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MASTERARBEIT | MASTER'S THESIS

Titel | Title

Effects of social Isolation on ZIF268 expression in selected striatal and limbic regions

verfasst von | submitted by

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angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien | Vienna, 2025

Studienkennzahl lt. Studienblatt | Degree programme code as it appears on the student record sheet:	UA 066 865
Studienrichtung lt. Studienblatt | Degree programme as it appears on the student record sheet:	Masterstudium Molecular Biology
Betreut von | Supervisor:	Dr. Daniela Pollak-Monje Quiroga

Acknowledgement

I would like to thank all those who supported and motivated me during my studies and during the preparation of this master thesis. First, I would like to thank Professor Daniela Pollak for being my first supervisor and giving me the opportunity to write my master thesis in her research group. I would also like to thank Dr. Anna Gundacker, who supervised me during my master's thesis and explained the methodology and implementation of the experiments to me. Special thanks go to my family, who supported me during a very challenging period in my life and motivated me to keep going in pursuit of my goals.

Abstract

The ability to recall past experiences and use them to influence our behaviour in the future is based on cognitive processes that enable our brain to form and save neural representations. These neural representations can be recalled and updated at will. These processes require mechanisms of neural plasticity triggered by cellular and molecular changes within the neurons that are activated during learning. The role of ZIF268, a transcription factor from the Early growth response protein gene family (EGR) that is involved in neural plasticity and memory formation, is relevant in this context. It is one of the ‘immediate early genes’ responsible for the induction of regulatory transcription factors in the cell, particularly in synaptic plasticity. It is assumed that the expression of ZIF268 occurs during synaptic activity, which is enhanced in response to physiological stimuli.

This study investigated the hypothesis that physiological stimuli and the associated triggering of ultrasonic vocalisations (USV) lead to specific activation of brain regions involved in social behaviour and stress responses. To this purpose, the expression of ZIF268 in mouse pups was examined in the hippocampus with the cornu ammonis 2 (CA2), hypothalamus, thalamus and corpus putamen. Seven-day-old mouse pups were separated from their mothers and placed individually in a soundproof box to record their ultrasonic vocalisations. The recordings were to classify the mice into vocalising and non-vocalising mice. This was used to illustrate differences in ZIF268 expression. After 3 minutes of recording the USVs, the brains of the young animals were removed and frozen. Cryostat brain sections 30 µm thick were prepared. These sections were then marked immunohistochemically using the indirect method and stained with 4' 6-diamidino-2-phenylindole-dihydrochloride (DAPI). The brain sections were photographed with a confocal microscope. The specific cells expressing ZIF268 were counted using the Fiji ImageJ programme. The results showed almost no difference between the two groups in the thalamus and hypothalamus, but differences were observed in the expression of ZIF268 forming cells in the cauda putamen and hippocampus regions of vocalising and non-vocalising mouse pups. The results can be explained by the social isolation of the mother and stress, but also by developmental plasticity processes. Furthermore, they point to the possible relevance of ZIF268 dysregulation for understanding neurological and psychiatric disorders.

Introduction

The subcortical, limbic and basal ganglia structures play a crucial role in processing behaviour, learning and emotions. Within these brain areas Important emotional homeostatic processes occur and form the basis for higher cognitive functions such as decision making, memory and motivated action (Foerde & Shohamy, 2011).

The hippocampus, hypothalamus, thalamus and CPU are nodal point in these networks. Especially the basal ganglia help with stimulus-response learning and habit formation, and they give reward related feedback through dopaminergic impulses, which are important for the selection of actions (Foerde & Shohamy, 2011). In parallel, the hippocampus, striatum, and prefrontal circuits function as a homeostatic network that integrates contextual, motivational, and cognitive signals to guide adaptive behaviour (Mizumori, 2013). This is a mechanism whose disruption is associated with psychiatric disorders (Sigurdsson & Duvarci, 2016).

This study investigates the expression of the transcription factor ZIF268 which is used as a molecular marker for neural activity and plasticity. ZIF268 is expressed in several brain regions, particularly in connection with learning processes, stress and social behaviour (Duclot & Kabbaj, 2017). The regional expression of ZIF268 facilitates the derivation of functional activations within the brain architecture and could provide new insights into the neurobiological basis of memory, habits and emotional responses (Balleine, 2007).

Brain area	System affiliation	Function	ZIF268 Expression
Hippocampus	Limbic system	Memory consolidation, spatial and episodic learning	High in LTP learning and memory consolidation
CA2- region	Part of the hippocampus	Social memory Context processing	Active in social learning and stress-dependent
Hypothalamus	Limbic system/diencephalon	Hormone control Stress response	Active during stress. Cortisol-induced expression
Thalamus	Subcortical integration centre	Attention Memory access	Increased expression after sensory stimulation
Caudate putamen	Basal ganglia	Habit formation, motor control, reward processing	Increases with dopaminergic stimuli, reward.

Figure 1. Overview of the brain regions examined.

ZIF 268 a transcription factor of the early growth response factor family

ZIF268 is a transcription factor of the early growth response factor family in mammals, it plays an important role in gene regulation during physiological and neurological processes (Duclot & Kabbaj, 2017).

Research indicates that the expression of ZIF268 is swiftly and temporarily elevated in response to diverse stimuli, including synaptic activity and stress (Khan, 2025; Duclot & Kabbaj, 2017). As a transcription activator the gene controls how strongly other target genes involved in different cellular processes, such as synaptic plasticity, cell growth, and cell survival, are expressed (Bozon, 2002) .Target genes for ZIF268 are diverse and have been found involved in many physiological and pathological processes, such as neuronal plasticity, learning and memory, and the development of different neurological diseases(Bozon, 2002). A diminished expression of ZIF268 has been associated with the development of several neurological disorders, such as depression and schizophrenia (Bristot, 2024).

ZIF268 is an immediate early gene of the MAPK/ERK signalling pathway (Ojea Ramos, 2022). This pathway plays a key role in the learning and memory process. Synaptic activity activates the MAPK/ERK cascade, which leads to the phosphorylation of CREB in the cell nucleus. Activated CREB recruits coactivators such as CBP/p300 and induces the expression of ZIF268, which regulates genes that are important for long-term potentiation, memory consolidation and synaptic plasticity (Ojea Ramos, 2022; Duclot & Kabbaj, 2017). Dysfunction of this pathway has consequences: it has been shown that blocking of ZIF268 in the hippocampus blocks long-term potentiation and the associated formation of long-term memory (Jones, 2001). In addition, another study showed that antidepressants effect the CREB/EGR1 by modulating ZIF268 expression. This improves the processing of emotions and neuronal plasticity (Slattery, 2005; Duclot & Kabbaj, 2017). These results showed that the MAPK/ERK signalling pathway provides a molecular basis for therapeutic treatments. This could be relevant for disorders such as depression and schizophrenia, which are characterised by reduced plasticity (Duclot & Kabbaj, 2017; Ojea Ramos, 2022).

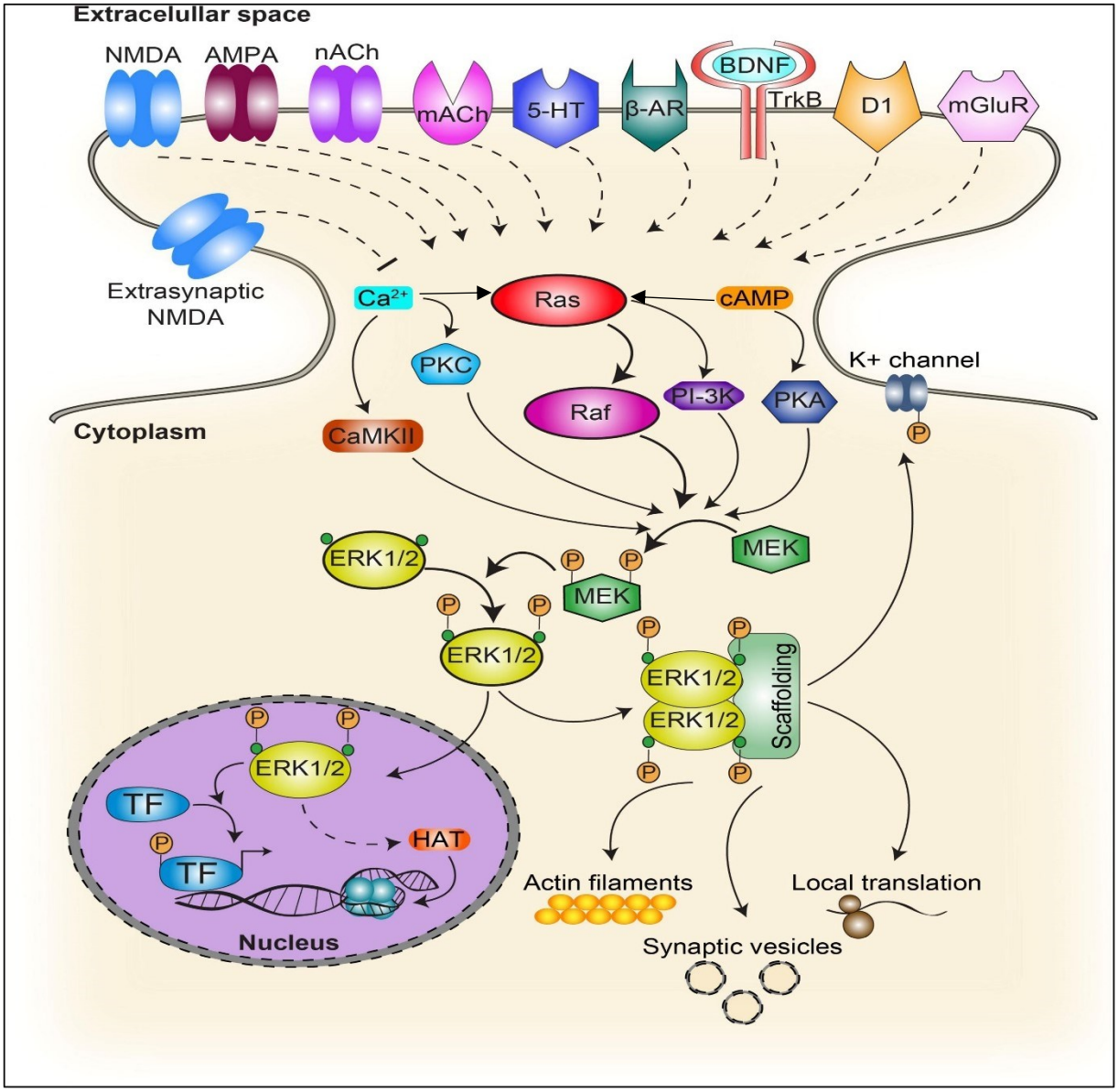

Figure 2. Signal pathway of MAPK/ERK, modified according to Ojea Ramos, (2022). Synaptic activation by neurotransmitters increases the intracellular concentration of Ca^{2+} and cAMP, which triggers the activation of Ras and the kinase cascade. Raf phosphorylates MEK, which activates ERK1/2. After phosphorylation, ERK1/2 translocate to the cell nucleus and phosphorylates transcription factors, including CREB. Activated CREB works with co-activators such as CBP/p300 and initiates the transcription of immediate early genes, including ZIF268. The induction of ZIF268 subsequently regulates genes that mediate synaptic plasticity, long-term potentiation, memory consolidation and adaptive behaviour (Ojea Ramos, 2022).

In summary, the ZIF268 gene is important for controlling gene expression of important processes in the brain in response to different stimuli. Additional research is essential to comprehensively explain the role of this gene in healthy brain function and in pathogenesis of neuronal and psychological disorders to identify the potential of therapeutic interventions (Duclot & Kabbaj, 2017).

The hippocampus and the CA2 region: consolidation of long-term memory

The hippocampus is part of the limbic system, which is important for the formation of long-term memory, especially when it comes to spatial, episodic and contextual learning (Bear, 2020). It is located in the medial temporal lobe and consists different areas, such as cornu ammonis with the substructures CA 1- 4, dentate gyrus and the Subiculum (Bear, 2020). These areas form a neural network that stores and processes information from the cortex (Bear, 2020; Alahmadi, 2023). The hippocampus is operationally involved in the structuring of temporal events, spatial orientation and the integration of experiences (Eichenbaum, 2001). The Papez circuit connects it to the thalamus, the hypothalamus and the limbic cortex, which connects emotions to learning motivations (Pessoa & Hof, 2015).

The CA2 region of the hippocampus is important for recognising people and for processing social information and receives increasing interest in research (Hitti & Siegelbaum, 2014). This region neurologically special because of its relative resistance to certain forms of long-term potentiation while responding to certain hormonal and neuromodulatory signals, such as vasopressin or stress hormones (Chafai, 2012). This underlines its importance for social interactions (Tzakis & Holahan, 2019).

After the developmental phase of learning spatial relationships, processing contexts and retrieving memories, ZIF268 is expressed more strongly in the hippocampus, particularly in the CA1 and CA3 areas coinciding with the late phase of long-term potentiation, a prerequisite for the formation of long-term memory (Jones, 2001). The activation of ZIF268 during and after the acquisition of social behaviour and related social skills in CA2 indicates an important role in the social memory network (Hitti & Siegelbaum, 2014).

The hypothalamus: control centre for physiological homeostasis

The hypothalamus is a major component of the diencephalon and is located below the thalamus. As the regulatory centre of the autonomic nervous system, it is responsible for maintaining physiological homeostasis (Bear, 2020). The hypothalamus regulates essential physically functions such as hunger, thirst, body temperature, sleep-wake cycles, reproductive behaviour and stress responses by processing neural and hormonal signals (Saper & Lowell, 2014).

It is connected to the pituitary gland and forms the hypothalamic-pituitary axis, which regulate the release of stress hormones. The hypothalamus is anatomically divided into several main groups(Herman, 2016).The paraventricular nucleus is very important because it sends neuroendocrine signals to the pituitary gland. The suprachiasmatic nucleus and other important nuclei control the circadian rhythm, while the lateral hypothalamus is associated with food intake and motivation (Swanson, 2000).

The hypothalamus has reciprocal connections with limbic structures such as the hippocampus, the amygdala and the ventral striatum. These connections enable the integration of emotional states, hormonal responses and behaviour. For example, an emotional stress stimulus (e.g., threat) can trigger activation of the amygdala which stimulus the Hypothalamus to initiate the Hypothalamic Pituitary Adrenal axis (HPA) and the release of stress hormones (Herman, 2016).

ZIF268 has been studied in the hippocampus and cortex in relation to learning and memory. Studies in the hypothalamus have shown an increase ZIF 268 expression after stress (Knapska & Kaczmarek, 2004). This suggests that ZIF268 acts as a molecular marker for stress induced neuronal activity.

The thalamus: the brain's information processing centre

The thalamus is a part of the diencephalon and is important for processing information in the brain (Bear, 2020). It serves as a central switching point and is the primary hub of sensory signals that are forwarded from the periphery to the cerebral cortex (Bear, 2020). Almost all sensory information is processed in the thalamus before it reaches the cortex, this includes visual, auditory, somatosensory, and proprioceptive stimuli (Bear, 2020). The thalamus filters signals selectively, which means that only essential information is shared, while other information is not further transmitted. This function is especially important for memory, consciousness, and attention (Sherman & Guillery, 2013).

The thalamus is also linked to the prefrontal cortex, the hippocampus, and the hypothalamus, which are all parts of the brain that control emotions and memory (Mitchell, 2015). Through these connections, it plays a role in controlling emotional, motivational, and cognitive processes, such as making decisions, sleeping, processing pain and learning (Mitchell, 2015). There are a few main compartments that can be distinguished according to their function. One of them is the anterior thalamic nucleus which is important for memory processes. It is linked to the hippocampus by the Papez circuit (Aggleton, 2011). This connection improves declarative memory, enabling consciously recall facts and events (Hamilton & Dalrymple-Alford, 2023).

A higher expression of ZIF268 has been observed in the thalamus after intense sensory stimulation or stress stimuli (Bristot, 2024).For example, a notable elevation in ZIF268 was detected in the thalamus after conditioned stimuli, indicating that these regions are actively involved in the processing and transmission of learning related information(Bristot, 2024). The correlation between ZIF268 expression and thalamic activity is increasingly being used to identify functional neural networks. ZIF268 expression in thalamus can be interpreted as a molecular indicator for the activation of memory and attention pathways (Hamilton & Dalrymple-Alford, 2023).

The caudate putamen: coordination of movement and motivation

The CPU is an important part of the basal ganglia, a group of subcortical nuclei that control movement and motivation. The caudate nucleus and the putamen are shaped differently and collectively form this structural entity, working together (Bear, 2020). This area receives information from the prefrontal, motor and limbic areas of the cortex. Simultaneously, the CPU nucleus sends signals back to the cortex via the pallidum and the thalamus, creating a cortico-striatal loop(Groenewegen, 1999). Through these circuits, the CPU is crucial for controlling arbitrarily movements, planning behaviour and selecting motor functions. (Graybiel, 2008). The CPU is not only responsible for motoric function but is also closely linked to reward processing and motivation driven behaviour. Particularly important is the dopaminergic projection from the substantia nigra pars compacta, which alters the function of the striatum (Sonne, Reddy, & Beato, 2024). This mechanism is active when a habit is learnt for example (Graybiel, 2008).

In the CPU, ZIF268 expression responds particularly strongly to dopaminergic stimuli. Studies have shown that ZIF268 mRNA levels increase significantly when animals display conditioned behaviour patterns(Hernandez, 2006). In the dorsolateral striatum, ZIF268 is more strongly activated during learning of habits and automatic behaviour (Maroteaux, 2014). This activation indicates that the molecular structure of synaptic connection changes, which is important for long-term alteration of behaviour. ZIF268 in the striatum shows that the brain is flexible when it comes to learning and behaviour (Maroteaux, 2014).

Hypothesis and Aims

The aim of this study was to investigate the neural correlates of ultrasonic vocalisations in seven days old mouse pups. It is hypothesised that the expression of ZIF268 is increased as an immediate early gene in synaptic activity triggered by physiological stimuli. The brain regions examined were the hippocampus including CA2, caudate putamen, thalamus and hypothalamus. ZIF268 was used as a marker of neural activity to investigate the relationship between vocal behaviour and neural activation. The hypothesis was that separation from the mother and the associated triggering of USVs would lead to specific activation of brain regions involved in social behaviour and stress responses.

Materials and Methods

C57BL/6N mice

For this study, C57BL/6N mice were obtained from Charles River (Sulzfield, Germany). The mice were paired. The males were housed individually, whereas females were kept in groups. During pregnancy, the females were shared cages in pairs, and shortly before birth of the offspring, they were separated from each other. Mice were bred and maintained under standard laboratory conditions with a room temperature of 22°C and a humidity of 22% in a 12-hour light-dark cycle with access to food and water. Each animal experiment was approved by the national ethics committee for animal welfare and animal use in Austria (2020-0.193.053-. Federal Ministry of Science and Research). All behavioural experiments were performed in accordance with the ARRIVE Guidelines and U.K. Animals (Scientific Procedures Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments). The experiments were carried out so that the suffering of the animals was minimized and the number of animals was kept to a minimum.

Timed mating

Before mating, female mice were maintained in groups to synchronize their oestrous cycle. To increase the likelihood of successful fertilization, the females were exposed to male mouse pheromones for 70 hours, by placing the females in the previously inhabited cage of a male. The males were kept in a new cage in the meantime. After 70 hours, the respective male was reintroduced into its cage with the preconditioned female for a 12-hour period overnight. Subsequently, the mice were separated again. The females were weighed every two days starting from E0.5 until E16.5 to confirm pregnancy.

Behavioural test (ultrasound vocalisation)

Ultrasonic vocalizations served as the qualitative indicator for stress stimuli. The mother and pups were placed in a room for an hour to acclimatize to the environment. Subsequently, the pups were separated from the mother and individually placed in a soundproof box to record their USV calls for three minutes. The recordings were conducted using the Observer XT program (Noldus Information Technology GmbH, Emmerich am Rhein). The recordings were used to distinguish between pups that vocalised, indicating stress and candidates for elevated ZIF268 levels, and those that did not (control group).

Molecular biology experiment

After completion of the behavioural experiment, the brains were collected after 90 min. The brains were then fixed in 4% Paraformaldehyde (PFA) for 24 hours at 4°C and stored in 30% saccharose at 4°C for 48 hours. The brains were then embedded in embedding medium (Tissue-Tek® O.C.T, Sakura Finetek), frozen, and stored at -80°C. The brains were sectioned into serial coronal free-floating 30 µm thick slices using a cryostat (Leica CM1950, Vienna).

Tissue preparation and immunostaining

The coronal brain sections were placed in 12 well plates that were previously filled with 1 ml of phosphate-buffered saline solution (PBS) (Thermo Fisher Scientific, Gibco). The sections were washed three times with 500 µl of PBS for 20 minutes. Then, the brain sections were washed with 500 µl of PBS + 0.3% Triton (Merck, Darmstadt, Germany) for 5 minutes. Subsequently, the tissue was incubated for 2 hours with a solution consisting of PBS with 0.1% Triton and 5% goat serum (Abcam, Cambridge, England) to block non-specific antibody binding sites. After 2 hours, the first antibody targeting EGR1 antigens (1:500 EGR1 /1577, Cell Signalling Technology, Danvers, USA) was applied in a 1:500 dilution in PBS with 0.1% Triton and 1% goat serum and incubated for 24 hours with gentle shaking at - 4°C. After 24 hours, the brain sections were washed three times with 500 µl of PBS for 20 minutes each. After washing, the

second antibody Alexa Fluor 647 (1:500 Alexa Fluor 647, Invitrogen, Waltham, USA) was added, diluted 1:500 in a solution of 1% goat serum and PBS with 0.1% Triton, which was incubated in darkness for 2 hours. Then, the sections were washed three times with PBS for 20 minutes each. Subsequently, DAPI (Sigma-Aldrich, St. Louis, Missouri) was added to the sections at a ratio of 1:2000 with PBS for 5 minutes. The sections were then mounted on slides. Images were captured with a Zeiss Axio Imager.Z2 fluorescence microscope (Zeiss, Jena, Germany) and confocal images for analysis were taken with an inverted Nikon Eclipse Ti microscope (Nikon, Tokyo, Japan).

Image analysis

Images were analysed descriptively and Fiji ImageJ was used for counting cells expressing ZIF268 in the brain areas. The threshold was manually set, and the field of analysis was manually determined. The images were taken at a 20X magnification. All recordings were taken to the same conditions to avoid any distortions.

Statistical analysis

Calculations were performed by using GraphPad PRISM and Microsoft Excel. All data was expressed as mean ± standard error of the mean. Statistical difference was determined via independent Students t-test with significance accepted at a confidence level set to 95% (p-value < 0.05).

Results

ZIF268 expression in CPU, hippocampus, thalamus and hypothalamus

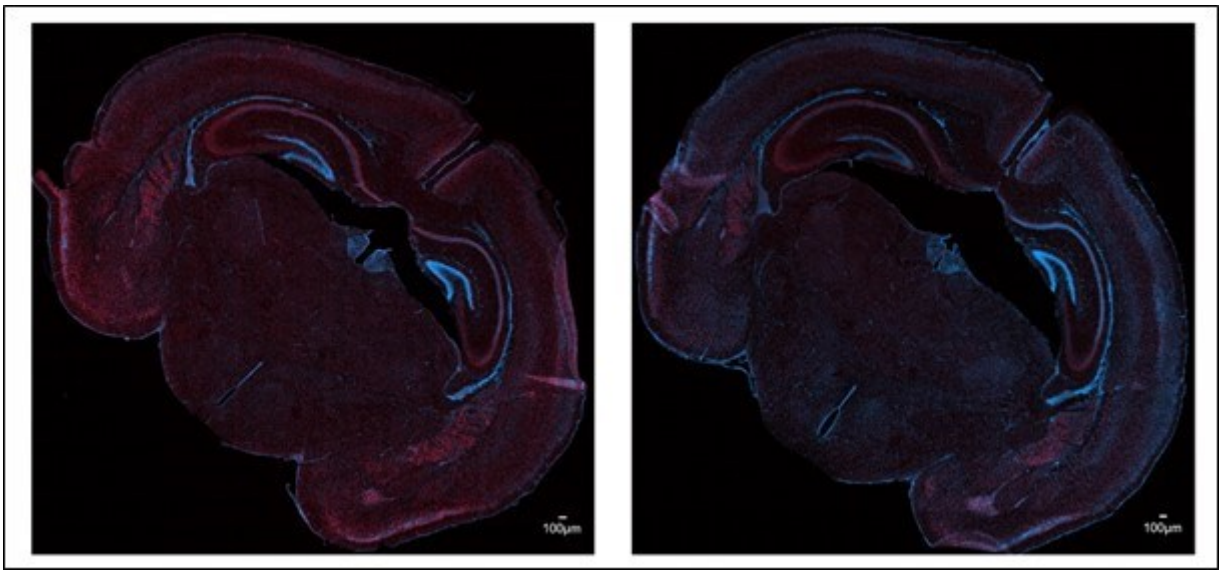

Figure 3. Fluorescence microscope images of coronary mouse brain sections showing ZIF268 expression (red). The left image shows a section from an animal in the vocalisation group, the right image from the non-vocalising control group. Cell nuclei are stained with DAPI. Increased expression can be seen in the CPU and hippocampus of the vocalising group.

Fluorescence microscopic image of coronary brain sections from seven days old mice. Figure 2 shows sections from the vocalisation group (left) and one from the control group (right). The subcortical and limbic brain regions are clearly visible in both sections. Cell density is visible through DAPI fluorescence. ZIF268 immunofluorescence shows differences in signal frequency between the groups. Increased ZIF268 immunofluorescence is observed in the CPU and hippocampus regions in the vocalising group. Regions with weak signal density are the thalamus and hypothalamus in both groups.

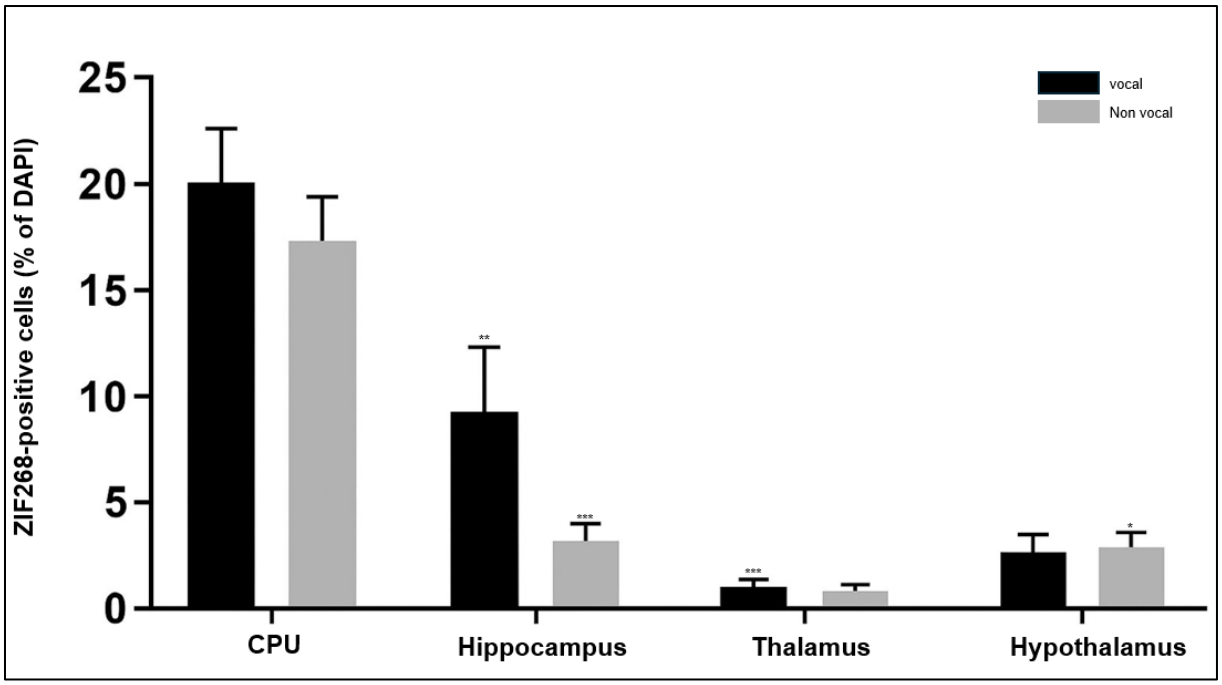

Figure 4. Comparison of ZIF268 expression between vocalising (n=7) and non-vocalising(n=7) seven days old mouse pups. Y-axis: percentage expression of ZIF268 relative to the number of DAPI-stained cell nuclei. The X-axis shows the brain areas examined. Black bars show the vocalising mouse pups, grey is the non-vocalising control group. Differences in ZIF268 expression can be seen in the CPU and hippocampus, with the vocalising groups showing higher values in the respective brain areas. No differences were found for the thalamus and hypothalamus. The groups were analysed using t-test [n.s. (not significant), *p < 0.05, **p < 0.01, ***p < 0.001].

The bar chart shows the results of the four brain areas examined and their ZIF268 expression in relation to DAPI staining. The vocalizing groups (black) were compared with the non-vocalizing control group (gray). The results in the CPU of the vocalizing group showed an average ZIF268/DAPI value of about 20 %, while the value in the control group was about 17 %. In the hippocampus, the expression of ZIF268-expressing cells for the vocalizing group was about 8 % to DAPI, compared to about 2 % in the control group. In the thalamus, the expression of ZIF268 relative to DAPI is lowest and about the same in both groups. In the hypothalamus, the two groups show comparable values.

ZIF268 expression in the CA2 region of the hippocampus

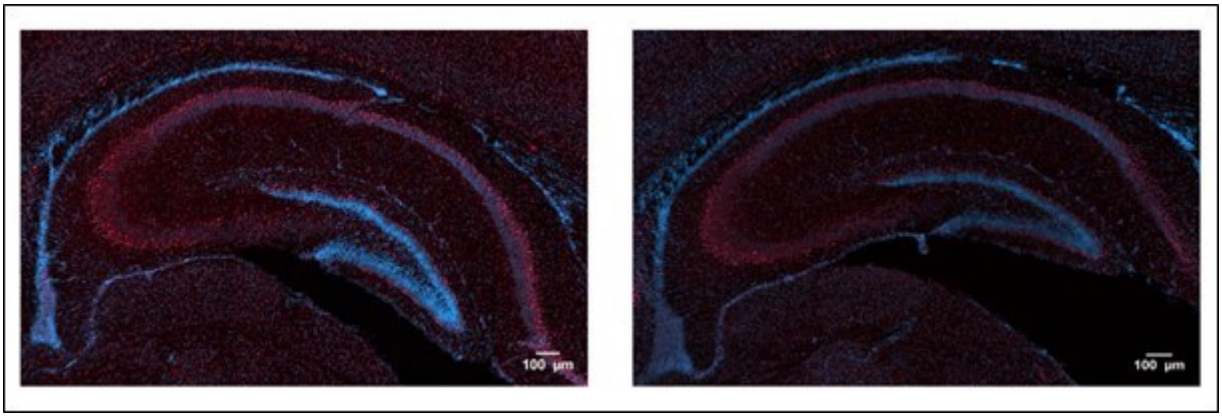

Figure 5. Fluorescence microscope image of CA2 sections from the cortex of seven days old mice. The images show two brain sections of parts of the hippocampus with a focus on the CA2 region. The left image shows the CA2 region of a vocalising mouse, the right image shows the CA2 region of a non-vocalising mouse. The ZIF268 signal intensity is higher in the CA2 region of the vocalising mouse than in the non-vocalising group.

The images show parts of the hippocampus with a focus on the CA2 region. The left image shows the CA2 region of a vocalising animal with increased ZIF268 expression. The right image shows the CA2 region of a non-vocalising mouse. In the right image, the CA2 region shows lower ZIF268 expression.

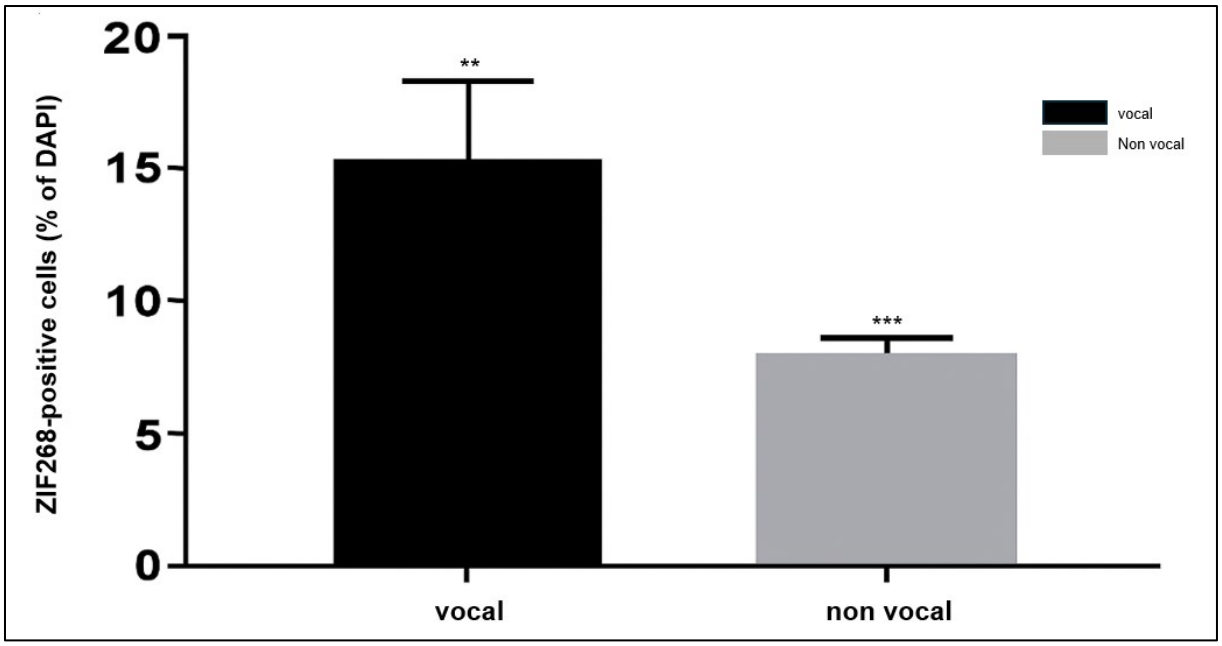

Figure 6. Comparison of ZIF268 expression between vocalising (n=7) and non-vocalising (n=7) 7-day-old mouse pups. Y-axis: percentage expression of ZIF268 relative to the number of DAPI-stained cell nuclei. The X-axis shows the CA2 region of the two groups. Black bars show the vocalising mouse pups, grey is the non-vocalising control group. Increased ZIF268 expression can be seen in the CA2 region of vocalising mouse pups. The groups were analysed using t-test [**$p < 0.01$, ***$p < 0.001$].

The bar charts show the expression of ZIF268 in relation to DAPI staining in the CA2 region of the hippocampus of mouse pups. The vocalising groups (black) were compared with the non-vocalising control group (grey). The CA2 region shows higher expression in vocalising mice, with approximately 15 %, This is more than twice as high as in non-vocalising animals, where it is around 7 %.

Discussion

This study examined the neural activation of 7 days old mouse pups after social isolation and the associated USVs. USVs are an important means of contact with the mother for the young animals. They are important for bonding with the mother and are therefore essential for survival. This behaviour is not purely reflexive, but also socially motivated. Group differences in ZIF268 expression therefore not only reflect general neural activation but also distinguish between behavioural types in the context of social communication. To record the neural activity, the expression of ZIF268, which is considered a marker of synaptic plasticity, was analysed. ZIF268 is upregulated in connection with learning processes, memory consolidation and the regulation of long-term potentiation (Duclot & Kabbaj, 2017). The focus was on the brain regions CPU, thalamus, hypothalamus and hippocampus with the CA2 region. These regions are involved in motor control, memory formation and emotional processing (Kamali, 2023). To validate the effect experimentally, the animals were isolated in a soundproof box for three minutes to trigger USVs. After isolation, the puppies' brains were removed, cut into 30 µm thick slices and immunostained. The analysed brain areas belong to a functional network of subcortical and limbic structures. These support sensory motor coordination, memory formation, emotional evaluation and social cognition(Kamali, 2023). The results show basic ZIF268 expression in all regions examined. However, differences between the groups were observed in the CPU, hippocampus and CA2. In the regions mentioned, higher ZIF268 expression was observed in the vocalisation group compared to the control group.

The CPU is involved in the planning, initiation and automation of movements (Graybiel, 2008). It integrates motor, motivational and cognitive signals and is essential for reward learning and choice of selection (Graybiel, 2008). In this study, vocalising mouse pups showed increased ZIF268 expression in the CPU compared to the non-vocalising control group. The higher activity in vocalising animals could indicate that this region is involved in the sensomotoric control of vocalisation, possibly in the sense of motor learning. This activation suggests that vocalisation at this age is not just a reflexive process but could be linked to conscious or at least motivationally guided action selection. This is supported by the fact that ZIF268 is considered a marker for

experience dependent gene activation (Guzowski, 2001). The results for the CPU region can be interpreted as follows: The stress caused by isolation stimulates the young animals to produce ultrasonic sounds, a communication signal that is important for survival. The CPU is active in initiating the relevant motor behaviour, which is selective and not purely reflexive (Graybiel, 2008). The associated high ZIF268 expression in the CPU signals neural activation and synaptic plasticity (Knapska & Kaczmarek, 2004).

In summary, isolation activates emotions and motivation and triggers a possible CPU controlled USV. This is associated with increased ZIF268 expression in the CPU. This sequence indicates that vocalisation is linked to motivated, complex motor behaviour and is not a pure reflex, but rather a learnable, motivation dependent process.

The hippocampus, a central element of the limbic system, plays a key role in memory consolidation, spatial orientation and the integration of contextual information (Bear, 2020). It is also relevant for stress management and the building of social memory (Bear, 2020). The vocalising animals showed a higher ZIF268 concentrations in the hippocampus than the non-vocalising group. This increased activity could be due to the processing of isolation.

As already mentioned, the CA2 region is located between CA1 and CA3.It is an independent anatomical and functional structure within the hippocampus. CA2 is essential for the formation and retrieval of social memory and contains a high density of vasopressin-1b receptors, which help modulate synaptic plasticity in response to social signals (Pagani, 2015; Hitti & Siegelbaum, 2014). In this study, vocalisation seems to reflect increased social stress, which may improve vasopressin binding, activate CA2, and trigger then ZIF268 expression. Altogether, these findings highlight CA2 as a hormone sensitive region that links social signals to memory processes during early life isolation.

The functional circuit can be represented as follows: isolation leads to emotional activation, which leads to vocalisation. This activates the hippocampus, especially the CA2 region, resulting in increased ZIF268 expression. The activation of ZIF268 could be a key mechanism that links emotional experiences, social interaction and memory formation. Figure 7 illustrates this functional cycle and showing the link between

isolation emotional and social activation and the ZIF268 expression and the long-term effects on social memory and motivation.

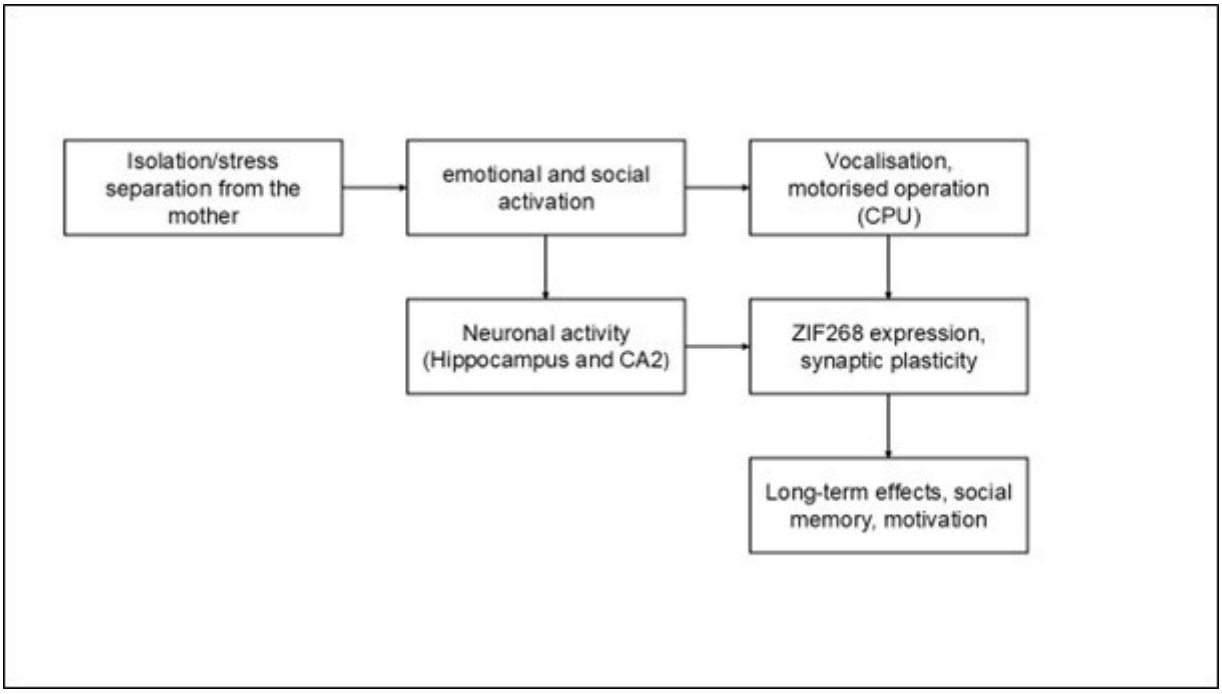

Figure 7. Schematic representation of the increase in ZIF268 expression after vocalisation in isolated mouse pups.1. Isolation/stress activates emotional and social centres such as the hypothalamus. 2. USVs are triggered, which are motorised via the CPU. 3. The hippocampus is activated in parallel, especially the CA2 region, which promotes social memory formation. 4. Increased ZIF268 expression occurs in these areas, resulting in synaptic plasticity. 5. In the long term, social motivation and memory traces can develop as a result.

The thalamus acts as a sensory relay centre that transmits information from the periphery to the cortical areas. (Bear, 2020). It is also involved in attention, emotional evaluation and memory consolidation, particularly via its mediodorsal and anteromedial nuclei (Kamali, 2023). In this study, no notable differences in the thalamus were found regarding to ZIF268 expression between the two groups. This indicates nonspecific activation through isolation and vocalisation. One possible interpretation would be that the thalamus, as a nonspecific ‘attention centre’, reacts to the changed environment (isolation) and is not activated by the communication behaviour of the young animals themselves.

The hypothalamus has a regulatory role on vegetative functions, it controls the regulation of hormones and emotions. It is closely linked to the HPA axis, which modulates stress responses (Herman, 2016). The measured ZIF268 expression showed no difference between the two groups. This could indicate a general activation of this region due to the isolation of the mice, regardless of vocalisation. It could also indicate a hormonal response to isolation, particularly in connection with the HPA axis. The small difference between the groups suggests that the main differences may be caused by stress and not by social communication processes.

The present results not only provide insights into the neural processing of social communication in pups. They could also be relevant for mental disorders such as schizophrenia, depression, anxiety disorders and autism spectrum disorders (Duclot & Kabbaj, 2017; Wöhr & Scattoni, 2013). These disorders are often associated with social and motivational deficits and reduced neural plasticity (Duclot & Kabbaj, 2017). The increased activity of ZIF268 observed here in the CPU, hippocampus, and CA2 region could be important for understanding these symptoms.

Ultrasonic vocalizations in mouse pups are socially important and trigger a response from the mother (Hofer, 2002). A reduced number or changed quality of these USVs has been documented in animal models for affective disorders (anxiety/depression), schizophrenia and especially autism spectrum disorder (Wöhr & Scattoni, 2013). These results also support the view that the MAPK/ERK signalling pathway provides a molecular approach for therapeutic treatments through its expression of early genes such as ZIF268. This pathway is important in psychiatric disorders such as depression and schizophrenia, which are associated with impaired neural plasticity (Duclot & Kabbaj, 2017; Ojea Ramos, 2022). The study results show that vocalizing in young animals have increased ZIF268 expression in the CPU and that early social communication is linked to neural activity and motor motivational control. The absence of this activation in animal models could indicate early motivational and social dysfunctions.

The CPU is also strongly associated with reward learning and choice of action. In schizophrenia, dysfunction of the basal ganglia is associated with amotivation and social passivity. The same pattern can also be seen in depression and anxiety disorders (Saleh, 2021).The hippocampus, especially the CA2 region, is crucial for social memory (Hitti & Siegelbaum, 2014). The increased ZIF268 expression in CA2 in vocal pups suggests that social signals induce synaptic plasticity in memory networks. Since dysfunction in the CA2 region could be discussed in autism spectrum disorders as well as in schizophrenia, the present results could be a relevant model for social dysfunctions. (Hitti & Siegelbaum, 2014; Pagani, 2015; Oliva., 2020). In addition, the results could indicate that molecular mechanisms such as ZIF268 regulation and comparable processes also play a role in depression and other affective disorders (Duclot & Kabbaj, 2017; Covington, 2010).

Since ZIF268 plays a role in the consolidation of long-term potentiation and memory processes, and since dysregulation of ZIF268 has been described in animal models of schizophrenia and depression, this dysregulation could be related to these disorders. (Jones, 2001; Knapska & Kaczmarek, 2004; Duclot & Kabbaj, 2017).

Outlook

In summary, this study suggests that the expression of ZIF268 in the CPU and hippocampus, with its CA2 region, could be used as a molecular indicator of social communication in the early development of mouse pups. Future work could investigate whether these mechanisms are changed in models of psychiatric disorders such as autism spectrum disorder, schizophrenia, and depression. Long-term studies could clarify whether early USV related neural activation has lasting effects on social memory and emotional regulation in adulthood. Furthermore, approaches targeting vasopressin signalling and ZIF268 regulation could provide valuable insights into potential intervention strategies for social and motivational dysfunction.

Abbreviations

USV…….Ultrasonic vocalizations
DAP……..4' 6-diamidino-2-phenylindole-dihydrochloride
EGR……Early growth response protein
PBS……Phosphate-buffered saline
PFA……..Paraformaldehyde
CA2……..Cornu Ammonis field 2
HPA……Hypothalamic Pituitary Adrenal

Table of figures

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Deutsches Abstract

Die Fähigkeit, vergangene Erfahrungen abzurufen und dass diese unsere Handlung in der Zukunft beeinflussen, basiert auf kognitiven Prozessen, die es unserem Gehirn ermöglichen, neuronale Repräsentationen zu bilden und zu speichern. Diese neuronalen Repräsentationen können nach Belieben abgerufen und aktualisiert werden. Diese Prozesse erfordern Mechanismen der neuronalen Plastizität, die durch zelluläre und molekulare Veränderungen innerhalb der Neuronen ausgelöst werden, die während des Lernens aktiviert werden. Die Rolle von ZIF268, ein Transkriptionsfaktor aus der EGR-Gen-Familie, der an der neuronalen Plastizität und Gedächtnisbildung beteiligt ist, ist in diesem Zusammenhang relevant. Es handelt sich um eines der „unmittelbaren Frühgene", die für die Induktion regulatorischer Transkriptionsfaktoren in der Zelle, insbesondere bei der synaptischen Plastizität verantwortlich sind. Es wird angenommen, dass die Expression von ZIF268 bei synaptischer Aktivität erfolgt, die in Reaktion auf physiologische Reize verstärkt wird. In dieser Arbeit wurde die Hypothese untersucht, wie sich ein physiologischer Reiz und die damit verbundene Auslösung von USVs zu einer spezifischen Aktivierung von Gehirnregionen führt, die an sozialem Verhalten und Stressreaktionen beteiligt sind. Dazu wurde die Expression von ZIF268 bei Mäusewelpen in den Regionen Hippocampus mit der CA2, Hypothalamus, Thalamus und Corpus Putamen untersucht. Es wurden sieben Tage alte Mäusewelpen von ihrer Mutter getrennt und einzeln in eine schalldichte Box gesetzt, um ihre Ultraschallvokalisation aufzuzeichnen. Die Aufzeichnungen wurden verwendet, um die Mäuse zu klassifizieren in Vokalisierende Mäuse und nicht Vokalisierende. Das diente dazu Unterschiede in der ZIF267-Expression zu veranschaulichen. Nach 3 Minuten Aufzeichnung der USVs wurden die Gehirne der Jungtiere entnommen und eingefroren. Es wurden 30 µm dicke Kryostat-Hirnschnitte angefertigt. Diese Schnitte wurden dann immunhistochemisch mit der indirekten Methode markiert und mit DAPI gefärbt. Die Hirnschnitte wurden mit einem Konfokal Mikroskop fotografiert. Die spezifischen Zellen, die ZIF268 exprimierten, wurden mit dem Programm Fiji ImageJ gezählt. Die Ergebnisse zeigten kaum Unterschiede in den Bereichen Thalamus und Hypothalamus zwischen den beiden Gruppen, jedoch wurden Unterschiede in der Expression von ZIF268-bildenden Zellen in den Regionen Cauda Putamen und

Hippocampus der vokalisierenden und nicht vokalisierenden Mäusewelpen beobachtet. Die Ergebnisse können durch die soziale Isolation der Mutter und stress erklärt werden, aber auch durch entwicklungsbedingte Plastizität Prozesse. Darüber hinaus weisen sie auf eine mögliche Relevanz von ZIF268 Dysregulation für das Verständnis neurologischer und psychiatrischer Erkrankungen hin.