

The role of PTH2 neuropeptide in social function – a study using PTH2 receptor KO mice

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Arrasz Nikolett

Biology MSc

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Supervisor:

DR. DOBOLYI ÁRPÁD

Department of Physiology and Neurobiology

Eötvös Loránd University



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Summary

Social behaviour is important to reproduce and survive for many species. The role of parathormon 2 neuropeptide (PTH2) has been reported in the social life of zebrafish, and there are evidences of its involvement in social grooming of rats. Therefore, we examined the role of the PTH2-PTH2 receptor (PTH2R) neuromodulator system in the social behaviour of mice. We investigated the role of PTH2R in different tests of social behaviours by comparing the behaviour of PTH2R KO and wild type mice. WT mice spent more time with an unfamiliar mouse than their cagemate. This expected behaviour disappeared in KO mice. Furthermore, KO mice exhibited an increased latency of sniffing in social environment, an introductory behaviour between mice. In turn, there was no detectable difference between WT and KO mice in the elevated plus maze test arguing against altered anxiety level in the absence of PTH2R. Furthermore, we aimed to investigate socially induced neuronal activation in WT and PTH2R KO mice. Using c-Fos immunohistochemistry, we found a reduced number of activated cells in KO compared to WT mice in medial prefrontal cortex, in the ventral part of the lateral septum and in the medial preoptic area, which regions are known to be involved in the control of social behaviour. These data suggest that PTH2 contributes to the neuronal activation in these brain regions in response to social interaction via the PTH2R. In conclusion, PTH2 may be have a role in the control of some aspects of social behaviours.

Abbreviations

AMY	amygdala
cAMP	cyclic adenosine monophosphate
HET	heterozygote
IL	infralimbic cortex
KO	knock-out
LS	lateral septum
LSv	ventral part of the lateral septum
MG	medial geniculate body
MPFC	medial prefrontal cortex
MPL	medial paralemniscal nucleus
MPOA	medial preoptic area
Ni-DAB	nickel sulphate + 3,3'-Diaminobenzidine
PFA	paraformaldehyde
PIL	posterior intralaminar complex of the thalamus
PL	prelimbic cortex
PTH	parathyroid hormone
PTH2	parathyroid hormone 2
PTH1R	parathyroid hormone receptor
PTH2R	parathyroid hormone 2 receptor
PVG	periventricular grey of the thalamus
PVN	paraventricular nucleus of the hypothalamus
SCN	suprachiasmatic nucleus
SN	substantia nigra
TIP39	tuberoinfundibular peptid of 39 residues
WT	wild type

1. Introduction

1.1. The social brain network

Social behaviour is important to reproduce and survive in many species. For species living in social groups, social interactions are essential for mental and therefore, physical well-being as well. Humans are highly sociable species, therefore, mapping the neuronal background of the process of perception, social cue integrations, cognition and behavioural response is a topic receiving a lot of attention. However, because of the invasiveness of some experimental methods, animal models are used to describe particular aspects of social behaviours. Since laboratory rodents are also social, they are often the choice of subject. Here, we briefly describe main parts of rodent social brain network.

Perception of social cues depends on the type of the sensation. Rodents often use olfactory information, which enters the brain on the site of olfactory bulb. Another important type of cues can be somatosensory signals. The posterior intralaminar complex of the thalamus (PIL) was recently demonstrated to play a possible role as a thalamic relay station in social somatosensory information processing (Keller et al., 2022). Further processing areas of social cues coding emotions are the amygdala (AMY), and the bed nucleus of stria terminalis, which also have roles in threat detection, including social threat (Emmons et al., 2020). Another important site is the hypothalamus. Paraventricular hypothalamic nucleus (PVN) has important role in oxytocin release in the pituitary gland. Oxytocin neurons also project to several part of the social brain network, such as the AMY and the lateral septum (LS). The other hypothalamic area participating in social interactions is the medial preoptic area, which has demonstrated functions in sexual, parental and general social responses between adults (Keller et al., 2022; Wang et al., 2019; Wu et al., 2014). On the site of motivation, nucleus accumbens, as the part of the reward circuit, has the role of promoting or inhibiting a social response by computing the valence and salience of a social stimuli. Executional functions have a higher level cortical modulator, medial prefrontal cortex (MPFC), which performs final decision-making, while being in connection with several above mentioned areas in the brain (Fernández et al., 2018).

Social brain network is a high complexity network in the brain with several, sometimes two-way connections. Throughout our project, we mainly focused on one type of neuropeptide of

this network, parathyroid hormone 2, and the possible projections of its neurons form a somatosensory relay centre, PIL, toward parts of the social brain network.

1.2. The parathyroid hormone 2 neuromodulatory system

Parathyroid hormone 2 was discovered in the 90's, from then on many investigations were performed to find the function of this peptide in the physiology of vertebrates. After finding its unique localisation in the brain, several discoveries revealed its diverse roles. In the last decade, this neuropeptide was proved to be an important member of the social brain network, which title was established by investigations on its function, first in maternal, and subsequently in general social behaviours.

1.2.1. General properties and appearance

Parathyroid hormone 2 (PTH2) neuropeptide, alternatively named as tuberoinfundibular peptid of 39 residues (TIP39) belongs to a small peptide family containing the parathyroid hormone (PTH) released by the parathyroid gland, which has importance in calcium homeostasis through actions on kidneys and bones. The presence of PTH2 in the periphery has also been demonstrated in the kidneys, and some cardiovascular and reproductive organs. Nevertheless, this peptide is most abundant in the central nervous system, forming a neuromodulatory system with its receptor, parathyroid hormone 2 receptor (PTH2R) (Dobolyi et al., 2010). This receptor belongs to the class B or secretin-like G-protein coupled type of receptors, and shows about 50% similarity with the parathyroid hormone receptor (PTH1R). The distribution of PTH2R's in the central nervous system overlaps with the distribution of PTH2 terminals, which is an evidence of their receptor-ligand relationship. Binding of the PTH2 to its receptor starts an excitatory response via Gs and Gq intracellular signalling pathways through increasing the levels of secondary messengers, cAMP and Ca²⁺, respectively (Keller et al., 2022a).

The presence of this neuropeptide and its receptor has been demonstrated in the brain of vertebrates. Neurons expressing PTH2 are located in the periventricular grey of the thalamus (PVG) and the posterior intralaminar complex of the thalamus (PIL). In addition, it is expressed in the medial paralemniscal nucleus (MPL) of the lateral pons (Keller et al., 2022a). The brain

areas expressing the PTH2 receptors are very diverse, containing limbic and hypothalamic areas and the median eminence (Matsumoto et al., 2010).

1.2.2 The diverse roles of parathyroid hormone 2

Generally, the role of PTH2 neuromodulatory system is to process sensory information and modulate hormone release, which will regulate behavioural responses. Some of the main functions of PTH2 is nociception (Matsumoto et al., 2010), thermoregulation (Dimitrov et al., 2011), coping with environmental stress, and maternal behaviour (Keller et al., 2022a).

Focusing on behavioural functions, one important role of PTH2 is to respond to environmental provocations. In normal conditions, mice lacking of PTH2 showed no anxiety-related response compared to wild type animals, but when the mice are faced to stress-generating environment, for example, too high illumination, the anxiety level of the animal is higher if the PTH2 is absent (Fegley et al., 2008). Related to this phenomenon, PTH2 neuropeptide was proved to be a regulatory factor of emotional processing of environmental challenges, and important factor of saving short term memory after facing an unknown environment in mice (Coutellier et al., 2011).

Many studies revealed the role of PTH2 in maternal behaviour, which is a specific form of social behaviour. From parturition to the end of lactation period, PTH2 expression levels are elevated in the brain of mother rats. The activity of PTH2 neurons in the posterior intralaminar complex of the thalamus (PIL) are increased in the case of suckling, as projections of this neuron enhance the release of prolactin through arcuate nucleus and oxytocin via the hypothalamic paraventricular nucleus (PVN). Both of them supports milk ejection. Further investigations proved that projections of PTH2 neurons to the medial preoptic area (MPOA) has impact on the motivation of the mothers to take care of their pups (Cservenak et al., 2017; Cservenák et al., 2014; A. Dobolyi et al., 2018).

1.2.2. The social aspects of parathyroid hormone 2 functions

In the case of zebrafish and rat, PTH2 was proved to respond to the mechanosensory inputs in social context through its expression level, and also to modulate outputs leading to behavioural answers to the social situation.

In group living fish species, zebrafish, PTH2 expression level was found to be decreased when the animal was in social isolation. This state turned out to be flexible, as following exposure to

conspecifics lead to sudden increase of PTH2 only after 30 minutes, as acute isolation from a social environment also decreased it. This time resolution of the response suggests that the level of PTH2 only reflects the current social environment of the animal, which is proved further by finding that prior housing state in the juvenile age of the animal has no effect on the answer of PTH2 levels neither in case of isolation, nor in social environment. The origin of the mechanosensation causing this phenomenon was proved to be the lateral line organ, which can detect the density of a group of conspecifics in the water. Lesion of its neuromast cells led to low PTH2 level, while mechanical imitation of the group via perturbation of the water caused the same results as the swimming motion generated waves by conspecifics (Anneser et al., 2020; Venuto et al., 2022). Subsequently, functions of PTH2 in social life of zebrafish began to be discovered in the case of lacking of functional PTH2R. While in juveniles, social responses of mutants was not different, in adulthood, social preference decreased in animals in the absence of PTH2R (Anneser et al., 2022).

Recently, it has also been proved that PTH2 has a social function in rodents. It was demonstrated that PTH2 expression is elevated in rats in case of social interactions between same age female conspecifics. Furthermore, it was shown that via PTH2 neurons projecting from the PIL to the MPOA, social grooming behaviour can be enhanced via the high amount of PTH2R present in the MPOA. The investigation used chemogenetic stimulation and inhibition of the PIL-MPOA pathway as well as a PTH2R antagonist to verify the role of the neuromodulator system in social interactions. Stimulation led to increased time spent with social grooming. Therefore, we can assume that neurons activated during social context modulate the behavioural outputs of the animal. PTH2 was proved to be involved in the regulation because administration of PTH2R antagonists reduced the time of social grooming. The connections between the two brain areas through PTH2 projections have been also demonstrated using retrograde tracers. Therefore, PIL PTH2 neurons seem relay social information leading to further direct social interactions (Keller et al., 2022b).

PIL PTH2 neurons have increased activity during social interactions, and are thought to receive sensory inputs from the spinal cord during physical social contact while projecting to the MPOA and also to oxytocin neurons in the PVN. Oxytocin is known to promote social behaviours via the targets of oxytocin neurons, which are limbic areas of the social brain network. The elevation of activation level was shown by the increased by elevated number of cells expressing immediate early gene c-Fos in the PIL. This experiment was performed on female rats, which had been separated before the test, then the cagemates spent 2 hours together. The reunion of

the animals caused significant increase is neuron activity in the brain compared to the rats, which were not given back their mates (Cservenak et al., 2017).

All these recent discoveries support the idea about the possible functions of PTH2 neuromodulatory system in social behaviours between conspecifics. Therefore, in the present study, we further investigated social behavioural aspects, in which this system plays potential roles.

2. Objectives

The central hypothesis of the study was that the PTH2-PTH2R neuromodulator system is involved in the control of social behaviours. Therefore, we examined mice lacking the PTH2R in different social contexts. The specific objectives were the following:

1. To investigate the social behaviour of PTH2R KO as compared to littermate WT mice. For that purpose, 3 chamber test was used and direct social interactions were examined.
2. To examine the possibly altered anxiety and depression-like behaviours of PTH2R KO mice in order to establish if these alterations might contribute to differences in social behaviours
3. To determine, where PTH2 exerts its actions in the brain by investigating socially induced neuronal activation in WT and KO mice.

3. Materials and Methods

3.1. PTH2R KO Transgenic Mice Line and Genotype Verification

In the study, we used C57BL/6 adult female virgin mice from the line PTH2R KO, which animals are lacking of the expression of parathyroid hormone 2 receptor. We bred heterozygote (HET) mice of this line, therefore, we always needed to verify the genotype of the descendants in every litter to determine their role in the subsequent tests. To investigate the genotype of the mice we got samples from the tails of the 2-weeks old animals. After DNA isolation, purification, and amplification of the DNA region of our interest, we carried out agarose gel electrophoresis to determine the genotype of the animals.

As mentioned above, all three genotypes of the line had specific role during the experiment: KO and WT mice were used as test animals and control animals, respectively, while HET littermates were the cagemates of the former genotypes, having the role of familiar or unfamiliar stimulus animals during the tests. For the behaviour tests, we used 9 KO and 7 WT animals.

3.2. Habituation Period

After the mice reached 2 months of age, they were separated into groups of two per cage, containing the KO or WT animal with a HET cagemate. Habituation to this housing lasted at least 3 weeks prior to the experiment.

3.3. Social Behaviour Tests

3.3.1. Direct Social Interaction Test

Direct Social Interaction Test was performed in an open field arena, where the test or control animal, as well as their cagemate could freely move and be in contact with each other. The test followed a 20-minute separation of the cagemates, during which we carried out the 2 times 10-minute-long habituation of the mice to the arena, the first ten minute for the KO or control WT animal, and the second for the cagemate. After habituation we immediately started the 30-minute test.

3.3.2. Sociability and Social Novelty Preference Test

Sociability and Social Novelty Preference Tests were performed in a Three Chamber Apparatus, which is a rectangle shaped arena separated to three equal sized zones. The two peripheral zone contains 1-1 cylinder shaped cages. During Sociability Test the KO or WT animal can freely explore the three zones. One of the two cage contains the HET cagemate, the other one is empty. Following this test, we immediately performed Social Novelty Preference test, which has the following setting modification: the previously empty cage is now contain an unfamiliar conspecific which also has HET genotype. The KO or WT animal can freely decide to explore the familiar and unfamiliar mice in the apparatus. The tests followed a habituation period for each the KO or WT animal and for the cagemate and unfamiliar animal. First,

habituation to the cages was performed with the last two animal parallel in time, for 5 minutes, to avoid increased stress level, triggered by the lack of space in the cylinder. This was followed by a 10-minute habituation of the KO or WT animal to the Three Chamber Apparatus, where the two cages were empty. When we changed the status of the cages, before and between the tests, the freely moving animal spent this transition times in the middle zones while the gateways between zones were closed. Both of the test lasted for 10 minutes.

3.4. Additional Behaviour Tests

We carried out additional behavioural tests to investigate the anxiety- and depression-like behaviours of mice. Open Field Test of the test and control animals was performed in an open field arena. The test was 10-minute-long and the animal could freely explore the arena.

Elevated Plus Maze is a four-arm maze, two of the arms are separated from the environment by walls, while two of them are only open plancks. The animal can freely explore these arms. Test and control animals was tested, each of them spent 10 minutes on the Elevated Plus Maze.

Forced Swim Test was performed in 2 l transparent beakers, with 1,6 l 26 °C tap water in it. With these parameters, mice can be in the water without reaching the bottom, therefore, they need to keep themselves on the surface. The test lasted for 6 minutes.

3.5. Evaluation of Behaviour Tests

Two evaluation methods were used for behavioural tests. Direct Social Interaction Test, Elevated Plus Maze Test and Forced Swim Test was evaluated by Solomon Coder software, in which we can create frames form every 0,20 s of the video recordings, and labelling them with one of the behavioural element types within the configuration setup according to the given type of test. The evaluation of the videos was done by a person who was unaware of the genotypes of the mice. Based on the behavioural element series, the software can calculate the duration, the latency, and the frequency of an element.

In case of Direct Social Interaction Test, labels are listed in *Table 1*.

<i>non social</i>	there is no physical contact or signs of a following social behaviour element in the next frame
<i>social partner</i>	only the partner animal is active socially for example by sniffing or mounting the test animal
<i>approach</i>	the test animal's nose is close to the partner and it is usually followed by a sniffing element with contact
<i>body contact</i>	the two animals are in physical contact, but it cannot be labelled as any of the other social elements
<i>body sniffing</i>	the test animal's nose is in contact with the partner animal's body
<i>anogenital sniffing</i>	when the body sniffing is presented on the anogenital parts of the partner animal
<i>tail sniffing</i>	the test animal's nose is in contact with the partner animal's tail
<i>head to head</i>	the animals sniffs each other on the head
<i>side to side</i>	the animals have a body-long physical contact on their side
<i>mounting</i>	the test animal is climbing on the back of the partner

Table 1. Labels of behaviour elements which were monitored during the 30-minute-long Direct Social Interaction Test.

During evaluation of the Elevated Plus Maze Test we used 3 labels, “open” when more than 50% of the animals’ body is on the open arm, and “close” or “middle” if the same situation was right in the close arm or the area connecting the arms, respectively.

Labelling elements of Forced Swim Test also included 3 categories, “climbing”, when the animal tried to climb the wall of the beaker, “swimming” when they tried actively staying on surface by swimming motions of at least one of their paws, and “floating”, when they were immobile on the surface of the water.

The other method of evaluation was using Smart software, which can track the movement of the animal within the arena real time or after recording.

3.6. Immunohistochemistry

Animals, which were used in this part of the study, were kept and habituated in the same way as they did before the behavioural tests. The pairs were separated for 18 hours, and half of each group – KO and WT animals – received their cagemate back. This way the experiment contained four groups. Animals, which received their cagemate, spent 1 and a half hours together, before sacrificing all the KO and WT mice. Animals were processed to perform c-Fos Ni-DAB immunohistochemistry: paraformaldehyde (PFA) perfusion was followed by post-

fixation of the brains in PFA for 24 hours. The brain was sliced into 50 µm sections with cryomicrotome. Within the two groups of the animals, based on their history before perfusion - received partner back and not received partner back - we paired KO and WT brain slices in one staining tube to provide the exactly same treatment during the procedure within pairs. This way, the result of quantitative comparison only reflects the genotype of the animals. WT brain slices was marked for the easy distinction of the origin of slices after the immunohistochemistry. Immunolabeling was performed using rabbit c-Fos primary antibody and donkey anti-rabbit secondary antibody. Pictures about the slices were taken at 4x magnification for determination of location and at 10x for quantitation.

4. Results and Discussion

4.1 Direct Social Interaction Test

During the 30 minutes of examination, the animals could freely move and behave. We distinguished 10 behavioural elements listed above in *Table 1*. In *Figure 1.*, we show a comparison about the durations of each element throughout the test.

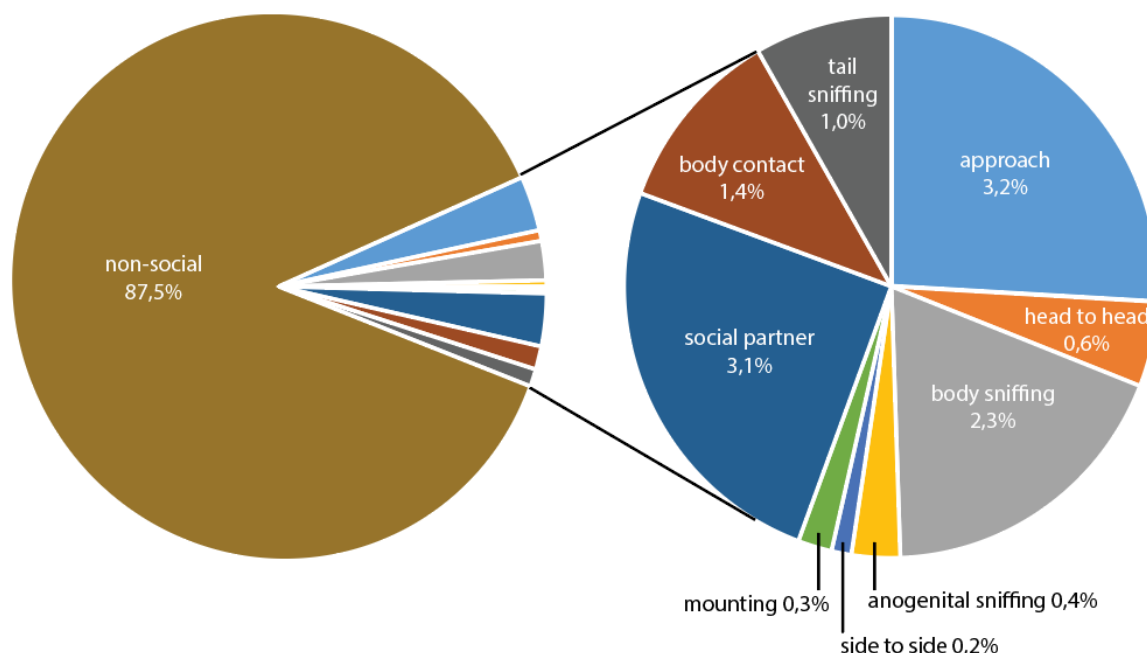


Figure 1. Percentages of durations, which animal spent showing different behavioural elements. On the right, independent pie-chart of the social behaviours can be seen.

In 87.5% of the 30-minute test, the animals did not show social interactions, that is only 12.5% of total duration was social interaction. From this, we can subtract the time, during which only the cagemate (social partner) was engaged in active social behaviour. Therefore, the test and control animals spent 9.4% of their time showing social behaviours. The two major behavioural element shown was the approach and the body sniffing. These behaviours we interpret as introductory behaviours, preceding other forms of social interactions with important role in the recognition of conspecifics. In case of the durations and frequencies of these behavioural elements, we did not find significant differences between the KO and WT animals, but significant differences were found in the latency in the case of this two behavioural element highlighted above, as we show it in *Figure 2*.

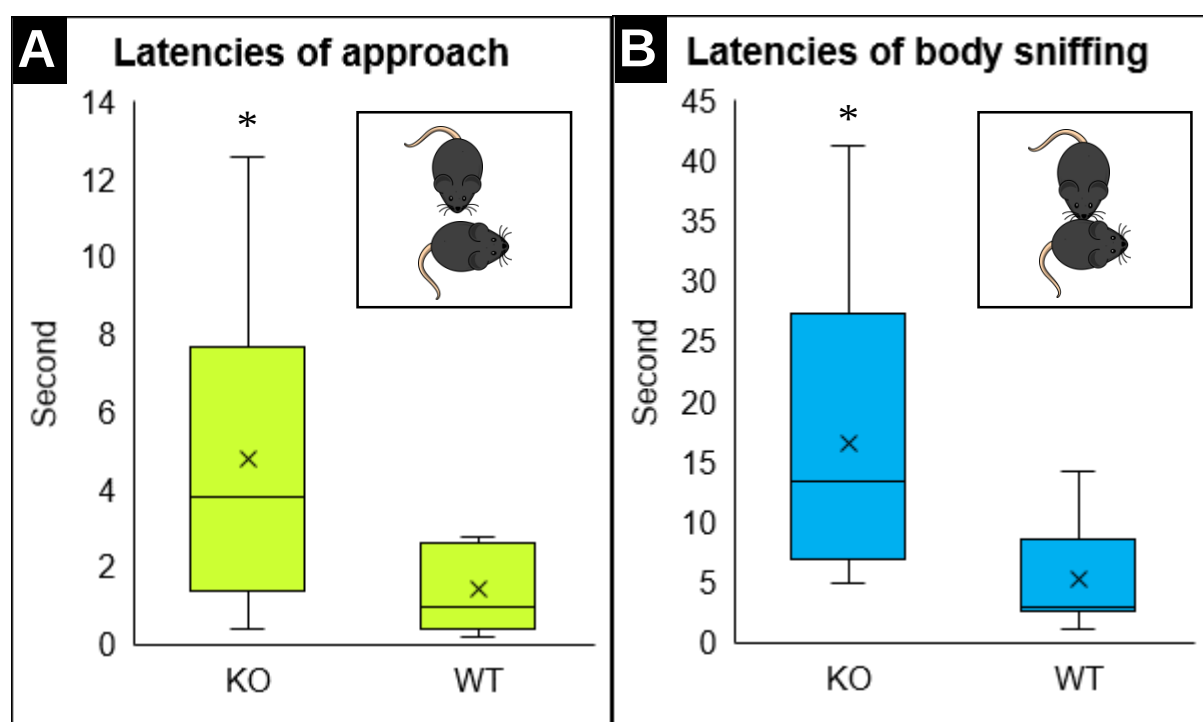


Figure 2. Latency values of approach (A) and body sniffing (B) element with illustrations about what we see on the video frames while labelling these behavioural elements.

As we see on the figures, KO animals started these introductory behaviours significantly later compared to WT animals, p values of differences are 0.039 for the approach and 0.029 for the body sniffing elements.

4.2. Sociability and Social Novelty Preference Tests

Sociability of KO animals did not differ from wild types, however, when we monitored their preferences between the chambers containing familiar cagemates and unfamiliar conspecifics,

we found that in the case of KO animal, natural preference towards the unknown mouse diminished significantly. These findings are shown in *Figure 3*.

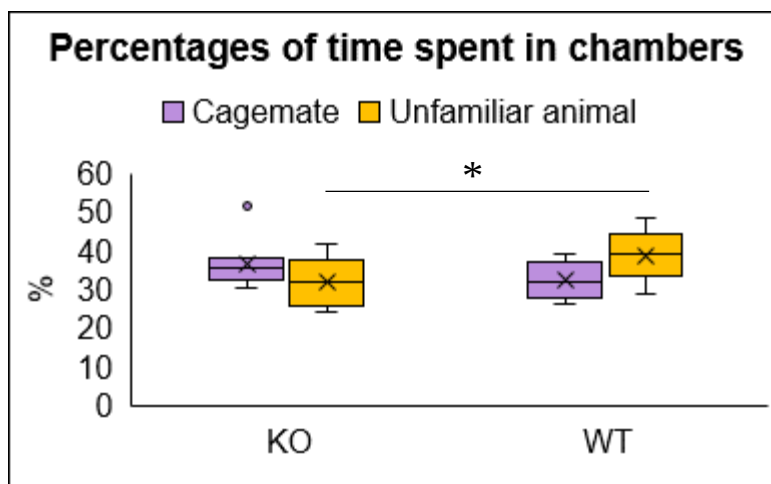


Figure 3. Comparison of durations, which KO and WT mice spent in the peripheral chambers containing their HET cagemate and a HET unfamiliar conspecific.

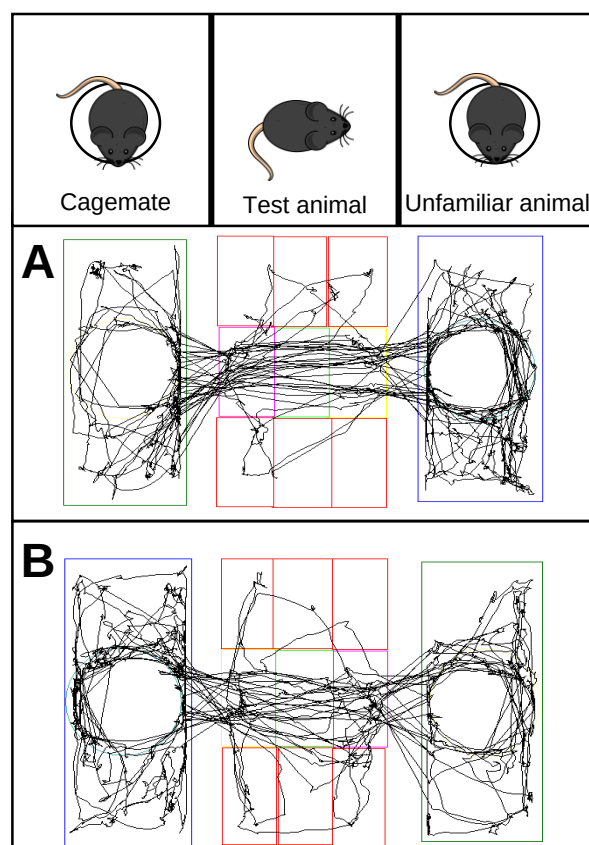


Figure 4. Examples of trajectories about the movement of animals in the three chamber apparatus. A) is one of the WT animal and B) is example for a KO animal.

Examples of trajectory maps from a KO and a WT animal with illustrations of the apparatus setting are shown in *Figure 4*. Differences between the preferences toward the unfamiliar animal of WT and KO animals were significant with a p value 0.019.

4.3. Additional Behavioural Tests

We carried out behavioural tests to investigate potential anxiety and depression-like behaviours of the animals. Anxiety-like behaviour was monitored by open field tests and elevated plus-maze tests. In the case of elevated plus-maze test we found no differences between the durations, frequencies or latencies. On *Figure 5*, we show the results regarding durations, how much time mice spent in each zone of the elevated plus-maze.

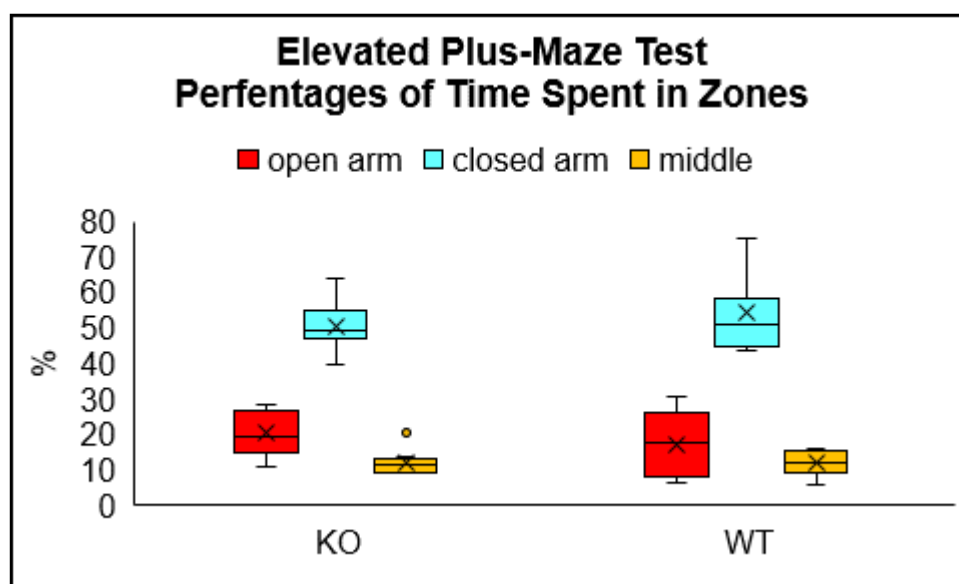


Figure 5. Percentages of time spent in different zones of the elevated plus-maze.

The result showed that the normal preferences of wild type animal toward the closed arm did not change in KO animal, which indicates that normally KO mice do not have a significantly different anxiety level than the WT animals.

Findings regarding the open field test were partly supporting what we concluded from the result of elevated plus-maze test. We data are shown in detail in *Figure 6*. We did not find any difference between WT and KO animals, when we monitored how much time they spent in each zone of the open field arena. The arena had 16 zones illustrated in *Figure 6*. They are divided to 3 types: green zones are corner zones, yellow ones are edges, and the red ones are the middle zones. The only significant difference what we found was the number of entries to

the zones in the middle (6,7,10 and 11). Avoiding these zones can mean higher anxiety level. The significance level of this difference between KO and WT animal is 0.014.

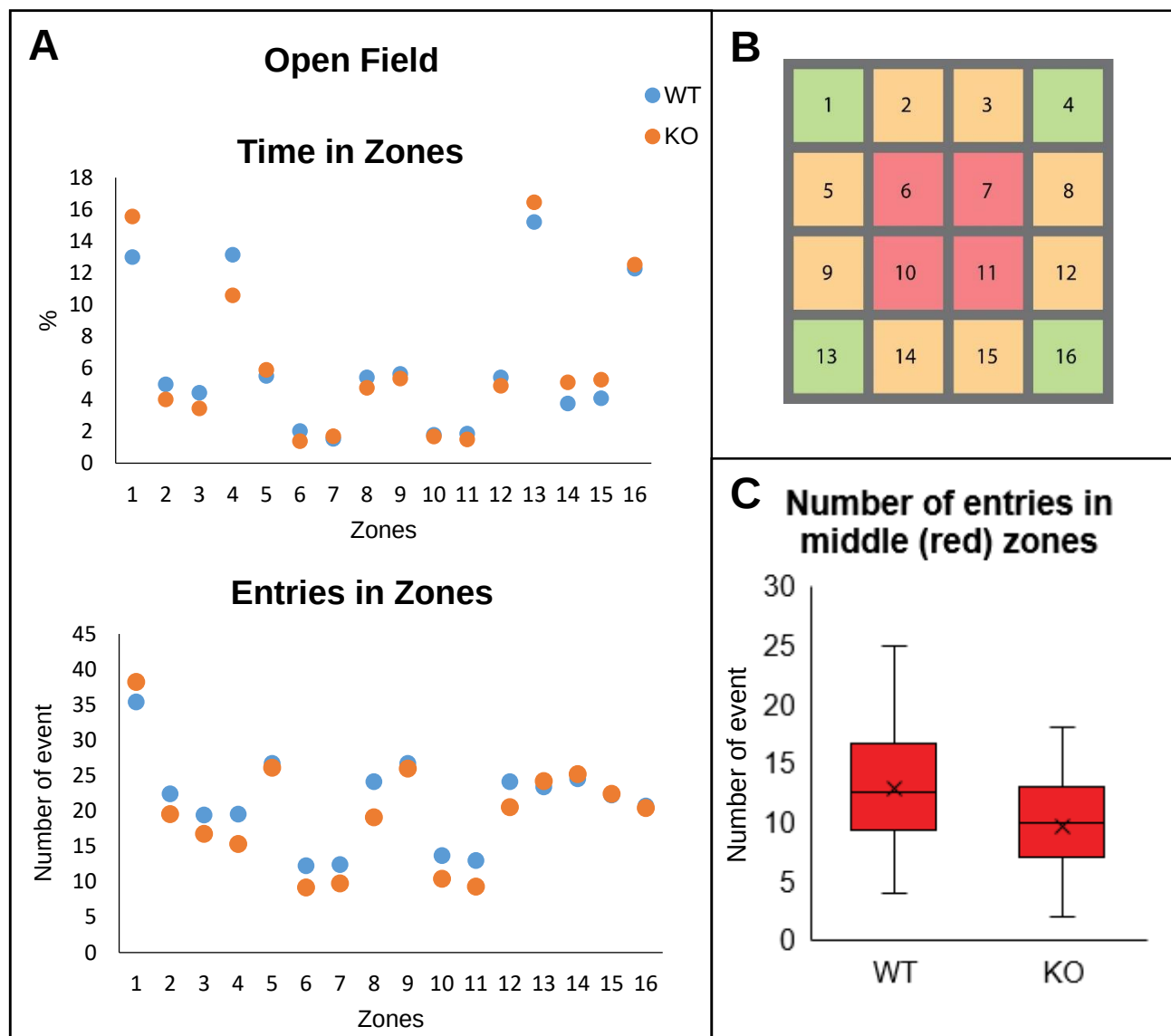


Figure 6. Results of the open field test. A) Average values of the two group of animals (KO and WT) in the case of time spent in each zones (on the top) and number of entries in each zones (on the bottom). B) Illustration about the zones within the open field arena. C) Number of entries in the middle zones shown with red in the panel B.

Depression-like behaviour was monitored by forced swim test, when we examined the behaviour of the animal in a situation, where it needs to actively strive to cope with the situation. In this case, it had to avoid sinking in deep water. If floating behaviour is increased, it can refer to elevated level of depression. However, in this experiment, we did not see any significant

difference between the WT and KO animals regarding the durations, latencies and frequencies of their behavioural elements in this situation. We show data on duration in *Figure 7*.

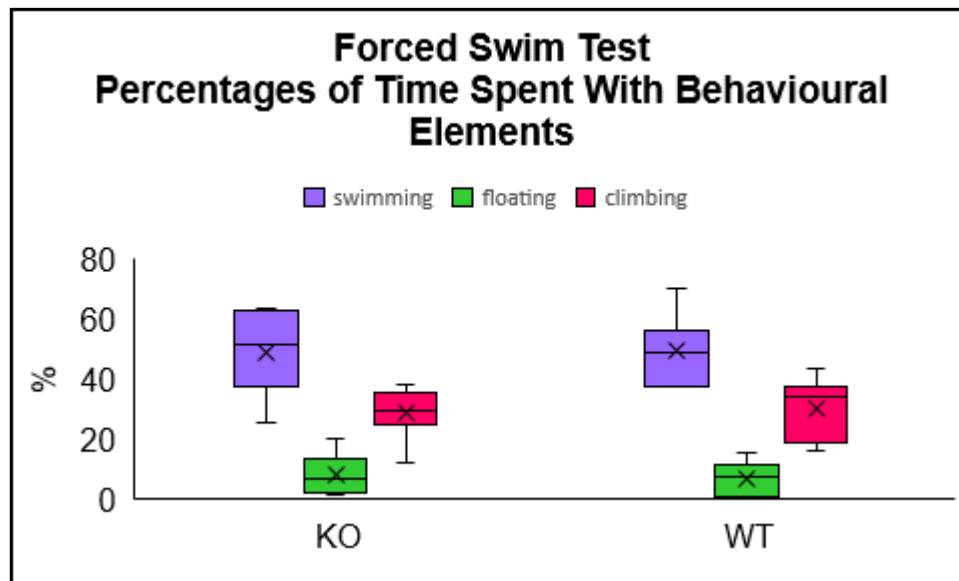


Figure 7. Percentages of behaviours which mice performed during Forced Swim Test.

To sum up the findings on the behavioural properties of animals lacking PTH2R in social environment, PTH2R KO animals delay investigating their cagemates after a short separation, and they prefer investigating unknown conspecifics less than wild type animals. These KO animals, did not show signs of anxiety and depression-like behaviour. The fewer number of entries to the middles zone of the open field can be referred to a higher level of anxiety, but we can see that these lower entry number occur also in the case of edge zones, and in one of the corner zones also, which refer to a lower activity level of the KO animals. We also monitored activity levels, but did not find differences between genotypes, therefore, elevated anxiety level of KO animals is not fully proved. We know from prior literature that KO animals show elevated anxiety level if the test was followed by or was performed during uncomfortable, fearful situation for the animal (Coutellier et al., 2011; Fegley et al., 2008). New environment can be stressful for animals; this is why habituation periods were performed prior to the tests. Direct social interaction test was followed a 10-minute habituation, while social novelty preference test was preceded by a 10-minute habituation, and a 10-minute sociability test, in the same apparatus. With these findings, we propose, that the functional disruption of PTH2-PTH2R system may have cause difficulties exclusively in some of the social behaviour aspects of the animal.

4.4. Immunohistochemistry

Ni-DAB-stained c-Fos immediate early gene expressing transcription factor can refer to activated neurons in a brain area. We compared 4 parts of brain, which are known to be involved in the social brain network: medial prefrontal cortex (MPFC), lateral septum (LS), medial preoptic area (MPOA) and posterior intralaminar complex of the thalamus (PIL). Microscopic analysis identified differences in the activation of first three brain sites between KO and WT animals, when they received their cagemates back. The number of activated neurons was lower in KO than in WT animal all 3 examined brain regions while in the PIL, there was no observed difference between the genotypes. Examples of this findings are shown on *Figure 8*.

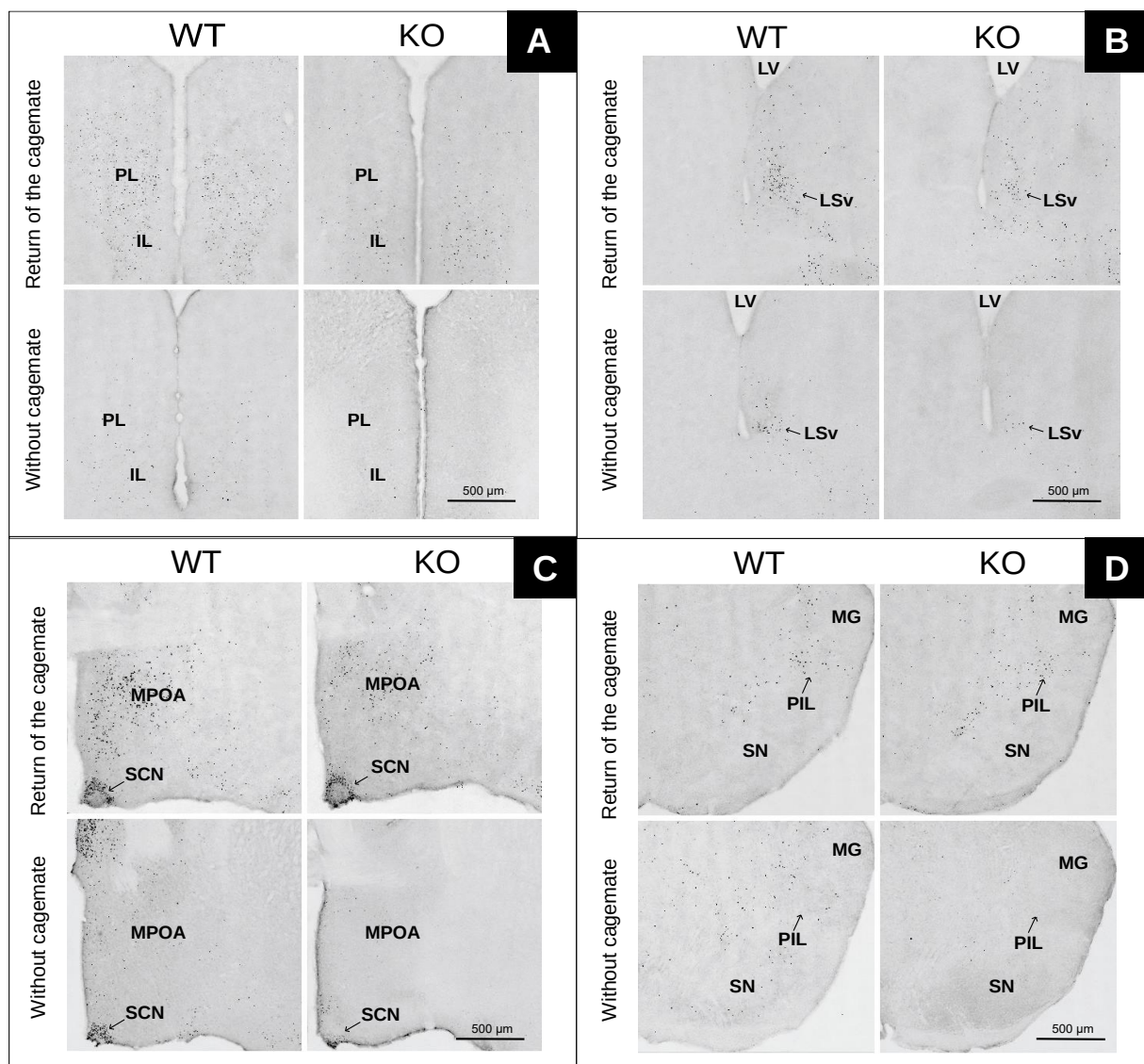


Figure 8. Results of immunohistochemistry on four area of the brain known to involved in social brain network. A) medial prefrontal cortex (Parts: PL – prelimbic cortex, IL – infralimbic cortex) B) ventral part of the lateral septum (LSv) C) medial preoptic area (MPOA) D) Posterior intralaminar complex of the thalamus (PIL). Further abbreviations: LV – lateral ventricle, SCN – suprachiasmatic nucleus, SN – substantia nigra, MG – medial geniculate nucleus.

We assume, that PIL contains mostly PTH2⁺ neurons, not its receptor, but on areas where PIL PTH2 neurons are projecting, differ in neuronal activity from the brain of WT animals, if the receptor PTH2R and its effect on Gs and Gq excitatory intracellular signalling pathways are absent. We suppose these areas listed above are receiving projections from PIL. Projections from PIL toward MPOA has been proved recently, which supports our hypothesis (Keller, Láng, et al., 2022b). We plan to examine quantitatively the differences, what we observed, to statistically establish it on higher sample numbers. Then, we plan to investigate, if the PTH2R⁺ neurons and activated neurons of these brain areas are overlapping.

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References

- Anneser, L., Alcantara, I. C., Gemmer, A., Mirkes, K., Ryu, S., & Schuman, E. M. (2020). The neuropeptide Pth2 dynamically senses others via mechanosensation. *Nature*, 588(7839), 653–657.
- Anneser, L., Gemmer, A., Eilers, T., Alcantara, I. C., Loos, A. Y., Ryu, S., & Schuman, E. M. (2022). The neuropeptide Pth2 modulates social behavior and anxiety in zebrafish. *IScience*, 25(3), 103868.
- Coutellier, L., Logemann, A., Kuo, J., Rusnak, M., & Usdin, T. B. (2011). TIP39 modulates effects of novelty-induced arousal on memory. *Genes, Brain and Behavior*, 10(1), 90–99.
- Cservenak, M., Keller, D., Kis, V., Fazekas, E. A., Ollos, H., Leko, A. H., Szabo, E. R., Renner, E., Usdin, T. B., Palkovits, M., & Dobolyi, A. (2017). A thalamo-hypothalamic pathway that activates oxytocin neurons in social contexts in female rats. *Endocrinology*, 158(2), 335–348.
- Cservenák, M., Szabó, É. R., Bodnár, I., Lékó, A., Palkovits, M., Nagy, G. M., Usdin, T. B., & Dobolyi, A. (2014). Thalamic neuropeptide mediating the effects of nursing on lactation and maternal motivation. *J Psychoneuroendocrinology*, 38(12), 1–23.
- Dimitrov, E. L., Kim, Y. Y., & Usdin, T. B. (2011). Regulation of hypothalamic signaling by tuberoinfundibular peptide of 39 residues is critical for the response to cold: A novel peptidergic mechanism of thermoregulation. *Journal of Neuroscience*, 31(49), 18166–18179.
- Dobolyi, A., Cservenak, M., & Young, L. J. (2018). Thalamic integration of social stimuli regulating parental behavior and the oxytocin system. *Frontiers in Neuroendocrinology*, 51, 102–115.
- Dobolyi, Á., Palkovits, M., & Usdin, T. B. (2010). The TIP39-PTH2 receptor system: unique peptidergic cell groups in the brainstem and their interactions with central regulatory mechanisms. *Prog Neurobiol*, 90(1), 1–65.
- Emmons, R., Sadok, T., Rovero, N. G., Belnap, M. A., Henderson, H. J. M., Quan, A. J., Del Toro, N. J., & Halladay, L. R. (2020). Chemogenetic manipulation of the bed nucleus of the stria terminalis counteracts social behavioral deficits induced by early life stress in C57BL/6J mice. *Journal of Neuroscience Research*, March, 1–20.
- Fegley, D. B., Holmes, A., Riordan, T., Faber, C. A., Weiss, J. R., Ma, S., Batkai, S., Pacher, P., Dobolyi, A., Murphy, A., Sleeman, M. W., & Usdin, T. B. (2008). Increased fear- and

- stress-related anxiety-like behavior in mice lacking tuberoinfundibular peptide of 39 residues. *Genes, Brain and Behavior*, 7(8), 933–942.
- Fernández, M., Mollinedo-Gajate, I., & Peñagarikano, O. (2018). Neural Circuits for Social Cognition: Implications for Autism. *Neuroscience*, 370, 148–162.
- (b) Keller, D., Láng, T., Cservenák, M., Puska, G., Barna, J., Csillag, V., Farkas, I., Zelena, D., Dóra, F., Küppers, S., Barteczko, L., Usdin, T. B., Palkovits, M., Hasan, M. T., Grinevich, V., & Dobolyi, A. (2022). A thalamo-preoptic pathway promotes social grooming in rodents. *Current Biology*, 1–14.
- (a) Keller, D., Tsuda, M. C., Usdin, T. B., & Dobolyi, Á. (2022). Behavioural actions of tuberoinfundibular peptide 39 (parathyroid hormone 2) – Enhanced Reader.pdf. *J Neuroendocrinol*, 34(9), e13130.
- Matsumoto, M., Kondo, S., Usdin, T. B., & Ueda, H. (2010). Parathyroid hormone 2 receptor is a functional marker of nociceptive myelinated fibers responsible for neuropathic pain. *J Neurochemistry*, 112(2), 521–530.
- Venuto, A., Smith, C. P., Cameron-pack, M., & Erickson, T. (2022). Alone in a crowd : effect of a nonfunctional lateral line on expression of the social hormone parathyroid hormone 2. *Biology Open*, 11(10), 1–9.
- Wang, L., Talwar, V., Osakada, T., Kuang, A., Guo, Z., Yamaguchi, T., & Lin, D. (2019). Hypothalamic Control of Conspecific Self-Defense. *Cell Reports*, 26(7), 1747-1758.e5.
- Wu, Z., Autry, A. E., Bergan, J. F., Watabe-Uchida, M., & Dulac, C. G. (2014). Galanin neurons in the medial preoptic area govern parental behaviour. *Nature*, 509(7500), 325–330.