The olfactory tract is one of our oldest major sensory systems, allowing us to detect the presence of volatile chemicals. The process of olfaction begins in the olfactory epithelium (OE) in the nasal cavity, whose sensory bipolar neurons (OSN) transmit the stimulus to the glutamatergic cells of the olfactory bulb (OB) (Lage-Rupprecht et al. 2020). Another important cell type in the OB is the group of GABAergic neurons that produce the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (Lage-Rupprecht et al. 2020). Axons deriving from glutamatergic neurons arrange in bundles to form tracts that terminate in primary and secondary olfactory centers (*e.g.*, anterior olfactory nucleus, olfactory tubercle, piriform cortex (PC) and entorhinal cortex). The most important primary olfactory field is the PC, which, after processing information, transmits it to secondary or association centers (Truex and Carpenter 1969).

Olfactory stimuli affect our social behaviour (Boesveldt and Parma 2021) and in most mammals play a role in social hierarchy formation, sexual partner preference and offspring care (Vosshall 2005; Zou et al. 2015; Oettl and Kelsch 2018). In addition to its effects on social behaviour, the functional integrity of the olfactory system is required to avoid predators (Zou et al. 2015), thus contributing to the innate fear response and associated anxiety. Predator-derived chemical stimuli inducing avoidance behaviour, mediated by the olfactory system, trigger the activation of certain limbic areas (amygdala, ventromedial nucleus of the hypothalamus, BNST, periaqueductal grey matter (PAG), PVN), thus shaping the innate fear response and anxiety (Pérez-Gómez et al. 2015; Janitzky et al. 2015). In addition, following the application of a predator odour, increased stress-related neuronal activation was also found in the locus coeruleus, which provides noradrenergic innervation to the most important limbic brain areas (hippocampus and PFC) involved in the pathogenesis of PTSD, respectively, demonstrating the importance of this structure in the development of fear responses during the stress adaptation (Janitzky et al. 2015). The above detailed data suggest that a possible dysfunction of these

limbic structures and associated neurotransmitter systems may contribute not only to the innate fear responses, but also to the development of some stress-related maladaptive disorders (*e.g.*, PTSD).

2. AIMS

2.1 Mapping and characterisation of TRPA1 expression in the olfactory tract

Our first aim was to map the expression of *Trpa1* mRNA in the olfactory epithelium and major primary olfactory areas (OB, PC) involved in the processing of olfactory information. To determine this, we performed immunofluorescence combined with RNAscope ISH, which allows the accurate characterization of cells containing *Trpa1* mRNA.

2.2 Investigation of the role of TRPA1 in the predator odour evoked fear responses

Using *Trpa1* gene-deficient (KO) animals, we investigated the effect of compounds imitating the odour of two different predators (fox and cat) on the innate fear responses of the animals. Here, we performed behavioural tests to determine the freezing behaviour of the mice, followed by hormone level measurements to investigate the response of the HPA axis to the predator odours, and finally, we determined the TRPA1 receptor activating ability of these odorants applying cell lines overexpressing the TRPA1 ion channel.

2.3 Investigation of the role of TRPA1 in the development of PTSD

Since TRPA1 expressed on EWcp/UCN1 neurons may influence the regulation of stress adaptation, it is hypothesized that EWcp/TRPA1/UCN1 cells may contribute to the pathomechanism of PTSD which disorder is also caused by disturbance of the stress adaptation. Therefore, we investigated the differences in the behaviour of *Trpa1* wild type (WT) and KO animals using two different PTSD models (single prolonged stress (SPS) and electric foot shock) and performed morphological analysis focusing on the urocortinergic neurons of the EWcp applying the RNAscope technique and immunofluorescence staining.

3. MATERIALS AND METHODS

3.1 Animals

We used 9-14 weeks old male C57BL/6 and *Trpa1* WT and KO mice. The animals were housed in standard polycarbonate cages at the Institute of Pharmacology and Pharmacotherapy, University of Pécs, Medical School in a 12-h day-night cycle at 24-25°C, with *ad libitum* drinking tap water and rodent food.

3.2 Experimental design

3.2.1 Investigation of *Trpa1* mRNA expression in the mouse olfactory tract

The expression of *Trpa1* in the olfactory tract was determined in the olfactory epithelium, OB and PC samples deriving from C57BL/6 mice. To characterize *Trpa1*-expressing cells, we performed RNAscope ISH combined with anti-β-tubulin III immunofluorescence on olfactory epithelium, while multiplex RNAscope ISH was carried out on brain samples containing OB and PC using probes specific for neuronal nuclear protein (*NeuN*), vesicular glutamate transporter 1 (*Vglut1*), and glutamate decarboxylase 1 (*Gad1*) mRNA.

3.2.2 Investigation of the role of TRPA1 in the predator odour-induced fear responses

To investigate the innate fear responses evoked by predator odour, we used compounds imitating fox (2-methyl-2-thiazoline (2-MT)) and cat (valeric acid) odours. During the experiment, we estimated primarily the predator odour-induced freezing responses of *Trpa1* WT and KO mice, followed by the measurement of stress hormone levels (adrenocorticotropic hormone (ACTH) and corticosterone) after the experiment.

3.2.3 Investigation of the TRPA1 activating ability of valeric acid and 2-MT

Using 2-MT and valeric acid, Ca^{2+} influx measurements were performed on Chinese Hamster Ovary (CHO) cell lines expressing mouse and human TRPA1 receptors to determine the TRPA1 receptor activating ability of these odorants.

3.2.4 Investigation of the role of TRPA1 in mouse models of PTSD

Two different experimental protocols were used to induce PTSD in *Trpa1* KO and WT animals. First, we performed the SPS model, in which animals were exposed to restraint stress, followed by forced swim test (FST) and finally by ether narcosis. During the behavioural experiments, we estimated the immobility of the mice. In the other model, we used repetitive foot shock combined with acoustic startle stimuli (ASS) in stressed animals and in the behavioural tests, we determined the freezing and jumping behaviour of mice. The PTSD models and the related behavioural tests were performed during the early dark phase between 18 and 22 h. In both

paradigms, animals were sacrificed 36 h after the behavioural tests and brain samples were collected for further histological examinations.

3.3 RNAscope *in situ* hybridisation combined with immunofluorescence

The coronal OB, PC and EWcp sections were subjected to RNAscope ISH analysis. Here, we performed the hybridization of the target genes with probes specific for mouse *Trpa1*, *NeuN*, *Vglut1*, *Gad1* and *Ucn1* mRNA. Upon channel development, fluorophores with different emission spectra were used to identify the targets (*Trpa1 -Cyanine 3* (Cy3), *NeuN - Cyanine 5* (Cy5), *Vglut1* - fluorescein, *Gad1* - Cy5, *Ucn1* - Cy3). UCN1 immunofluorescence was also performed on EWcp sections staining with recombinant rabbit anti-urocortin 1 antibody followed by donkey anti-rabbit secondary antibody conjugated to Alexa Fluor 488. On the transversely sectioned olfactory epithelial samples we performed *Trpa1*-specific RNAscope ISH followed by β-tubulin III immunofluorescence staining with polyclonal rabbit anti-β-tubulin III antibody followed by donkey anti-rabbit secondary antibody conjugated to Alexa Fluor 488.

3.4 Microscopy, morphometry

For imaging, we used an Olympus Fluoview FV-1000 laser scanning confocal microscope and FluoView FV-1000S-IX81 image analysis software. Morphometry was performed on raw images obtained from slices containing EWcp using ImageJ software (version 1.52a). Upon this procedure, we manually evaluated the fluorescence specific signal density (SSD) determined in arbitrary units (au) in 5-10 cell bodies per slice based on images of four EWcp slices per animal for *Ucn1* mRNA and UCN1 peptide. For the *Trpa1* mRNA signal, we also manually counted the number of copies per cell in 5-10 neurons per slice in four slices per animal.

3.5 Predator tests

To investigate the innate fear responses induced by predator odours, we examined the freezing responses in adult male *Trpa1* WT and KO mice (n = 12 in both groups) triggered by 2-MT and valeric acid.

3.6 Radioimmunoassay

Blood was collected from the animals immediately after the odour aversion tests, and after centrifugation, ACTH and corticosterone levels were measured in the obtained serum samples using antibodies developed by the Institute of Experimental Medicine (Budapest, Hungary) (Zelena et al. 2003).

3.7 Measurement of Ca^{2+} influx by flow cytometry

The calcium responses induced by 2-MT and valeric acid were investigated by flow cytometry in CHO cell lines overexpressing mouse and human TRPA1.

3.8 The SPS model of PTSD and the related behavioural tests

The SPS protocol was carried out on one set of adult male *Trpa1* KO (n=12) and WT (n=9) mice, while another group of KO (n=10) and WT (n=10) animals were used as non-stressed controls. After two weeks, each group was exposed to 15 min of restraint and 6 min of FST, and the immobility of the animals was assessed.

3.9 The electric foot shock model of PTSD and the related behavioural tests

In the electric foot shock paradigm, a group of *Trpa1* KO (n=10) and WT (n=10) mice was exposed to foot shock combined with ASS (phase of fear conditioning), while the non-stressed control KO (n=9) and WT (n=11) groups received ASS alone. Four weeks after the fear conditioning, animals were replaced to this original context (situation reminder), but without shocking, we used only ASS in each group and examined the behaviour of mice, focusing mainly on the freezing response and jumping.

3.10 Statistical analysis

Statistical analysis was performed using Statistica 13.5.0 software. We used two-sample t-test to compare the behaviour of WT and KO mice in the odour aversion tests as well as the *Trpa1* mRNA expression of WT animals in the PTSD models. In the other cases, main effects were tested by two-way analysis of variance followed by Tukey's *post hoc* test. Results were considered statistically significant if the p-value was less than 0.05.

4. RESULTS

4.1 Analysis of *Trpa1* mRNA expression in the mouse olfactory tract

In the olfactory epithelium, *Trpa1* mRNA was detected in very low copy numbers and colocalization with β-tubulin III was not revealed. In the OB samples, *Trpa1* mRNA was showed only on *NeuN*-containing neuronal elements, among which moderate expression of *Trpa1* was confirmed on GABAergic inhibitory neurons containing *Gad1* mRNA, whereas co-localization with *Vglut1* positive excitatory cells was barely detectable. In contrast, in the PC, *Trpa1* mRNA was showed exclusively in glutamatergic stimulatory neurons.

4.2 Investigation of the role of TRPA1 in the predator odour-induced fear responses

In *Trpa1* KO mice, using 2-MT, the duration of freezing was significantly decreased and in parallel the time spent with sniffing of the odour source enhanced compared to WT animals. Using valeric acid, a similar significant difference was observed between the two genotypes in terms of sniffing duration, however investigating the freezing behaviour, only the tendency could be revealed. Upon the hormone level measurements, we observed significantly elevated ACTH levels in KO mice only after 2-MT exposure, whereas no difference in corticosterone levels was detected between the genotypes after application of either predator odour.

4.3 Investigation of the TRPA1-activating ability of valeric acid and 2-MT

Using 2-MT, a concentration-dependent increase of the fluorescent signal was detected in both human and mouse cell lines compared to unstimulated cells, whereas none of the TRPA1-expressing cell lines responded to valeric acid.

4.4 Investigation of the role of TRPA1 in mouse models of PTSD

4.4.1 SPS model

In behavioural tests, increased immobility was observed in both stressed groups compared to control (non-stressed) animals applying restraint stress. In the FST, WT mice exposed to stress showed significantly higher levels of immobility compared to their control counterparts, whereas similar differences were not detected in KO animals.

In the morphological analyses, significantly reduced *Trpa1* mRNA expression was revealed in WT mice using the SPS model compared to the control group. Investigating the changes in *Ucn1* mRNA levels, both basal and SPS-induced *Ucn1* expression was significantly elevated in KO animals compared to their WT counterparts, however significant changes in the expression were not detected in response to stress in either genotype. Significantly increased UCN1 peptide content was found in WT animals upon SPS, but similar SPS-induced changes

were not observed in KO mice, as supported by a significant interaction between stress-genotype variables.

4.4.2 Electric foot shock model

In the electric foot shock model, significantly increased freezing was observed in both stressed groups compared to control (non-shocked) animals during the situation reminder, whereas no significant main effect was observed for genotype. However, the application of the reminder sound effect resulted in a significantly increased frequency of jumping in stressed KO animals compared to their control counterparts, while no similar difference was detected in WT mice, as confirmed by a significant interaction between stress-genotype variables.

Morphological analyses revealed significantly reduced *Trpa1* mRNA expression in the WT animals exposed to shock compared to the control group. In the further RNAscope studies, significantly higher *Ucn1* mRNA expression was observed in WT animals after the application of foot shock, whereas similar shock-induced changes were not observed in KO mice, as confirmed by the significant interaction between the two variables. In addition, the tendency of shock-induced increase in UCN1 peptide content during the UCN1 immunofluorescence, which was only observed in WT animals, was associated with a significant difference in the genotype main effect.

5. DISCUSSION

We firstly demonstrated the expression of *Trpa1* mRNA in the olfactory tract. Here, we showed that *Trpa1* copies are mainly found in the OB GABAergic and PC glutamatergic cells, respectively. Furthermore, odour aversion tests in *Trpa1* WT and KO animals confirmed the role of TRPA1 ion channel in the development of predator odour-induced innate fear responses, which is supported also by the literature (Wang et al. 2018).

A previous publication reported that fox odour can act as a direct activator on TRPA1 receptors in the olfactory epithelium (Wang et al. 2018), which was confirmed also by our research group in both human and mouse TRPA1-overexpressing CHO cell lines using 2-MT. On the other hand, using valeric acid (a cat odour imitating compound), no enhanced Ca^{2+} influx was showed in any CHO cell line, which could be explained by the fact that valeric acid do not have electrophilic properties, therefore it is unable to directly activate TRPA1 receptors. Moreover, the difference between the behaviour of the two genotypes during the application of cat odour was only manifested in the longer and more frequent sniffing behaviour of KO animals compared to their WT counterparts, whereas the freezing responses were not affected at all. One possible explanation for the different behavioural responses to the two different predator odours may be that the odour of valeric acid is generally less fearful to mice. However, it should be emphasized that the absence of TRPA1 ion channel does not generally reduce the degree of fear responses, which was supported by the enhanced freezing response of *Trpa1* KO animals in a previous study investigating the conditioned fear responses in mice (Lee et al. 2017). Our results suggest that the role of TRPA1 receptors located in the central parts of the olfactory system (*e.g.*, OB or PC) may be more pronounced in inducing the interesting behaviour towards the odour stimuli, while in the OE, the ion channel, expressed mainly on trigeminal nociceptive afferents, may play a more prominent role in mediating the pain sensation associated with irritant odours (Talavera et al. 2020), thus shaping the behavioural responses of animals. Furthermore, the generated pain signal as well as the direct connection between the PC and PVN (Kondoh et al. 2016) may increase the neuronal activity in stress-sensitive brain areas (*e.g.*, PAG and PVN) (Wang et al. 2018). The *Trpa1* KO animals responded to 2-MT treatment with elevated ACTH levels, which is seemingly a contradictory observation that may be explained by the increased locomotor activity measured in KO mice during the predator test (White-Welkley et al. 1995). However, applying valeric acid, no detectable difference in the levels of any of the stress hormones was found between the two genotypes, which may be

explained by the different activation pattern of the neuronal pathways during the perception of different odours.

To confirm the importance of TRPA1 in olfaction, we showed its presence in certain parts of the olfactory tract. However, in the OE, *Trpa1* mRNA was detected only at low copy numbers and was not co-localized with the immunomarker specific for OSN, suggesting that the receptor may be expressed in other OE structures (*e.g.*, trigeminal terminals) (Koike et al. 2021). In the OB, moderate expression of *Trpa1* mRNA was detected mainly on GABAergic inhibitory neurons, which is confirmed also by a previous study (Dong et al. 2012). In this area, TRPA1 receptors may presumably contribute to the modulation of the effects of aversive odorants. Our research group showed firstly that *Trpa1* mRNA is also expressed on the main neuron type (glutamatergic cells) of the PC, which receive stimuli from other olfactory structures and limbic areas (*e.g.*, BLA), while the diverse afferentation of these cells determines the fine-tuning of the information (Wang et al. 2020).

The above detailed behavioural studies, as well as morphological results, suggest a role for the TRPA1 receptor in the central processing of odour information and in the shaping of behavioural patterns induced by different odours.

In the previous experiments of our group, we observed a decrease in *Trpa1* mRNA expression on EWcp/UCN1 neurons in the mouse model of CVMS, as well as in human samples from suicidal individuals (Kormos et al. 2022). This observation raises the possibility that TRPA1 receptors expressed on UCN1 neurons may play a role in the pathomechanism of certain stress adaptation disorders, such as PTSD. This hypothesis is supported by the results of previous studies that revealed a direct link between EWcp and the most relevant brain structures involved in the development of PTSD (PFC, amygdala, and hippocampus) (Zuniga and Ryabinin 2020; Topilko et al. 2022).

In the validation of the PTSD models, both experimental paradigms (SPS and electric foot shock) were found to be reliable for modelling the disease, as *Trpa1* WT mice showed increased immobility/freezing characteristic for the disorder upon both FST after SPS and during the situation reminder after foot shock (Török et al. 2019). Upon FST, we showed that the lack of TRPA1 ion channels may prevent the development of some stress-related behavioural changes consistent with former results in the literature (De Moura et al. 2014; Kormos et al. 2022). Using the shock model, no difference in the duration of freezing was detected between the two genotypes during the situation reminder. However, an increased jumping frequency of KO mice

was revealed in this paradigm, which parameter similarly to freezing, can be used to characterize the conditioned fear responses induced by the CeA (Hitchcock et al. 1989; Davis 1992; Lee et al. 2017), and can be ultimately considered as an increased arousal state (hyperarousal) mediated by locus coeruleus activation (Hitchcock et al. 1989; Davis 1992; Gresack and Risbrough 2011). Based on the above detailed data, it can be assumed that the CeA-mediated fear conditioning may be amplified and/or its extinction may be impaired by the functional ablation of TRPA1 receptors, which, in addition to the more pronounced passive avoidance behaviour described in the literature (Lee et al. 2017), leads to a more expressed or persistently existing arousal state in KO animals. The behavioural differences detected in the two models may thus provide evidence for a role of TRPA1 ion channels in the pathomechanism of PTSD.

During the morphological studies performed in both the SPS and the electroshock paradigms, we confirmed that *Trpa1* mRNA expression was significantly reduced in WT animals following stressor application, which is consistent with our previous results in the CVMS model (Kormos et al. 2022). A plausible mechanism underlying the decrease in *Trpa1* mRNA copy number could be the downregulation of the ion channel induced by agonist-receptor interaction, a process which may be mediated by reactive free radicals deriving from surrounding glial cells and by mediators released during oxidative stress and neuroinflammation (Hori and Kim 2019; Oroian et al. 2021). However, further pharmacological studies are needed to exactly define the putative regulatory effects of these inflammatory mediators. In WT mice, we demonstrated in both PTSD models that the decrease in *Trpa1* mRNA expression was accompanied by an increase in UCN1 peptide content. On the other hand, we detected increased *Ucn1* mRNA expression in WT animals using electric foot shock compared to the control group. In contrast, in gene-deficient animals, no significant increase in neuronal *Ucn1* mRNA or UCN1 peptide content was observed in either model compared to the control group.

It should be emphasised that the lack of *Ucn1*/UCN1 expression enhancement observed in SPS-exposed KO animals is consistent with the unchanged duration of immobility detected during FST. This altered stress response may be due to a reduced adaptive capacity of EWcp/UCN1 cells in KO animals, as reflected both by higher basal *Ucn1* mRNA expression levels compared to WT mice and by absent behavioural and urocortinergic responses to SPS. In contrast, using foot shock, missing enhancement of *Ucn1* mRNA expression and UCN1 peptide content was observed in gene-deficient animals associated with increased jumping frequency during the situation reminder compared to the WT mice, which parameter may be considered as an

equivalent behavioural pattern to hyperarousal in PTSD (Hitchcock et al. 1989; Gresack and Risbrough 2011). According to the results of former publications, the EWcp/UCN1 neurons may contribute to the recovery of normal homeostasis and the termination of stress responses in the late phase of stress adaptation (Hsu and Hsueh 2001; Gaszner et al. 2004). This data raises the possibility that the persistently increased arousal state observed in KO mice during the shock model could be explained by the reduced response of UCN1 neurons to stress leading to an impaired regulatory function on the stress axis and therefore to an inappropriate extinction of some stress-related behavioural responses. A further interesting observation was that on the one hand, the differences in the *Ucn1* mRNA expression described in both genotypes during the SPS were very similar to our previous results obtained in the CVMS model (Kormos et al. 2022), while in the electroshock model, we detected substantially different *Ucn1* mRNA expression dynamics. These apparent discrepancies could be explained by the fact that in the SPS and CVMS paradigms we used partially similar stressors (*e.g.*, restraint stress, FST), while the foot shock-induced PTSD is based on the nociceptive effect (Sawchenko et al. 2000; Dayas et al. 2001).

In addition to its effects on *Ucn1* mRNA expression, TRPA1 receptor may also enhance UCN1 release by increasing intracellular Ca^{2+} levels and therefore it may regulate stress adaptation (Kormos et al. 2022; Ujvári et al. 2022; Casello et al. 2022). In this context, it is suggested that stress-induced decreases in *Trpa1* mRNA expression may aim to compensate the excessive UCN1 release and associated behavioural responses, although further studies are needed to confirm this hypothesis. However, there was an apparent contradiction between the low EWcp/UCN1 peptide levels in stressed TRPA1 KO animals and the increased *Ucn1* mRNA expression during the SPS model, which may be explained by the altered dynamics of UCN1 peptide release. In addition, it cannot also be excluded that other cation channels on EWcp/UCN1 neurons (Zuniga and Ryabinin 2020) may compensate the lack of TRPA1 in KO mice.

Based on these findings, EWcp/UCN1 neurons may contribute to the development of certain stress adaptation disorders (Kormos et al. 2022), thus may also play a role in the pathomechanism of PTSD.

6. CONCLUSION, SUMMARY OF RESULTS

The expression of TRPA1 receptors at the different levels of the olfactory tract suggests that this ion channel may play a role in the perception of aversive/irritant odours as well as the central processing of odour stimuli, thus contributing to the development of the associated behavioural patterns. The decrease in *Trpa1* mRNA expression in the EWcp neurons of WT animals with the parallel increase in UCN1 peptide content during PTSD models raises the possibility that TRPA1 ion channels may influence stress adaptation responses, thus contributing to the pathomechanism of PTSD. The reduced immobility of KO animals in the SPS model and the increased jumping frequency detected during the electroshock protocol may suggest the role of TRPA1 in the development of depression-like behaviour as well as in the extinction of hyperarousal during PTSD.

New results:

- We were the first to demonstrate the presence of *Trpa1* mRNA in the glutamatergic neurons of the PC.
- We showed that the absence of *Trpa1* influences behavioural responses to predator odours (fox and cat).
- We detected reduced expression of *Trpa1* mRNA in the mouse models of PTSD.
- We demonstrated that *Trpa1* deficiency influences the function of EWcp urocortinergic neurons, *Ucn1* mRNA and UCN1 peptide content, and behavioural responses in the mouse models of PTSD.
- We first suggested the possible role of EWcp/TRPA1/UCN1 neurons in the pathomechanism of PTSD.

7. REFERENCES

1. Achenbach J, Rhein M, Gombert S, Meyer-Bockenkamp F, Buhck M, Eberhardt M, Leffler A, Frieling H and Karst M. Childhood traumatization is associated with differences in TRPA1 promoter methylation in female patients with multisomatoform disorder with pain as the leading bodily symptom. Clinical Epigenetics. 2019 Aug;28;11(1):126.
2. Adams HA and Hempelmann G. Die endokrine Streßreaktion in Anästhesie und Chirurgie-Ursprung und Bedeutung. AINS - Anästhesiologie · Intensivmedizin · Notfallmedizin · Schmerztherapie. 1991; 26(6): 294-305.
3. Al-Omari A, Kecskés M, Gaszner B, Biró-Sütő T, Fazekas B, Berta G, Kuzma M, Pintér E and Kormos V. Functionally active TRPA1 ion channel is downregulated in peptidergic neurons of the Edinger-Westphal nucleus upon acute alcohol exposure. Frontiers in Cell and Developmental Biology. 2023 Jan;10;10:1046559.
4. Auxéméry Y. Post-traumatic psychiatric disorders: PTSD is not the only diagnosis. Presse Medicale. 2018 May;47(5):423-430.
5. Bale TL, Picetti R, Contarino A, Koob GF, Vale WW and Lee KF. Mice deficient for both corticotropin-releasing factor receptor 1 (CRFR1) and CRFR2 have an impaired stress response and display sexually dichotomous anxiety-like behavior. Journal of Neuroscience 2002 Jan;1;22(1):193-9.
6. Bale TL, Lee KF and Vale WW. The role of corticotropin-releasing factor receptors in stress and anxiety. Integrative and Comparative Biology. 2002 Jul;42(3):552-5.
7. Boesveldt S and Parma V. The importance of the olfactory system in human well-being, through nutrition and social behavior. Cell and Tissue Research. 2021 Jan;383(1):559-567.
8. Casello SM, Flores RJ, Yarur HE, Wang H, Awanyai M, Arenivar MA, Jaime-Lara RB, Bravo-Rivera H and Tejeda HA. Neuropeptide System Regulation of Prefrontal Cortex Circuitry: Implications for Neuropsychiatric Disorders. Frontiers in Neural Circuits. 2022 Jun;21;16:796443.
9. Davis M. The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. Trends in Pharmacological Sciences. 1992 Jan;13(1):35-41.
10. Dayas CV, Buller KM, Crane JW, Xu Y and Day TA. Stressor categorization: acute physical and psychological stressors elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. European Journal of Neuroscience. 2001 Oct;14(7):1143-52.
11. Dong HW, Davis JC, Ding S, Nai Q, Zhou FM, and Ennis M. Expression of transient receptor potential (TRP) channel mRNAs in the mouse olfactory bulb. Neuroscience Letters. 2012 Aug;22;524(1):49-54.
12. Fekete EM and Zorrilla EP. Physiology, pharmacology, and therapeutic relevance of urocortins in mammals: ancient CRF paralogs. Frontiers in Neuroendocrinology. 2007 Apr;28(1):1-27.
13. Gaszner B, Csernus V and Kozicz T. Urocortinergic neurons respond in a differentiated manner to various acute stressors in the Edinger-Westphal nucleus in the rat. Journal of Comparative Neurology. 2004 Dec;6;480(2):170-9.
14. Goodnight JRM, Ragsdale KA, Rauch SAM and Rothbaum BO. Psychotherapy for PTSD: An evidence-based guide to a theranostic approach to treatment. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2019 Jan;10;88:418-426.
15. Gresack JE and Risbrough VB. Corticotropin-releasing factor and noradrenergic signalling exert reciprocal control over startle reactivity. The International Journal of Neuropsychopharmacology. 2011 Oct;14(9):1179-94.
16. Hitchcock JM, Sananes CB and Davis M. Sensitization of the startle reflex by footshock: blockade by lesions of the central nucleus of the amygdala or its efferent pathway to the brainstem. Behavioral Neuroscience. 1989 Jun;103(3):509-18.
17. Hori H and Kim Y. Inflammation and post-traumatic stress disorder. Psychiatry and Clinical Neurosciences. 2019 Apr;73(4):143-153.
18. Hsu SY and Hsueh AJ. Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. Nature Medicine. 2001 May;7(5):605-11.
19. Julius D. TRP channels and pain. Annual Review of Cell and Developmental Biology. 2013;29:355-84.
20. Im E. Multi-facets of Corticotropin-releasing Factor in Modulating Inflammation and Angiogenesis. Journal of Neurogastroenterology and Motility. 2015 Jan;1;21(1):25-32. doi: 10.5056/jnm14076. PMID: 25540945.
21. Janitzky K, D'Hanis W, Kröber A and Schwegler H. TMT predator odor activated neural circuit in C57BL/6J mice indicates TMT-stress as a suitable model for uncontrollable intense stress. Brain Research. 2015 Mar;2;1599:1-8.

22. Kamiya K and Abe O. Imaging of Posttraumatic Stress Disorder. Neuroimaging Clinics of North America. 2020 Feb;30(1):115-123.
23. Koike K, Yoo SJ, Bleymehl K, Omura M, Zapiec B, Pyrski M, Blum T, Khan M, Bai Z, Leinders-Zufall T, Mombaerts P and Zufall F. Danger perception and stress response through an olfactory sensor for the bacterial metabolite hydrogen sulfide. Neuron. 2021 Aug;4;109(15):2469-2484.e7.
24. Koivisto A, Hukkanen M, Saarnilehto M, Chapman H, Kuokkanen K, Wei H, Viisanen H, Akerman KE, Lindstedt K and Pertovaara A. Inhibiting TRPA1 ion channel reduces loss of cutaneous nerve fiber function in diabetic animals: sustained activation of the TRPA1 channel contributes to the pathogenesis of peripheral diabetic neuropathy. Pharmacological Research. 2012 Jan;65(1):149-58.
25. Kondoh K, Lu Z, Ye X, Olson DP, Lowell BB and Buck LB. A specific area of olfactory cortex involved in stress hormone responses to predator odours. Nature. 2016 Apr;7;532(7597):103-6.
26. Kormos V, Kecskés A, Farkas J, Gaszner T, Csernus V, Alomari A, Hegedüs D, Renner É, Palkovits M, Zelena D, Helyes Z, Pintér E and Gaszner B. Peptidergic neurons of the Edinger-Westphal nucleus express TRPA1 ion channel that is downregulated both upon chronic variable mild stress in male mice and in humans who died by suicide. Journal of Psychiatry and Neuroscience. 2022 May;4;47(3):E162-E175.
27. Lage-Rupprecht V, Zhou L, Bianchini G, Aghvami SS, Mueller M, Rózsa B, Sassoè-Pognetto M and Egger V. Presynaptic NMDARs cooperate with local spikes toward GABA release from the reciprocal olfactory bulb granule cell spine. Elife. 2020 Nov;30;9:e63737.
28. Lee DJ, Schnitzlein CW, Wolf JP, Vythilingam M, Rasmusson AM and Hoge CW. Psychotherapy versus pharmacotherapy for posttraumatic stress disorder: systemic review and meta-analyses to determine first-line treatments. Depression and Anxiety. 2016 Sep;33(9):792-806.
29. Lee KI, Lin HC, Lee HT, Tsai FC and Lee TS. Loss of Transient Receptor Potential Ankyrin 1 Channel Deregulates Emotion, Learning and Memory, Cognition, and Social Behavior in Mice. Molecular Neurobiology. 2017 Jul;54(5):3606-3617.
30. Logashina YA, Korolkova YV, Kozlov SA and Andreev YA. TRPA1 Channel as a Regulator of Neurogenic Inflammation and Pain: Structure, Function, Role in Pathophysiology, and Therapeutic Potential of Ligands. Biochemistry (Mosc). 2019 Feb;84(2):101-118.
31. Marone IM, De Logu F, Nassini R, De Carvalho Goncalves M, Benemei S, Ferreira J, Jain P, Li Puma S, Bunnett NW, Geppetti P and Materazzi S. TRPA1/NOX in the soma of trigeminal ganglion neurons mediates migraine-related pain of glyceryl trinitrate in mice. Brain. 2018 Aug;1;141(8):2312-2328.
32. Meents JE, Ciotu CI and Fischer MJM. TRPA1: a molecular view. Journal of Neurophysiology. 2019 Feb;1;121(2):427-443.
33. Miller MW, Wolf EJ, Sadeh N, Logue M, Spielberg JM, Hayes JP, Sperbeck E, Schichman SA, Stone A, Carter WC, Humphries DE, Milberg W and McGlinchey R. A novel locus in the oxidative stress-related gene ALOX12 moderates the association between PTSD and thickness of the prefrontal cortex. Psychoneuroendocrinology. 2015 Dec;62:359-65.
34. Miller MW, Lin AP, Wolf EJ and Miller DR. Oxidative Stress, Inflammation, and Neuroprogression in Chronic PTSD. Harvard Review of Psychiatry. 2018 Mar/Apr;26(2):57-69.
35. de Moura JC, Noroes MM, Rachetti Vde P, Soares BL, Preti D, Nassini R, Materazzi S, Marone IM, Minocci D, Geppetti P, Gavioli EC and André E. The blockade of transient receptor potential ankirin 1 (TRPA1) signalling mediates antidepressant- and anxiolytic-like actions in mice. British Journal of Pharmacology. 2014 Sep;171(18):4289-99.
36. Oettl LL and Kelsch W. Oxytocin and Olfaction. Current Topics in Behavioral Neurosciences. 2018;35:55-75.
37. Oroian BA, Ciobica A, Timofte D, Stefanescu C and Serban IL. New Metabolic, Digestive, and Oxidative Stress-Related Manifestations Associated with Posttraumatic Stress Disorder. Oxidative Medicine and Cellular Longevity. 2021 Dec;20;2021:5599265.
38. Perczel-Forintos D and Lisincki A. „A PTSD kezelése NICE irányelvek alapján 1". 2020. https://www.nice.org.uk/guidance/ng116.
39. Pérez-Gómez A, Bleymehl K, Stein B, Pyrski M, Birnbaumer L, Munger SD, Leinders-Zufall T, Zufall F and Chamero P. Innate Predator Odor Aversion Driven by Parallel Olfactory Subsystems that Converge in the Ventromedial Hypothalamus. Current Biology. 2015 May;18;25(10):1340-6.
40. Ritter JM, Flower RJ, Henderson G, Loke YK, MacEwan D and Rang HP. Rang & Dale's Pharmacology, 9th Edition. London: Elsevier. 2018.
41. Sawchenko PE, Li HY and Ericsson A. Circuits and mechanisms governing hypothalamic responses to stress: a tale of two paradigms. Progress in Brain Research. 2000;122:61-78.

42. Schrader C and Ross A. A Review of PTSD and Current Treatment Strategies. Missouri Medicine. 2021 Nov-Dec;118(6):546-551.
43. Talavera K, Startek JB, Alvarez-Collazo J, Boonen B, Alpizar YA, Sanchez A, Naert R and Nilius B. Mammalian Transient Receptor Potential TRPA1 Channels: From Structure to Disease. Physiological Reviews. 2020 Apr;1;100(2):725-803.
44. Tan KS, Nackley AG, Satterfield K, Maixner W, Diatchenko L and Flood PM. Beta2 adrenergic receptor activation stimulates pro-inflammatory cytokine production in macrophages via PKA- and NF-kappaB-independent mechanisms. Cell Signal. 2007 Feb;19(2):251-60.
45. Topilko T, Diaz SL, Pacheco CM, Verny F, Rousseau CV, Kirst C, Deleuze C, Gaspar P and Renier N. Edinger-Westphal peptidergic neurons enable maternal preparatory nesting. Neuron. 2022 Apr;20;110(8):1385-1399.e8.
46. Török B, Sipos E, Pivac N and Zelena D. Modelling posttraumatic stress disorders in animals. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2019 Mar;2;90:117-133.
47. Truex RC and Carpenter MB. Human neuroanatomy, 6th edition. Williams and Wilkins. 1969.
48. Ujvári B, Pytel B, Márton Z, Bognár M, Kovács LÁ, Farkas J, Gaszner T, Berta G, Kecskés A, Kormos V, Farkas B, Füredi N and Gaszner B. Neurodegeneration in the centrally-projecting Edinger-Westphal nucleus contributes to the non-motor symptoms of Parkinson's disease in the rat. Journal of Neuroinflammation. 2022 Feb;2;19(1):31.
49. Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S, Chan R, Turnbull AV, Lovejoy D, Rivier C, and mtsai. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature. 1995 Nov;16;378(6554):287-92.
50. Vosshall LB. Social signals: the secret language of mice. Current Biology. 2005 Apr;12;15(7):R255-7.
51. White-Welkley JE, Bunnell BN, Mougey EH, Meyerhoff JL and Dishman RK. Treadmill exercise training and estradiol differentially modulate hypothalamic-pituitary-adrenal cortical responses to acute running and immobilization. Physiology & Behavior. 1995 Mar;57(3):533-40.
52. Wang Y, Cao L, Lee CY, Matsuo T, Wu K, Asher G, Tang L, Saitoh T, Russell J, Klewe-Nebenius D, Wang L, Soya S, Hasegawa E, Chérasse Y, Zhou J, Li Y, Wang T, Zhan X, Miyoshi C, Irukayama Y, Cao J, Meeks JP, Gautron L, Wang Z, Sakurai K, Funato H, Sakurai T, Yanagisawa M, Nagase H, Kobayakawa R, Kobayakawa K, Beutler B and Liu Q. Large-scale forward genetics screening identifies Trpa1 as a chemosensor for predator odor-evoked innate fear behaviors. Nature Communications. 2018 May;23;9(1):2041.
53. Wang L, Zhang Z, Chen J, Manyande A, Haddad R, Liu Q and Xu F. Cell-Type-Specific Whole-Brain Direct Inputs to the Anterior and Posterior Piriform Cortex. Frontiers in Neural Circuits. 2020 Feb;7;14:4.
54. Zelena D, Mergl Z, Foldes A, Kovács KJ, Tóth Z and Makara GB. Role of hypothalamic inputs in maintaining pituitary-adrenal responsiveness in repeated restraint. American Journal of Physiology. Endocrinology and Metabolism. 2003 Nov;285(5):E1110-7.
55. Zou J, Wang W, Pan YW, Lu S and Xia Z. Methods to measure olfactory behavior in mice. Current Protocols in Toxicology. 2015 Feb;2;63:11.18.1-11.18.21.
56. Zuniga A and Ryabinin AE. Involvement of Centrally Projecting Edinger-Westphal Nucleus Neuropeptides in Actions of Addictive Drugs. Brain Sciences. 2020 Jan;26;10(2):67.

8. LIST OF PUBLCATIONS THE THESIS IS BASED ON

Konkoly J[*], Kormos V*, Gaszner B, Sándor Z, Kecskés A, Al-Omari A, Szilágyi A, Szilágyi B, Zelena D, Pintér E

The role of TRPA1 channels in the central processing of odours contributing to the behavioural responses of mice

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Transient receptor potential ankyrin 1 ion channel expressed by the Edinger-Westphal nucleus contributes to stress adaptation in murine model of posttraumatic stress disorder

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