RESEARCH SUMMARY

Background and aims

Aggression-related psychopathologies have consistently been associated with a history of maltreatment in early life (Carr et al., 2013**). Neglect is the most prevalent subtype of child maltreatment** and often receives less attention even though its negative consequences are comparable to, if not more detrimental than those of sexual or physical abuse (Gilbert et al., 2009). Considering the individual and societal burden of aggression-related disorders and violent crimes stemming from adversities experienced in childhood, the **identification of neurobiological changes induced by early-life social neglect** remains a crucial issue.

In line with human findings, our prior research modelling child neglect shows that post-weaning social isolation (PWSI) until adulthood in rats induces abnormal aggression and disturbances in social behavior (Toth et al., 2008, 2011, 2012) as well as structural changes in brain areas relevant for aggression control, especially the prefrontal cortex that is known for its enhanced sensitivity to environmental perturbations due to prolonged maturation processes (Biro et al., 2017). The aim of the current study was to delineate mechanisms through which long-term effects of early adversities on brain function and behaviour exerted.

We proposed to investigate i) brain **plasticity-related** and **epigenetic mechanisms** and ii) **serotonergic system-related mechanisms mediating the effects of early social stressors on adult aggressiveness**. This study is justified by the role of early adversities in engendering adult aggressiveness on one side (Turgay, 2004; Kempes et al., 2005; Crowe and Blair, 2008) and by the role of plasticity-related and epigenetic mechanisms and the serotonergic system in programming brain development and aggressiveness on the other side (Veenema, 2012; Champagne, 2018; Provencal et al., 2015; Larsen and Luna, 2018). Albeit the importance of plasticity and epigenetic mechanisms in aggression is widely recognized (Tremblay, 2018; Tremblay et al., 2018), the phenomenon was rather poorly studied earlier.

Results

1. Behavioral effects of post-weaning social isolation

We have extended our knowledge regarding PWSI-induced long-term behavioural effects. We confirmed that post-weaning social isolation (PWSI – a laboratory model of childhood neglect in humans) results in **abnormal forms of aggression** in rats, characterized by more frequent and more violent biting attacks that often target the more vulnerable body parts of the opponents (head, throat and belly) (data not shown). Moreover, as mice present a large selection of transgenic lines, offering an important toolkit for studies on brain mechanisms, we also aimed to characterize long-term behavioural effects of PWSI in mice. Our results showed that PWSI, but not isolation in adulthood causes robust disturbances in social behaviour of male CD1 mice as well, with a marked increase in defensive behaviours and the emergence of abnormal aggression, as characterized by abnormal attack patterns (significantly more frequent and violent bites that often target vulnerable body parts). Abnormal forms of aggression were accompanied by reduced social exploration and disorganized, fragmented behavioural patterns (**Fig.1.**, Biro, Miskolczi et al., under review). PWSI also induced social deficits in the social interaction test, and significant but milder alterations in emotional domains including reduced sucrose preference, reduced motivation in operant learning and delay discounting tasks (data not shown).

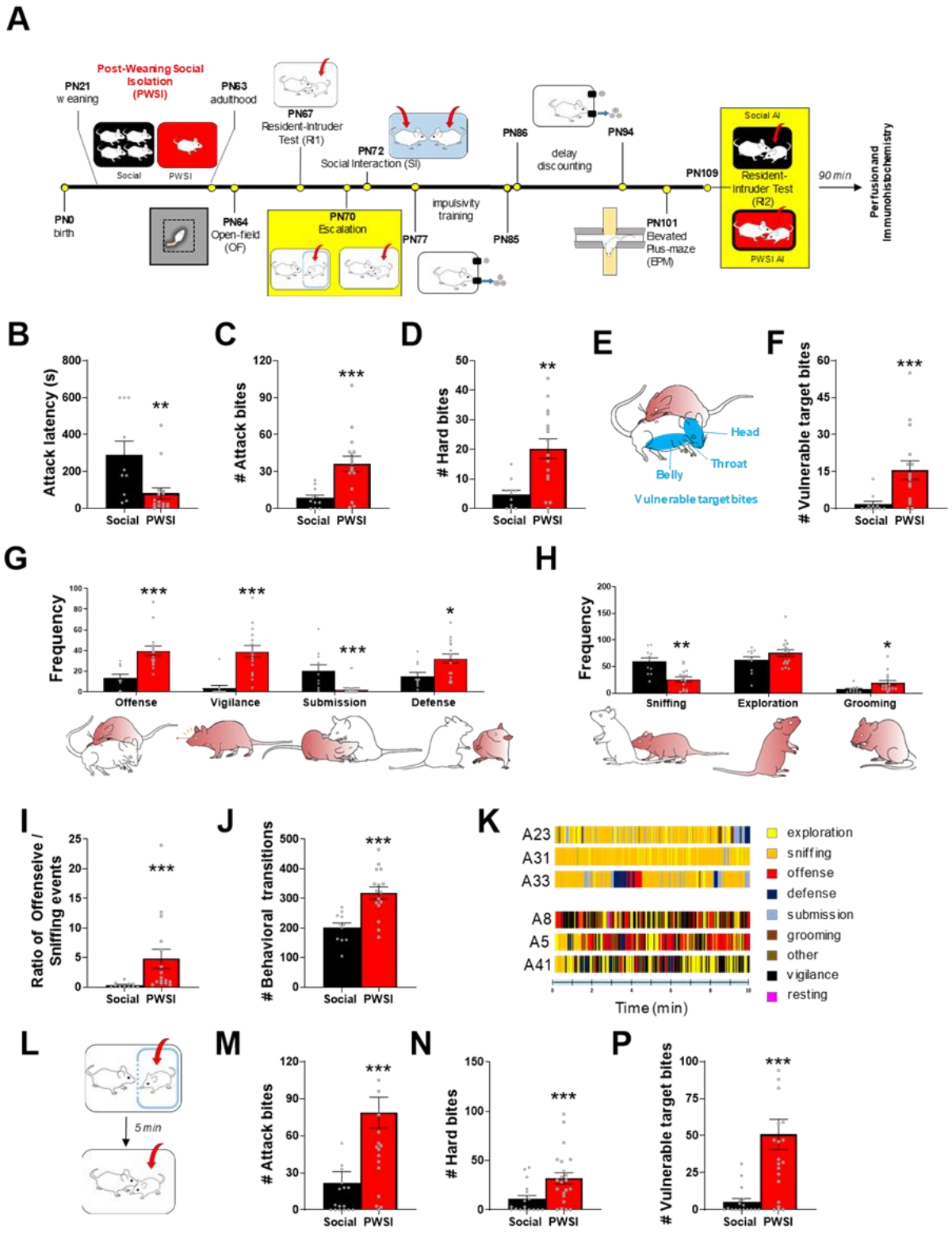

Fig. 1. Post-weaning social isolation (PWSI) induced abnormal aggression in conjunction with behavioral fragmentation. ***A***, Experimental design depicting rearing conditions and behavioral test battery starting in adulthood at PN64. Following weaning, mice were housed individually (PWSI) for 6 weeks or were housed in groups of four (SOC). Yellow shading indicates the resident-intruder test at P109 from which data are shown in ***B-K***. PN, postnatal day. ***B-D***, PWSI mice displayed shorter attack latencies, increased number of attack bites and hard bites compared to SOC mice in the resident-intruder test. ***E-F***, Schematic drawing depicting the vulnerable body parts (head, throat, belly) which were targeted preferentially by PWSI mice. ***G***, Frequency of agonistic behaviors recorded during the resident-intruder test. ***H***, Frequency of non-agonistic behaviors recorded during the resident-intruder test. ***I***, Ratio of offensive/sniffing events in the resident-intruder test. ***J***, Behavioral fragmentation indexed by the number of behavioral transitions in the resident-intruder test. ***K***, Representative temporal raster plots showing the fragmented behavioral organization of three SOC (top panels) and three PWSI (bottom panels) mice. ***L***, Schematic drawing of the social escalation procedure and the subsequent resident-intruder test at P70. ***M-O,*** Escalation procedure further shortened attack latencies and increased the overall and vulnerable area-targeting attack bites in PWSI mice. All data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001

2. Altered network operations in the prefrontal cortex following early adversities

Childhood and the adolescent period represent time windows during which brain regions modulating social behavior undergo major network reorganization. Adverse social experience during these sensitive periods can lead to disrupted maturation of such brain regions, especially the prefrontal cortex, known for its extended developmental trajectory. The PFC plays a primary role in the regulation of aggressive behavior (Siegel et al., 2007; Takahashi et al., 2014; Biro et al., 2018; Mikics et al., 2018; van Heukelum et al., 2019a, Halász et al., 2006) but its function appears to be modulated PWSI (Biro et al., 2017). However, it is unclear which prefrontal subregions are involved in these changes and how PWSI alters neuronal co-activation patterns across the highly-interconnected subnetworks. Therefore, we investigated **neuronal activation of PFC subregions using c-Fos mapping** in PWSI and socially reared (SOC) mice under baseline conditions and after aggressive interaction. According to our results (**Fig.2.,** Biro, Miskolczi et al, under review), at the network level, along with previous findings in rats and humans, we found that aggressive behavior is associated with increased neuronal activation of the PFC in SOC mice (Toth et al., 2012; Biro et al., 2017; Cupaioli et al., 2021). Additionally, in PWSI mice we observed even greater densities of active neurons following aggressive interaction, which were specific to medial prefrontal cortical (mPFC) subregions, as no such changes were found in the orbitofrontal cortex (OFC). Based on the marked behavioral phenotype induced by PWSI and on studies reporting that the PFC exhibits high interconnectivity between its various subregions (Ährlund-Richter et al., 2019), here we also aimed to assess **functional connectivity patterns across PFC subregions**. Using our c-Fos dataset, we calculated correlational matrices between prefrontal subregions to decipher interactions across the mPFC and observed aggressive interaction-specific correlation patterns, implicating the existence of finely balanced network operations between prefrontal subregions during normal agonistic interactions. In contrast, in the PFC of PWSI mice, we found that neuronal activation across all subregions correlated with each other. It is a plausible explanation that mPFC-specific neuronal hyperactivation heavily impacts brain-wide network operations, thus contributing to the disrupted co-activation profile seen in PWSI mice.

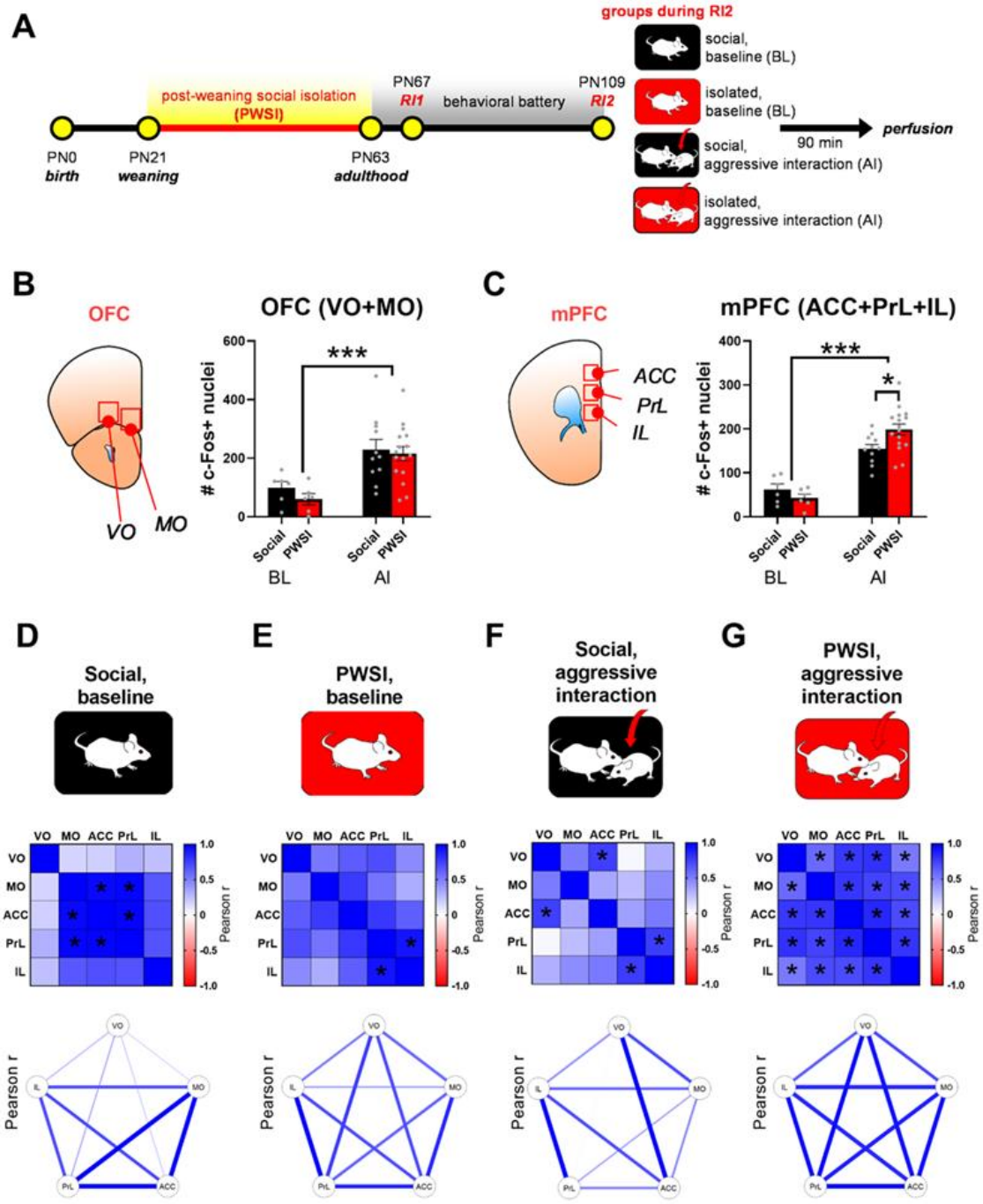

Fig. 2. PWSI-induced abnormal aggression is associated with subregion-specific neuronal hyperactivation in the mPFC and disrupted co-activation patterns across mPFC and OFC subregions. ***A***, Experimental design. Upon weaning, mice were housed individually (PWSI) or were housed in groups of four (SOC). After reaching adulthood mice were subjected to a behavioral test battery. On PN109, mice were perfused under baseline conditions (BL) or 90 minutes after the aggressive interaction (AI) followed by c-Fos immunostaining to investigate the impact of PWSI on the neuronal activation of the PFC. ***B-C***, *Aggressive interaction induced an mPFC-specific enhanced c-Fos expression in PWSI mice compared to SOC mice.* Panels depict the Bregma levels of the investigated orbitofrontal (OFC) and medial prefrontal (mPFC) subregions. ***B***, Bar graphs showing the number of c-Fos immuno-positive nuclei within the subregions of the OFC, the ventral orbitofrontal (VO) and medial orbitofrontal cortices (MO). ***C***, Bar graphs showing the number of c-Fos immuno-positive nuclei within the subregions of the mPFC, the anterior cingulate (ACC), the prelimbic (PrL) and the infralimbic cortices (IL) ***D-G***, *PWSI induced disrupted functional connectivity across PFC subregions.* Cross-correlation and network connectivity analysis of c-Fos expression in experimental groups (upper row), computed as covariance across subjects to reveal interactions between PFC subregions. The middle row represents the complete set of interregional correlations in each experimental group, whereas the lower row depicts node graphs where the nodes represent brain regions and the width of connections between nodes indicate the higher correlations coefficients. Colors indicate Pearson correlation coefficients (scale, right) and labels within squares correspond to P values of correlations. All data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001. ACC, anterior cingulate cortex; AI, aggressive interaction; RI2, second resident-intruder test; BL, baseline condition; IL, infralimbic cortex; MO, medial orbitofrontal cortex, mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; PN, postnatal day; PrL, prelimbic cortex, VO, ventral orbitofrontal cortex.

3. Altered inhibitory function in the mPFC contributes to behavioral deficits following early adversities.

Experience-dependent development of the prefrontal cortex during sensitive early periods is tied to the **refinement of inhibition via the maturation of parvalbumin-expressing interneurons** (PV+) and the **appearance of perineuronal nets** (PNNs) (Hensch, 2004). PV+ neurons are the most abundant interneuron-subtype in the neocortex and play a key role in maintaining excitatory/inhibitory (E/I) balance and neuronal plasticity (Tremblay et al., 2016). PNNs are

specialized extracellular matrix components that enwrap the perisomatic region of a great portion of PV+ neurons and are involved in the stabilization of synapses, restricting plasticity and promoting the closure of experience-dependent development (Dityatev et al., 2010). In our study, we aimed to elucidate how different PV+ interneuron populations, i.e. those without (PV+PNN-) or those enwrapped by PNNs (PV+PNN+) influence PFC network function and behavior in individuals undergoing early social adversities.

3.1. PWSI induces enhanced parvalbumin and perineural net intensities in the mPFC

Analyses of the mPFC and OFC subregions revealed that the **density of different PV+ neuronal populations** and the ratio of PV+PNN+ and PV+PNN- neurons shows **large subregion-specific differences**, but the densities are not affected by PWSI (data not shown). As exposure to adverse experiences has been linked to aberrant PV soma and PNN intensities in the PFC (Ueno et al., 2017b; Spijker et al., 2020), using high resolution confocal microscopy, we also investigated how PWSI affects PV soma and PNN intensities of PV+ neurons and PV+PNN+ neurons, respectively. We observed that OFC and mPFC PV+ neurons in **PWSI mice had significantly greater PV soma intensity** than SOC mice. Intriguingly, this neuronal population also showed **greater PNN intensity** in the mPFC but not OFC of PWSI mice (**Fig.3.**, Biro, Miskolczi et al., under review).

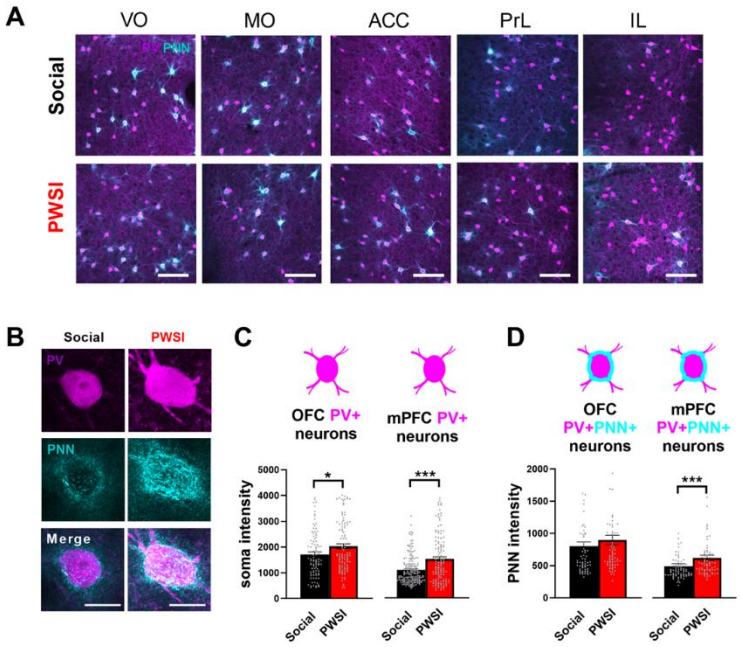

Fig. 3. PWSI increased PV and PNN intensities in the mPFC. ***A,*** Representative confocal maximal intensity projection images showing subregion-specific differences in the density of neurons co-labeled with PV (magenta) and WFA, a plant-based lectin stain that is commonly used to label perineuronal nets (PNN, cyan) in the PFC subregions from SOC and PWSI mice, respectively. Density of PV+PNN+ neurons increased in ACC and PrL compared to the ventrally located IL. Scale bar, 150 µm. ***B***, Representative high resolution confocal images of PV+PNN+ neurons in the mPFC showing increased PV and PNN intensities in PWSI mice compared to SOC mice. Scale bar, 20 µm. ***C-D,*** *Subregion-specific differences in the density of PV+ neuronal populations.* ***C,*** Graphs showing PV soma intensities of PV neurons (OFC, n = 92 neurons from 4 SOC mice and n = 106 neurons from 4 PWSI mice; mPFC, n = 176 neurons from 4 SOC mice and n = 127 neurons from 4 PWSI mice). ***D***, Graphs showing the PNN intensities of PV+PNN- neurons (OFC, n = 63 neurons from 4 SOC mice and n = 68 neurons from 4 PWSI mice; mPFC, n = 75 neurons from 4 SOC mice and n = 70 neurons from 4 PWSI mice). All data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001. ACC, anterior cingulate cortex; IL, infralimbic cortex; MO, medial orbitofrontal cortex, mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; PNN, perineuronal net, PrL, prelimbic cortex, PV, parvalbumin; VO, ventral orbitofrontal cortex.

3.2. PWSI-induced abnormal aggression correlates with mPFC-specific heightened activity of PV+PNN+ neurons

We also found that upon aggressive interaction **PV+ neurons showed marked c-Fos activation** in both the OFC and the mPFC in both SOC and PWSI mice, possibly reflecting a general increase in network activity and thus an elevation in the recruitment of PV+ neurons (**Fig. 4.,** Biro, Miskolczi et al., under review). Interestingly, we observed that fighting evoked a further elevated density of activated PV+ neurons in the mPFC of PWSI mice compared to SOC mice, lending additional support for the argument that **maladaptive developmental programming of PV+ neurons might contribute to social behavioral deficits in adulthood**.

Moreover, we uncovered an **increase in the density of activated PV+PNN+**, but not PV+PNN- neurons **in the mPFC of abnormally aggressive PWSI mice**, indicating that PV+PNN+ neurons might be more deeply embedded in the mPFC microcircuitry and thus play a pronounced role in circuit operations related to agonistic behaviors. Indeed, in PWSI mice the **activity of PV+PNN+ neurons negatively correlated with hallmark features of aggressive behavior**, while in SOC mice, prefrontal PV+ neuronal activity – mostly involving the PV+PNN- population – positively correlates with non-aggressive sniffing behavior (**Fig. 4.**). This suggests that different PV+ subpopulations may adjust individual behavioral responses during social challenges. Noteworthy, in PWSI mice, these changes were specific to the mPFC, proposing that early-life adversity results in region-specific alterations within frontal cortical regions. The overall

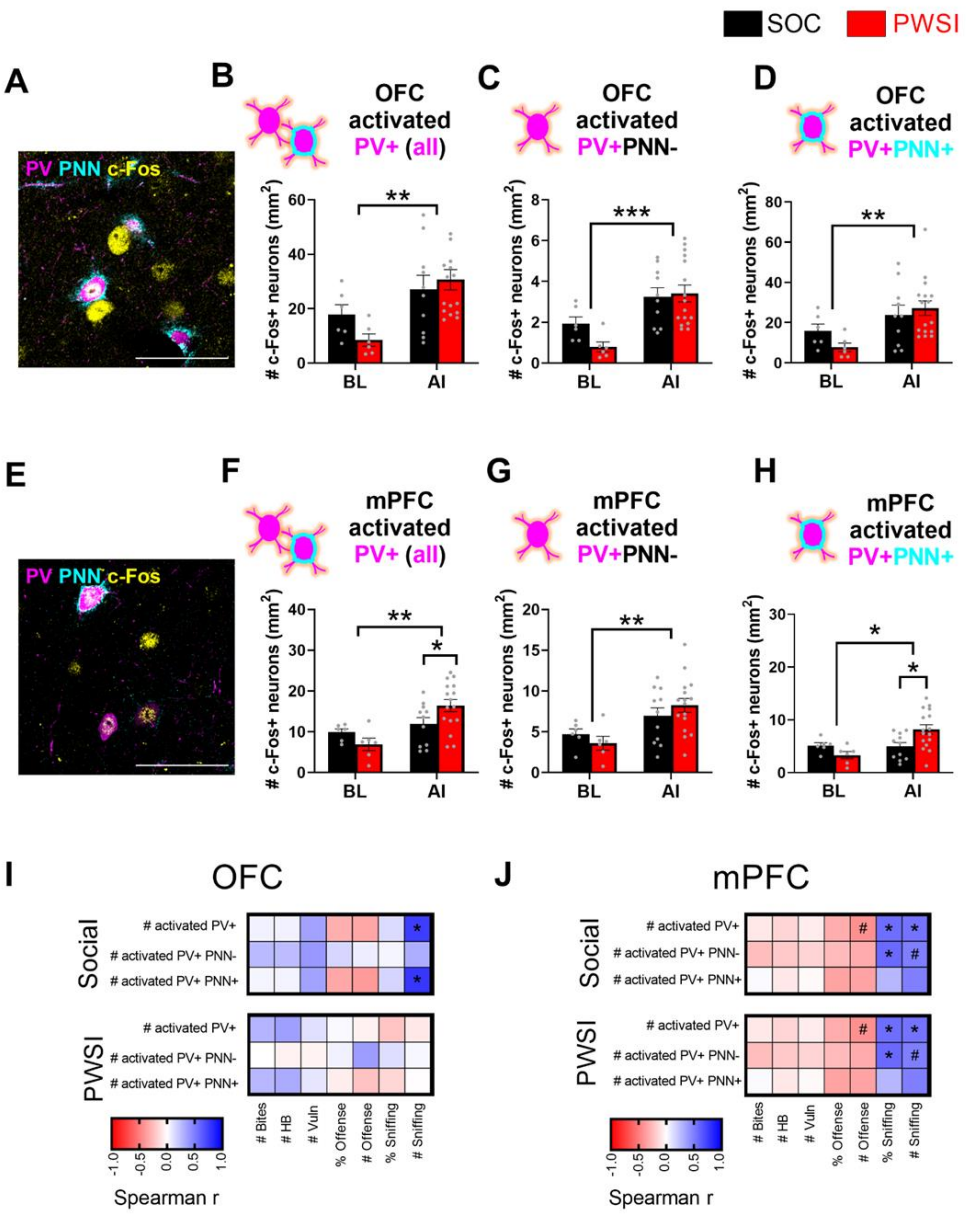

Fig. 4. PWSI-induced abnormal aggression correlates with mPFC-specific heightened activity of PV+PNN+ neurons. ***A-D***, *Aggressive interaction increased the activity of PV+ neurons in the OFC.* ***A***, Representative single plane confocal image showing activated (c-Fos-immunpositive, yellow) PV neurons (magenta) surrounded by PNNs (stained with WFA, cyan) from the OFC of a PWSI mouse. ***B***, Aggressive interaction (AI) increased the number of activated PV+ neurons compared to baseline, i.e. resting controls (BL). Graphs show the density of activated PV neurons (PV+cFos+ double immunopositive) in the OFC. Aggressive interaction increased the activation of both PV+PNN- (***C***) and PV+PNN+ (***D***) neuronal populations in the OFC of SOC and PWSI mice. ***E-H***, *PWSI induced hyperactivation of PV+ neurons in the mPFC following aggressive interaction.* ***E***, Representative single plane confocal image showing activated (c-Fos+, yellow) PV neurons (PV+, magenta) with PNN (stained with WFA, cyan) and without PNN from the mPFC of a PWSI mouse. ***F***, Aggressive interaction-specific significant increase in the density of activated PV+ neurons (PV+cFos+, double immunopositive) in the mPFC of PWSI mice compared to SOC mice. The density of activated PV+PNN- neurons (***G***) and PV+PNN+ neurons (***H***) in the mPFC revealed a PWSI-specific hyperactivation of PV+PNN+ neurons. ***I-J***, *PWSI altered correlations between behavioral variables during aggressive interaction and activation patterns of distinct PV+ neuronal populations in the PFC.* ***I***, Spearman correlation matrices for SOC mice (upper panel) and PWSI mice (lower panel) showing relationships between OFC PV+ neuron activation and behavior during AI. ***J***, Spearman correlation matrices for SOC mice (upper panel) and PWSI mice (lower panel) showing relationships between mPFC PV+ neuron activation and behavior during AI. Each square shows the Spearman's correlation coefficient as calculated by correlating mean densities of activated PV+ neurons, PV+PNN- neurons or PV+PNN+ neurons with hallmark aggression features shown in Fig. 1. Colors indicate Spearman correlation coefficients, and labels within squares correspond to P values of correlations. All data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.005, #p<0.06. AI, aggressive interaction; BL, baseline i.e. resting conditions; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; PV, parvalbumin; PV+PNN-, parvalbumin neurons without perineuronal nets; PV+PNN+, parvalbumin neurons with perineuronal nets.

activity changes in PV+ neuronal populations suggest a **dysregulated inhibitory tone within the mPFC**, which might contribute to the observed network disturbances and neuronal hyperactivation.

3.3. PWSI increased the density of excitatory cortical and subcortical boutons impinging on the perisomatic region of PV+ neurons

We detected more Bassoon immunoreactive (Bassoon+, presynaptic release site marker) puncta at the perisomatic region of PV+PNN+ neurons versus PV+PNN- neurons (**Fig. 5.,** Biro, Miskolczi et al., under review). Noteworthy, independently of PNN coverage, PWSI caused an overall increase in the density of Bassoon+ puncta surrounding the somata of PV+ neurons. We also observed a strong positive correlation between PNN fluorescence intensity and perisomatic Bassoon+ fluorescence intensity (**Fig. 5.).** These findings corroborate and extend previous findings from the visual cortex and the hippocampus indicating that **PNNs play an essential role in the organization and stability**

of the synaptic circuitry (Favuzzi et al., 2017; Lensjø et al., 2017b; Carceller et al., 2020, Dityatev et al., 2010).

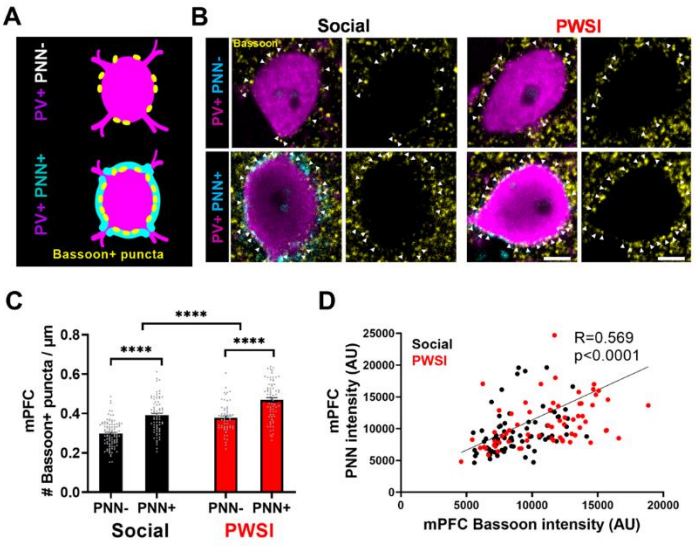

Fig. 5. PWSI increased the density of synaptic inputs opposed to the perisomatic region of PV+ neurons in the mPFC. ***A,*** Schematic drawings depicting parvalbumin neurons (PV+, magenta) without perineuronal net (PNN) or with PNN (cyan, PNN+), targeted by Bassoon presynaptic release site marker immunoreactive puncta (yellow). ***B***, Representative single plane confocal images showing immunoreactivity for PV (magenta) and Basson (yellow) as well as Wisteria floribunda agglutinin staining for PNNs (cyan) for a PNN- (upper panel) and a PNN+ (bottom panel) PV+ neuron within the mPFC of SOC and PWSI mice, respectively. White arrowheads indicate close appositions of Bassoon+ puncta surrounding the perisomatic region of PV+PNN- (top panel) and PV+PNN+ (bottom panel) neurons from the mPFC of SOC and PWSI mice. Scale bar, 4 µm. ***C***, Bar graphs showing that PV+PNN+ neurons receive more Bassoon+ puncta densities at their perisomatic region compared to PV+PNN- neurons and PWSI caused an overall increase in the density of Bassoon+ puncta at the perisomatic region of PV+ neurons (n = 101 PNN- neurons and n = 75 PNN+ neurons from 4 SOC mice; n = 57 PNN- neurons and n = 70 PNN+ neurons from 4 PWSI mice). ***D***, Scatter plots with regression lines represent a significant positive correlation of the perisomatic PNN intensity and perisomatic Bassoon intensity of PV+ neurons. The inset shows Spearman's correlation coefficient (R). All data are represented as mean ± SEM. ****p < 0.0001. mPFC, medial prefrontal cortex.

PV+ neuronal function highly depends on their synaptic inputs. PV+ neurons receive by far the most excitatory glutamatergic inputs compared to other interneuron populations (Gulyas et al. 1999) and PV+ neuron-specific changes in glutamatergic neurotransmission are implicated in neurodevelopmental disorders (Rotaru et al., 2012) and in aggression-regulation (Mikics et al., 2018; Newman et al., 2018; Guyon et al., 2021). To investigate how PWSI may affect the synaptic inputs of PV neuronal subpopulations in the PFC, we compared the density of perisomatic puncta expressing different synaptic markers labeling GABAergic inhibitory synapses (vGAT+), glutamatergic cortical (vGluT1+) and glutamatergic extra-cortical (vGluT2+) synapses, respectively. We found that **PWSI increased the density of both vGluT1+ and vGluT2+ inputs targeting the somata of mPFC PV+ neurons** (**Fig. 6.**, Biro, Miskolczi et al., under review), while vGAT+ inputs remained unaffected**. PV+PNN+ neurons received significantly higher densities** of both vGluT1+ and vGluT2+ inputs compared to PV+PNN- neurons (data not shown). Based on this one could argue that **abnormal aggressive behavior coupled with higher overall mPFC activity involved the recruitment of PV+PNN+ neurons, which was exacerbated by an enhanced excitatory drive emanating from both cortical and subcortical sources**, respectively.

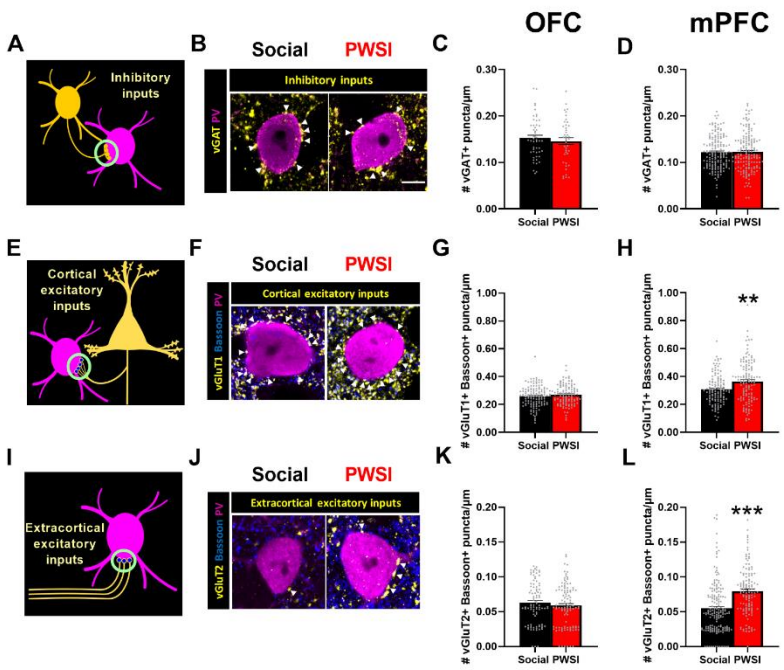

Fig. 6. PWSI increased the density of excitatory cortical (vGluT1+) and subcortical (vGluT2+) inputs impinging on the perisomatic region of PV+ neurons in the mPFC. ***A,*** Schematic drawing showing a local inhibitory neuron (yellow) targeting the perisomatic region of a PV+ neuron (magenta). Yellow circles indicate the vesicular GABA transporter (vGAT+) immunoreactive synapses analyzed in this experiment. ***B,*** Representative single plane confocal images showing close appositions of vGAT+ puncta (white arrowheads) surrounding a PV+ neuron (magenta) from the mPFC of SOC and PWSI mice. Scale bar, 5 µm. ***C-D*** *PWSI did not affect the density of inhibitory inputs targeting the perisomatic region of PV+ neurons.* Bar graphs showing the density of vGAT+ puncta targeting the perisomatic region of PV+ neurons from the OFC (**C**) and mPFC (**D**), respectively (OFC, n = 50 neurons from 4 SOC mice and n = 42 neurons from 4 PWSI mice; mPFC, n = 163 neurons from 4 SOC mice and n = 162 neurons from 4 PWSI mice). ***E-L***, *PWSI increased the density of excitatory inputs targeting the perisomatic region of PV+ neurons in the mPFC.* ***E,*** Schematic drawing showing a local putative pyramidal neuron (yellow) targeting the perisomatic region of a PV+ neuron (magenta). Yellow circles indicate the vesicular glutamate transporter type 1 (vGluT1+) immunoreactive synapses coupled with blue dots signaling Bassoon immunoreactive presynaptic active zones analyzed in this experiment. ***F***, Representative single plane confocal images showing close appositions of vGluT1+Bassoon+ puncta (white arrowheads) surrounding a PV+ neuron from the mPFC of SOC and PWSI mice. Same magnification as in **B**. Bar graphs showing the density of vGluT1+Bassoon+ puncta targeting the perisomatic region of PV+ neurons from the OFC (**G**) and mPFC (**H**), respectively (OFC, n = 129 neurons from 4 SOC mice and n = 109 neurons from 4 PWSI mice; mPFC, n = 121 neurons from 4 SOC mice and n = 128 neurons from 4 PWSI mice). ***I***, Schematic drawing showing extracortical excitatory inputs (yellow) targeting the perisomatic region of a PV+ neuron (magenta). Yellow circles indicate vesicular glutamate transporter type 1 (vGluT1+) immunoreactive synapses with blue dots signaling Bassoon immunoreactive presynaptic active zones analyzed in this experiment. ***J,*** Representative single plane confocal images showing close appositions of vGluT2+Bassoon+ puncta (white arrowheads) surrounding a PV+ neuron from the mPFC of SOC and PWSI mice. Similar magnification as in **B.** Bar graphs showing the density of vGluT2+Bassoon+ puncta targeting the perisomatic region of PV+ neurons from the OFC (**K**) and mPFC (**L**), respectively (OFC, n = 92 neurons from 4 SOC mice and n = 106 neurons from 4 PWSI mice; mPFC, n = 176 neurons from 4 SOC mice and n = 127 neurons from 4 PWSI mice). All data are represented as mean ± SEM. ***p < 0.0001. mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; vGAT, vesicular GABA transporter; vGluT1, vesicular glutamate transporter type 1; vGluT2, vesicular glutamate transporter type 2.

3.4. PWSI induces less effective perisomatic inhibition onto excitatory pyramidal neurons of the mPFC

Since PV+ interneurons constitute one of the main sources of perisomatic inhibition onto excitatory pyramidal neurons, providing a synchronized modulation of cortical activity through the regulation of oscillations (Freund and Katona, 2007; Kubota, 2014), we measured vGAT+ PV+ inhibitory puncta impinging onto the soma of pyramidal cells in the mPFC. We observed a marked reduction in the inhibitory output of PV+ interneurons, characterized by a reduced number of inhibitory puncta surrounding mPFC L5 pyramidal neurons (**Fig.7.**, Biro, Miskolczi et al., under review), suggesting **less effective perisomatic inhibiton in the mPFC**, which might contribute to the observed hyperactivation of the mPFC.

Taken together, we found that in PWSI mice aggressive interaction-evoked increased PV+ neuronal activation co-occurred with enhanced glutamatergic drive on PV+ neurons and less effective inhibition of pyramidal neurons. Our results provide support for the claim that **early life adversity perturbs the programming of glutamatergic neurotransmission at PV+ neurons in the mPFC that leads to altered network operations in the prefrontal cortex**.

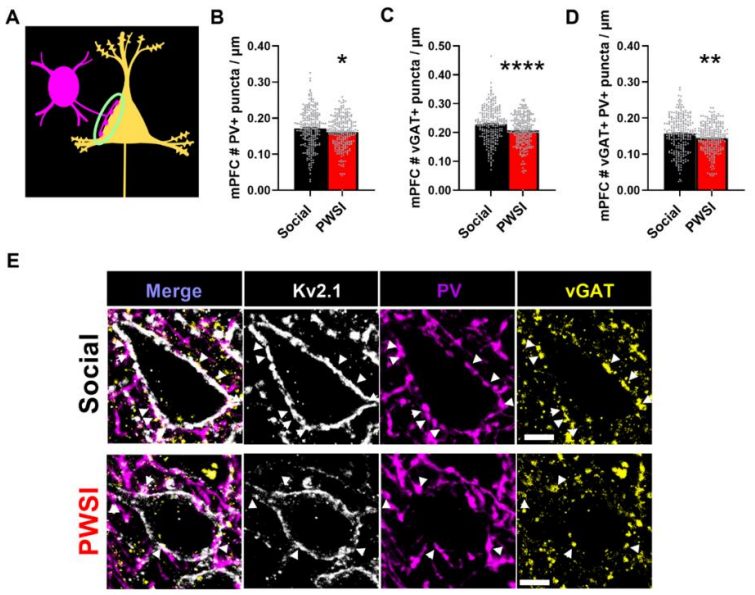

Fig. 7. PWSI reduced the density of PV+ inhibitory boutons (baskets) targeting the perisomatic compartment of pyramidal cells in the mPFC. ***A***, Schematic drawing depicting the inhibitory axons of PV+ neurons (magenta) targeting the perisomatic region of a pyramidal neuron (yellow) forming PV immunoreactive basket-like innervation. ***B-E***, *PWSI reduced the inhibitory output of PV+ neurons*. Bar graphs showing the density of PV+ puncta (***B***), vGAT+PV+ puncta (***C***), and vGAT+ puncta (***D***) targeting the perisomatic region of putative pyramidal neurons (n = 239 neurons from 4 SOC mice and n = 243 neurons from 4 PWSI mice). ***E***, Representative single plane confocal images from the mPFC showing immunoreactivity for PV (magenta) and vGAT (yellow) as well as voltage-gated potassium channel type 2.1 (Kv2.1, white, labels the perisomatic membrane of neurons) for SOC (top panel) and PWSI (bottom panel) mice, respectively. White arrowheads indicate vGAT+PV+ puncta opposing the perisomatic compartment of putative pyramidal neurons. Scale bar, 1 µm. All data are represented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.005.

4. Disturbed neuronal representation of conspecifics in the prefrontal cortex following early-life social stress

To assess abnormal behaviour-linked activity patterns in the medial prefrontal cortex, we performed fiber photometry experiments using the virally expressed Ca2+ sensor GCaMP6s that is suitable to measure the population activity of pyramidal neurons in freely moving mice. By combining fiber photometry measurements with synchronized, frame-by-frame behavioural analysis, we found that PWSI induced an altered activity patterns during agonistic interaction of mice, with significant increases evoked by defensive behaviour in PWSI mice and no significant increase during social exploration (sniffing) as observed in socially reared mice (**Fig. 8.** Szebik et al., preliminary data), confirming our hypothesis that **medial prefrontal activity patterns *during* aggressive interaction are related to early-life social adversity-induced altered behaviour**.

5. Serotonergic system-related mechanisms underlying early social adversities-related behavioural outcome

Serotonin (also known as 5-hydroxytryptamine, 5-HT) is likely **the most important neurotransmitter system in the regulation of human and animal aggression**, moreover, a majority of pharmacotherapeutic compounds in the treatment of social deficits and aggression-related problems target elements of the serotonergic system (Lesch et al., 2012; Coccaro et al., 2011; Olivier, 2004). The serotonergic system plays a key role in mediating the effects of stress and HPA functional alterations on emotionality (Zoratto et al., 2013; Strüber et al., 2014; Haller et al., 2005), and it seems that isolation has a major impact on the serotonergic system (Poeggel and Braun, 2003; Gos et al, 2006). Thus, the serotonergic system might constitute a link between early social deprivation and the occurrence of aggressive behavior. Here we aimed to uncover potential 5-HT signalling-related mechanisms in the aggression circuitry through which abnormal behavioural consequences of early adversities are transmitted.

5.1. Altered gene expression patterns in the aggression circuitry following early social adversities

PWSI resulted in selective alterations in mRNA expression levels of multiple genes involved in 5-HT-signalling, HPA-axis regulation, and neural plasticity in the aggression circuitry. To decrease the dimensions of our multivariate data and identify significant consequences of PWSI in gene expression, we applied the semi-supervised machine learning method, Random Forest (RF) (Breiman, 2001). In the mPFC and MeA areas, the most important predictors of previous early social isolation were the expression levels of Slc22a3 and Slc6a4 genes (**Fig 9**, Miskolczi et al., in preparation), i.e. serotonin transporter (SERT) and organic cation transporter3 (OCT3) gene expression level were highly associated with early life adversity in two brain regions directly involved in aggression control. A reduced model could classify subjects with above 75% accuracy to their assigned treatment group, indicating their potential association with PWSI. This is the first study that revealed such **complex interactions between monoamine transporters, early life adversity and acute exposure to social challenge**. While the role of SERT in the regulation of emotional and social behavior as well as in the transmission of early-life events has been previously indicated, the

OCT3 has not been identified earlier as potential mediator of the effects, though due to its enhanced sensitivity to glucocorticoids, its abundant expression in stress- and emotion-related brain structures and its potential role in the intracellular disposition of monoamines (Gasser et al. 2017, Scholl et al. 2022), it may be of particular interest for future studies.

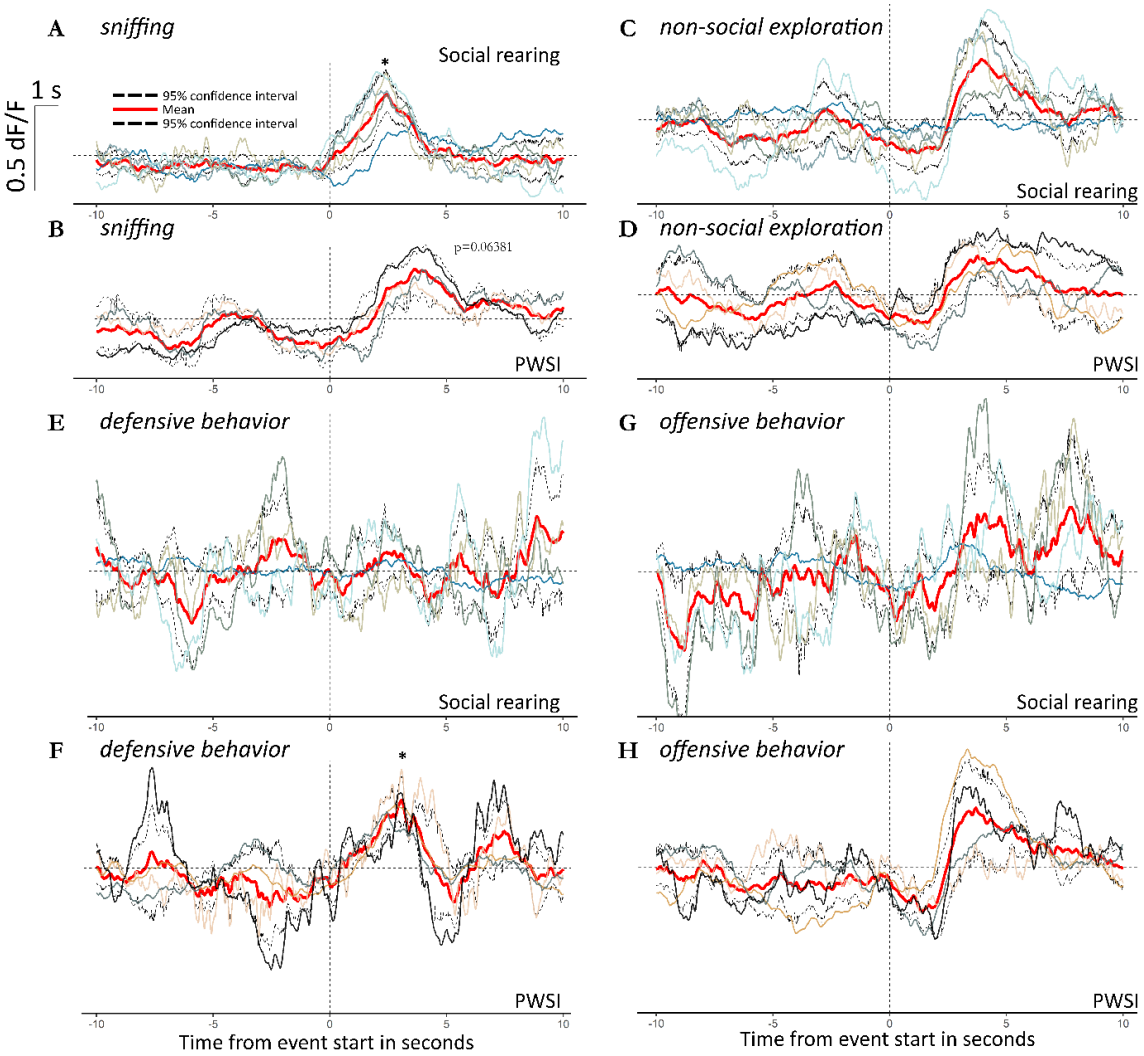

Fig. 8. Behavioral deficits caused by early social isolation are associated with altered behavior-linked medial prefrontal activity. According to our preliminary results obtained by fiber photometry recordings in the medial PFC, both social and PWSI (isolated) animals show increased population activity upon sniffing (A and B), which in turn is not detectable following non-social exploration (C and D). Social animals do not show increased activity at the start of defensive behavior (E), whereas we measured a significant population activity increase in PWSI mice (F). Offensive behavior shows biphasic responses in both experimental groups (G and H), indicating complex dynamic processes in prefrontal regulation of aggressive behavior. Coloured lines indicate measurements of individual animals (averaged over multiple displays of the given behavioural parameter). *p<0.05

5.2. Abnormal social behavior induced by early-life adversity is associated with a hyporeactivity of SERT expression in the prefrontal cortex

To further assess the involvement of mPFC serotonin (5-HT) system in the regulation of aggressive behaviour at the protein level, we performed immunohistochemical labelling against 5-HT and SERT. Using confocal microscopy, we observed a rapid, **fight-induced SERT upregulation in the prelimbic cortex** of mice, that was associated with **increased 5-HT immunoreactivity** (5-HT-IR) 90 min after aggressive interaction (**Fig. 10.**, Szebik et al. in preparation). Such **rapid, experience-dependent effect on SERT expression** has not been published before. This increased SERT expression was further confirmed using STORM superresolution microscopy to exclude potential changes in immunoreactivity caused by altered subcellular distribution (**Fig. 11.**, Szebik et al. in preparation). Importantly, SERT upregulation in the mPFC was limited to social challenges, as exposure to novelty (open-field test) only induced elevations in 5-HT but not in SERT levels. To address whether fighting-induced upregulation of SERT expression shows region-specific effects, we also performed immunolabeling at the level of key subcortical nodes of the aggression circuitry. According to our results, SERT and 5-HT expression is rapidly changing upon social challenge in a region-dependent manner. In PWSI animals, this fighting-induced expression increase was absent in a subregion-specific manner (**Fig. 12.**, Szebik et al. in preparation), indicating **a potential hyporeactive state of the serotonergic system following early-life adversity**.

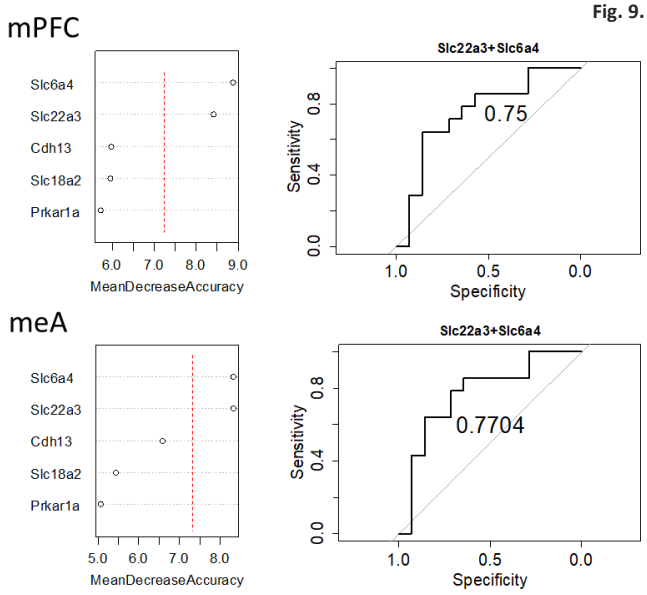

Fig. 9. mRNA expression patterns predict previous isolation with high accuracy. Using 384 well microfluidic Taqman array cards, qPCR mRNA expression analysis of genes selected for their involvement in 5-HT, HPA-axis and plasticity-related pathways was performed in socially reared and PWSI rats using brain micropunches from the medial prefrontal cortex, basolateral amygdala, medial amygdala and dorsal raphe, gained under baseline conditions or 60min after resident-intruder test. PWSI resulted in altered gene expression patterns in the studied regions (data not shown). To decrease the dimensions of our multivariate data and identify significant consequences of PWSI in gene expression, we applied the semi-supervised machine learning method, Random Forest (RF) (Breiman, 2001). RF can rank each variable's importance in classifying experimental subjects into treatment groups. In our analysis, i) we implemented a saturated model using the expression data of 45 potentially relevant genes in different brain areas, ii) ranked these genes according to their importance in the classification of social and PWSI rats, and iii) created subset models possessing the two most relevant genes. Finally, iv) we determined and displayed the specificity and selectivity of this final model using the receiver operating curve approach. In the mPFC and MeA areas, the most important predictors of previous early social isolation were the expression levels of Slc22a3 and Slc6a4 genes), i.e. serotonin transporter and organic cation transporter3 gene expression level were highly associated with early life adversity in two brain regions directly involved in aggression control. A reduced model, which only consists of the data of the above genes, could classify subjects with above 75% accuracy to their assigned treatment group, indicating their potential association with such environmental perturbation.

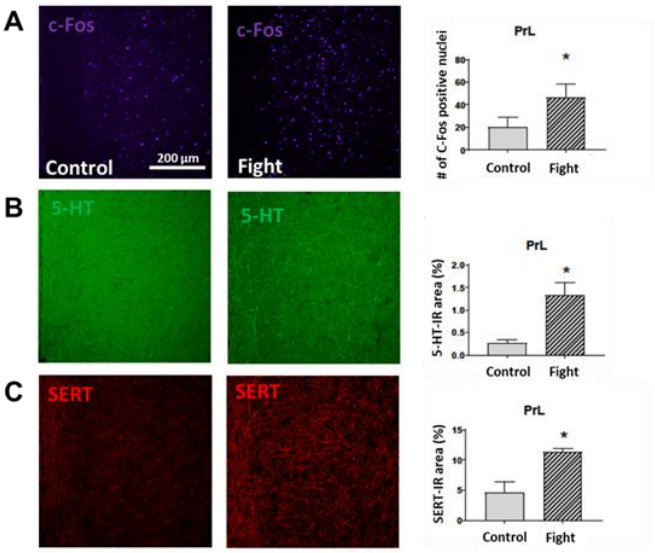

Fig. 10. Exposure to a conspecific male intruder leads to acute increases in SERT and 5HT immunreactivity in the prefrontal cortex. Socially reared male mice were perfused 90min after aggressive interaction ('Fight') or under baseline conditions ('Control'). (A) Number of activated neurons, as well as (B) 5-HT and (C) SERT immunoreactivity was significantly increased in the prelimbic cortex (PrL) of fighting mice. *p<0.05

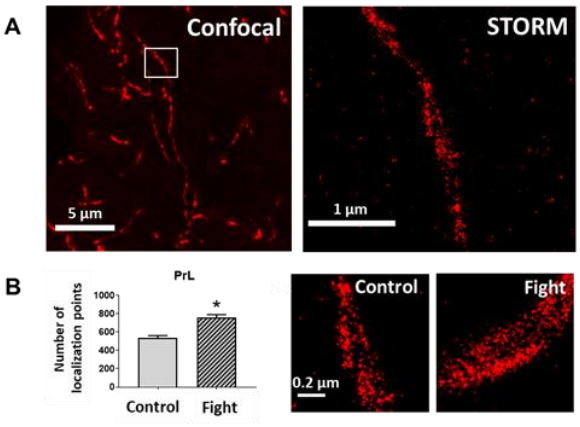

Fig. 11. Intruder exposure increases the number of SERT localization puncta measured by STORM superresolution microscopy. A, Representative images showing the increased resolution of STORM microscopy (right) compared to confocal fluorescent imaging (left). B, Mice perfused 90min after aggressive interaction show an increased number of SERT localization puncta in the prelimbic subregion of the prefrontal cortex. *p<0.05

5.3. Serotonergic modulation of early social isolation-induced behavioral changes in zebrafish

To strengthen translational validity of our approach and model, we also investigated **early isolation-induced behavioural changes in zebrafish** (Danio rerio), a species suitable for high-throughput behavioural and pharmacological studies. We have established and validated a **novel test for measuring emotional state and anxiety-like behaviour**, the swimming plus-maze test (Varga et al., 2018). For the first time, we have characterized a period during behavioural metamorphosis in which zebrafish are highly reactive to their environment (for details see Varga et al., 2020). Absence of social stimuli during this phase - established by isolated rearing - fundamentally altered the behavioral phenotype of postmetamorphic zebrafish in a challenge-specific manner, partially due to reduced responsiveness and an inability to develop stress-associated arousal state (**Fig 13.**, Varga et al., 2020). In line with this, isolation differentially affected whole-brain serotonergic signaling in resting and stress-induced conditions, an effect that was localized in the dorsal pallium and was negatively associated with responsiveness. Administration of the serotonin receptor 1A partial agonist buspirone prevented the isolation-induced serotonin response to novelty in the level of the whole brain and the forebrain as well, without affecting catecholamine levels, and rescued stress-induced arousal along with challenge-induced behaviors, which together indicates functional connection between these changes (**Fig. 14.**, Varga et al., 2020). These results imply a **conserved serotonergic mechanism that**

context-dependently modulates environmental reactivity and is highly sensitive to experiences acquired during a specific early-life time window.

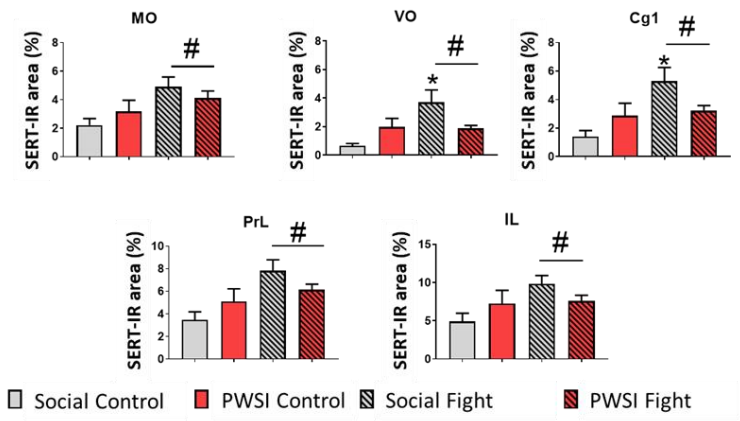

Fig. 12. Abnormal social behaviour induced by early-life adversity is associated with a hyporeactivity of SERT expression in the prefrontal cortex. Socially reared mice or mice reared in social isolation (PWSI) mice were perfused 90min after aggressive interaction ('Fight') or under baseline conditions ('Control'). SERT immunoreactivity was significantly increased in all prefrontal subregions of social mice, however, this fight-induced increase was absent in ventral orbitofrontal and anterior cingulate cortices of PWSI mice. #p<0.05 effect of fighting; *p<0.05 compared to 'Social control'. MO, medial orbitofrontal cortex; VO: ventral orbitofrontal cortex; Cg1: anterior cingulate cortex; PrL: prelimbic cortex; IL: infralimbic cortex

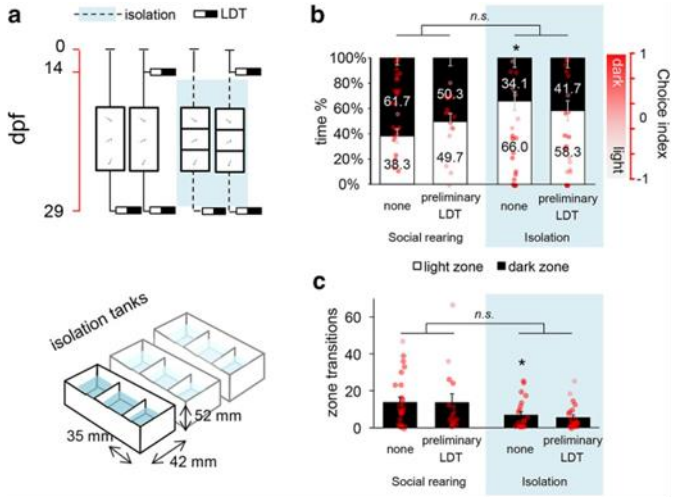

Fig.13. Social isolation during the early sensitive period disrupts later behavioral responsiveness of zebrafish. *a*, Protocol of Experiment (top) and schematic drawing of the isolation tanks (bottom). Red timeline represents the age of larvae expressed in days post fertilization (dpf). *b*, Avoidance behavior of postmetamorphic zebrafish 2 weeks following a single LDT challenge and/or after 2 weeks of chronic social isolation. Left axis indicates the percentage of time. Right axis indicates choice index in which 1 indicates 100% time in the dark zone, whereas −1 represents 100% time in the light zone. The reference levels of the linear mixed model were "social rearing" and "treatment none." *Significant differences between rearing conditions at the level "treatment none." n.s., No significant differences between the relation of the treatment levels in socially reared and isolated groups. *c*, Number of transitions between light and dark zones of postmetamorphic zebrafish 2 weeks following a single LDT challenge and/or after 2 weeks of chronic social isolation. LDT: light/dark tank.

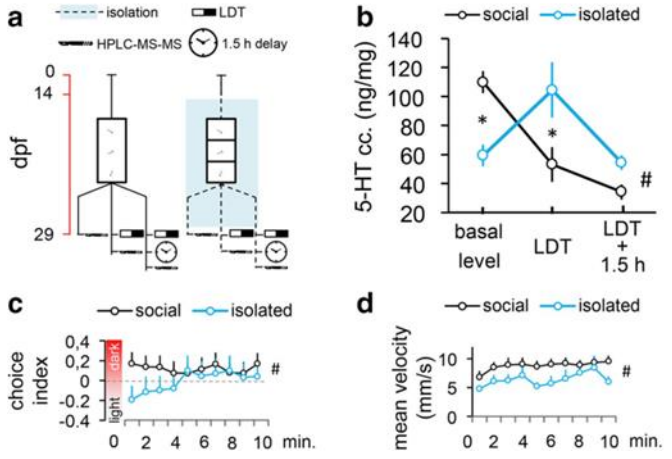

Fig. 14. Early social isolation differentially affects central 5-HT levels of zebrafish under resting and novel conditions. *a*, Protocol of Experiment. Red timeline represents the age of larvae expressed in days post fertilization (dpf). *b*, Whole-brain 5-HT concentration of postmetamorphic zebrafish in resting conditions, and immediately and 1.5 h after LDT challenge. *Significant differences between groups at the level of a single condition (basal level or LDT or LDT + 1.5 h). #Significant group × condition interaction. *c*, Minute-by-minute resolution analysis of avoidance measured by choice index. *d*, Velocity. #Significant group × condition interaction. LDT: light/dark tank.

6. Changes in epigenetic markers after chronic fluoxetine treatment combined with adult re-socialization

Using PWSI rats, we found earlier that re-socialization, a laboratory analogue of behavioural therapy, failed to correct the PWSI-induced escalation of aggression (Tulogdi et al., 2014) confirming the assumption that this paradigm induces persistent changes in emotions and suggesting that the treatment of developmentally induced aggression problems requires novel approaches. As some mental disorders including anxiety and depression have been shown to benefit from synergistic effects of parallel pharmaco- and psychotherapy we have recently investigated the **aggression-corrective efficacy of adult re-socialization in combination with the SSRI fluoxetine**. We found that fluoxetine, in combination with re-socialization, counteracted pathological aggression escalated by early adverse experiences, while neither treatment alone was effective (Mikics et al., 2018). We found that the

combined treatment counteracted with the PWSI-induced decrease in Bdnf1,4 and identified TrkB receptors, the major receptor of mature brain-derived neurothrophic factor (BDNF), in the infralimbic cortex (IL) as main mediators of this effect (Mikics et al, 2018). Our studies during the grant period aiming to understand epigenetic and plasticity-related mechanisms revealed that **Dnmt1, a key epigenetic enzyme** that directly influences the transcription of target genes, **shows decreased mRNA expression in the infralimbic cortex** (IL) and the MeA - but not in other aggression-controlling brain regions - of PWSI rats compared to socially reared controls. Interestingly, chronic fluoxetine treatment (independently of re-socialization) was able to increase Dnmt1 expression in the MeA, whereas only **the combined (fluoxetine+re-socialization) treatment was sufficient to restore Dnmt1 expression in the IL of PWSI rats** (**Fig 15.,** Miskolczi et al, in preparation). These data indicate the involvement of epigenetic mechanisms in pharmacological treatments in psychiatric conditions induced by early-life adversities.

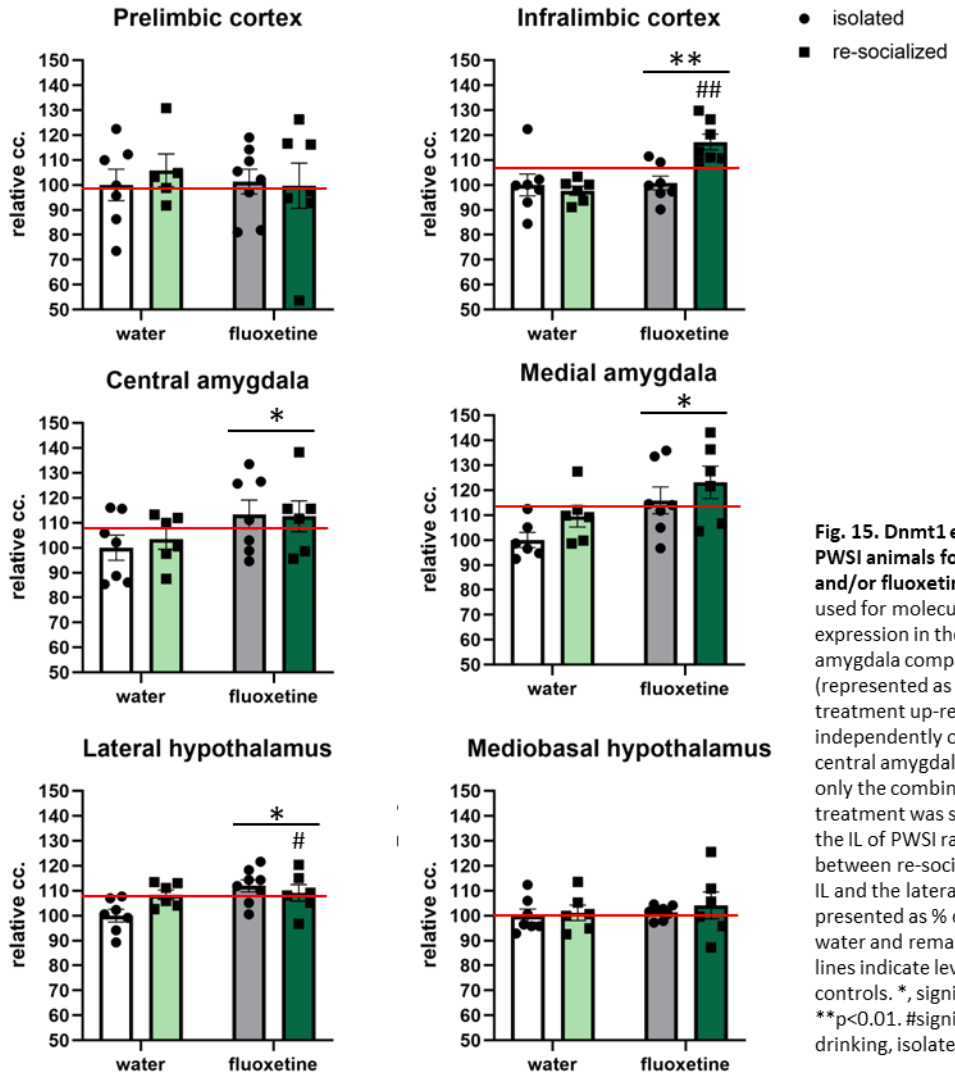

Fig. 15. Dnmt1 expression in the aggression circuitry in PWSI animals following 3-week long re-socialization and/or fluoxetine treatment. Within the brain punches used for molecular analysis, PWSI downregulated Dnmt1 expression in the infralimbic cortex (IL) and medial amygdala compared to socially reared controls (represented as red lines), $p<0.05$. Chronic fluoxetine treatment up-regulated Dnmt1 expression independently of resocialization in the medial and central amygdalae and lateral hypothalamus, whereas only the combined (fluoxetine+re-socialization) treatment was sufficient to restore Dnmt1 expression in the IL of PWSI rats. There was a significant interaction between re-socialization and fluoxetine treatment in the IL and the lateral hypothalamus of PWSI rats. Data are presented as % difference from PWSI animals received water and remained in isolation as mean ± SEM. Red lines indicate levels of socially reared, water-drinking controls. *, significant effect of fluoxetine, *$p<0.05$; **$p<0.01$. #significant difference from PWSI, water drinking, isolated animals. #$p<0.05$; ##$p<0.01$

7. Plasticity-related mechanisms underlying interneuron function and associated behavioural changes

The neurotrophin brain-derived neurothrophic factor (**BDNF**) is a key mediator of the neuronal activity-dependent processes in the brain that have a major impact on **neuronal development and plasticity** (for review, see [65]). Impaired control of BDNF expression has been implicated in transmitting early-life adversities (Miskolczi et al., 2019). The activation TrkB receptors, the major receptor type of mature BDNF signaling, mediates cortical network activity through the regulation of PV plasticity states across critical periods and in adulthood and have been recently implicated in behavioural regulation and antidepressant function (Winkel et al. 2021, Guyon et al, 2021). In order to understand plasticity-related mechanisms underlying PWSI-induced behavioural outcome, we first generated PV-TrkB CKO mice (strains received as courtesy of our collaborators E.Castren and J. Umemori, Univ. of Helsinki, Finland) in which TrkB receptors cannot be activated in PV+ neurons to prove that BDNF-signaling in PV+ neurons is crucial for the restoration of normal social/aggressive behavior, and pave subsequent studies that target PV+ neurons in the mPFC. PV-TrkB CKO mice turned out to show alterations in motor function and are therefore not ideally suitable to use them in social test paradigms. Therefore, in a side project directly linked to the grant, we investigated the role of BDNF signaling in another major, yes in this respect less characterized cortical interneuron population and used

somatostatin-TrkB CKO mice (SST_Cre mice crossed with floxed TrKB CKO line on C57BL6 background). We performed a series of behavioural tests and found that **BDNF signaling in somatostatin positive neurons is crucial in the regulation of forming active/passive coping responses in a series of challenging situations** but social behavior remained unaffected (**Fig. 16.,** preliminary data). Our analysis on differentially activated brain areas in CKO mice during stressful situations and selective molecular changes in SST+ interneurons (measured by single cell qPCR) is currently in progress, and a novel grant application (NKFIH FK#142171 to Mate Toth) was based on these findings in 2022.

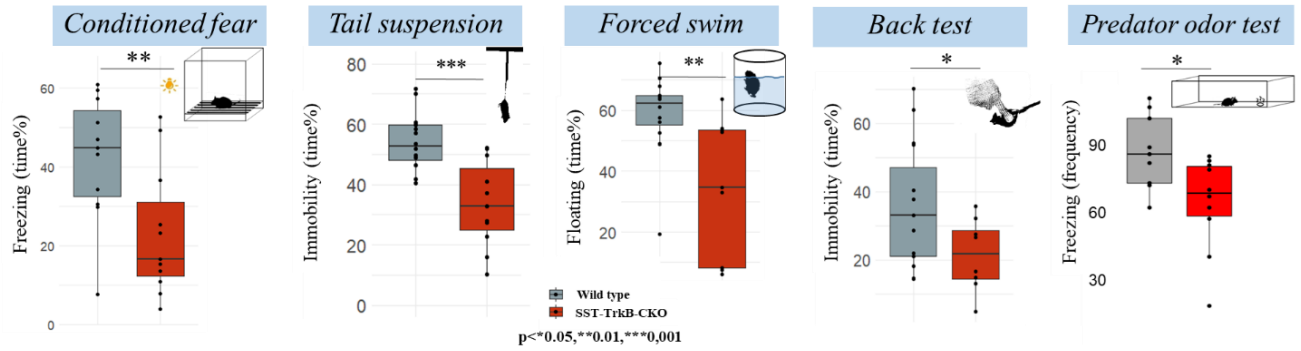

Figure 16. Alteration in coping behaviour induced by TrkB receptor knockdown in SST neurons (SST-TrkB-CKO). TrkB-CKO mice displayed altered coping responses to stressful challenges, characterized by a shift toward more active coping in relevant tests. Importanty, behavioural changes were highly selective, as other domains including neuromotor function, cognitive function and social behaviour remained unaffected (data not shown).

8. Post-transcriptional modification implied in the transmission of anxiety-related outcome

In this experiment we focused on emotional consequences of early adversities and investigated mPFC-function in rodents showing high- and low anxiety traits. To outline the neural underpinnings of trait anxiety, we performed RNASeq, an unbiased whole-transcriptome analysis in mPFC tissue, using our recently developed test paradigm that can reliably measure trait (rather than state) anxiety (Varga et al., in preparation), a condition more reliably predicting pathological anxiety than transient anxiety states. In contrast to our findings associated with state anxiety, we have detected **an enhanced number of neuronal correlates in association with trait anxiety**, including differential expression of several genes involved in the regulation of transcriptional processes, post-transcriptional modification of mRNA (Bc1) (**Fig 17.,** Varga et al. in preparation), neurotransmitter release-regulation (TASK-3), and perineuronal net organization (Adamts4). An extended literature analysis of gene function revealed that i) in **trait-like anxiety we detected 4-7x more gene-correlates** than we did using single conventional tests representing anxiety states, and ii) genes with reported function in the stress response were more represented in state-like anxiety testing, and **plasticity-related genes were more associated with anxiety trait** in our paradigm (**Fig, 17.,** Varga et al. in preparation). This suggests that our paradigm is highly suited to investigate and understand brain mechanisms underlying early adversities-induced emotional alterations. Based on these results, a novel grant application was initiated to achieve funding for causal investigations.

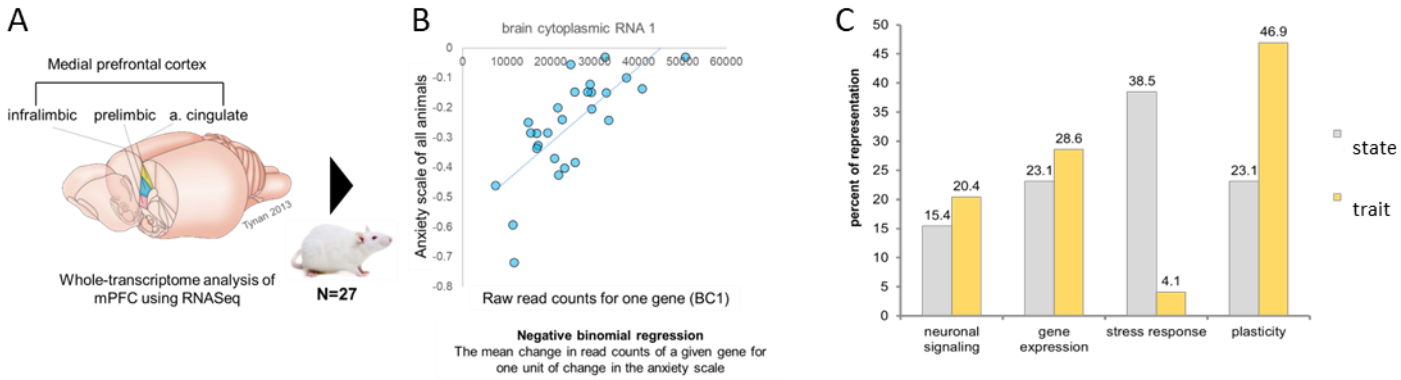

Fig. 17. State anxiety is related to plasticity-related gene expression patterns in the medial prefrontal cortex. ***A***, mPFC punches were collected from 27 male rats representing differences in anxiety traits based on our recently developed paradigm and RNASeq was performed. **B,** Correlation between relative anxiety scale (based on z-scores of multiple tests and time points individually) and BC1 gene expression. p<0,001, BC1 is a gene involved in post-translational modifications. **C,** Extended literature analysis of gene function revealed that genes with reported function in the stress response were more represented in state-like anxiety testing, and plasticity-related genes were more associated with anxiety trait in our paradigm.

Summary

We have successfully completed the planned studies. Using post-weaning social isolation (PWSI), an animal model of early social neglect, we characterized long-term deficits in social and emotional functioning. We observed altered network operations within the prefrontal cortex of PWSI animals and identified developmental changes in inhibitory interneuron function that underly network activity alterations and contribute to behavioural abnormalities. Our translational approaches, using etiological models of early-life adversities in rats, mice and zebrafish, revealed serotonergic system-related, epigenetic and plasticity-related changes within the aggression- and emotion-circuit of the brain, that serve as potential targets of novel treatment strategies.

We presented our results at several international and domestic congresses in forms of oral and poster presentations. We published parts of the results in two peer-reviewed high-ranking journals and published a review paper in the project's topic, partly based on our findings. An additional publication has already been submitted for publication and is under review, whereas two additional publications are short before submission. Some of the data have been included in a PhD thesis successfully defended in 2020, moreover two PhD works are still ongoing. Several prestigious awards were won by our undergraduate students presenting results of the project. With the help of this grant we have identified brain mechanisms that may transmit effects of early social adversities on long-term behavioural deficits that could help to test effective therapeutic approaches towards clinical testing.

Published international peer-reviewed articles based on the project

Miskolczi C, Halász J, Mikics É. Changes in neuroplasticity following early-life social adversities: the possible role of brain-derived neurotrophic factor. *Pediatr Res.* 2019; 85(2):225-233. doi: 10.1038/s41390-018-0205-7.

Varga ZK, Zsigmond Á, Pejtsik D, Varga M, Demeter K, Mikics É, Haller J,Aliczki M. The swimming plus-maze test: a novel high-throughput model for assessment of anxiety-related behaviour in larval and juvenile zebrafish (Danio rerio). *Sci Rep.* 2018; 8(1):16590. doi: 10.1038/s41598-018-34989-1

Varga ZK, Pejtsik D, Biró L, Zsigmond Á, Varga M, Tóth B, Salamon V, Annus T, Mikics É, Aliczki M. Conserved Serotonergic Background of Experience-Dependent Behavioral Responsiveness in Zebrafish (Danio rerio). *J Neurosci.* 2020; 40(23):4551-4564. doi: 10.1523/JNEUROSCI.2178-19.2020.

Submitted article (under review)

Laszlo Biro[#], Christina Miskolczi[#], Huba Szebik, Biborka Bruzsik, Zoltan Kristof Varga, Laszlo Szente, Mate Toth, Jozsef Halasz, Eva Mikics. #equal contribution. Post-weaning social isolation leads to abnormal aggression and disrupted network organization in the prefrontal cortex.

References

1: Ährlund-Richter S, Xuan Y, van Lunteren JA, Kim H, Ortiz C, Pollak Dorocic I, Meletis K, Carlén M. A whole-brain atlas of monosynaptic input targeting four different cell types in the medial prefrontal cortex of the mouse. Nat Neurosci. 2019 Apr;22(4):657-668. doi: 10.1038/s41593-019-0354-y.

2: Biro L, Toth M, Sipos E, Bruzsik B, Tulogdi A, Bendahan S, Sandi C, Haller J. Structural and functional alterations in the prefrontal cortex after post-weaning social isolation: relationship with species-typical and deviant aggression. Brain Struct Funct. 2017 May;222(4):1861-1875. doi: 10.1007/s00429-016-1312-z.

3: Biro L, Sipos E, Bruzsik B, Farkas I, Zelena D, Balazsfi D, Toth M, Haller J. Task Division within the Prefrontal Cortex: Distinct Neuron Populations Selectively Control Different Aspects of Aggressive Behavior via the Hypothalamus. J Neurosci. 2018 Apr 25;38(17):4065-4075. doi:10.1523/JNEUROSCI.3234-17.2018.

4: Carceller H, Guirado R, Ripolles-Campos E, Teruel-Marti V, Nacher J. Perineuronal Nets Regulate the Inhibitory Perisomatic Input onto Parvalbumin Interneurons and γ Activity in the Prefrontal Cortex. J Neurosci. 2020 Jun 24;40(26):5008-5018. doi: 10.1523/JNEUROSCI.0291-20.2020.

5: Carr CP, Martins CM, Stingel AM, Lemgruber VB, Juruena MF. The role of early life stress in adult psychiatric disorders: a systematic review according to childhood trauma subtypes. J Nerv Ment Dis. 2013 Dec;201(12):1007-20. doi: 10.1097/NMD.0000000000000049.

6: Coccaro EF, Sripada CS, Yanowitch RN, Phan KL. Corticolimbic function in impulsive aggressive behavior. Biol Psychiatry. 2011 Jun 15;69(12):1153-9. doi:10.1016/j.biopsych.2011.02.032.

7: Crowe SL, Blair RJ. The development of antisocial behavior: what can we learn from functional neuroimaging studies? Dev Psychopathol. 2008 Fall;20(4):1145-59. doi: 10.1017/S0954579408000540.

8: Cupaioli FA, Zucca FA, Caporale C, Lesch KP, Passamonti L, Zecca L. The neurobiology of human aggressive behavior: Neuroimaging, genetic, and neurochemical aspects. Prog Neuropsychopharmacol Biol Psychiatry. 2021 Mar 2;106:110059. doi: 10.1016/j.pnpbp.2020.110059.

9: Dityatev A, Schachner M, Sonderegger P. The dual role of the extracellular matrix in synaptic plasticity and homeostasis. Nat Rev Neurosci. 2010 Nov;11(11):735-46. doi: 10.1038/nrn2898.

10: Favuzzi E, Marques-Smith A, Deogracias R, Winterflood CM, Sánchez-Aguilera A, Mantoan L, Maeso P, Fernandes C, Ewers H, Rico B. Activity-Dependent Gating of Parvalbumin Interneuron Function by the Perineuronal Net Protein Brevican. Neuron. 2017 Aug 2;95(3):639-655.e10. doi: 10.1016/j.neuron.2017.06.028.

11: Freund TF, Katona I. Perisomatic inhibition. Neuron. 2007 Oct 4;56(1):33-42. doi: 10.1016/j.neuron.2007.09.012.

12: Gasser PJ, Hurley MM, Chan J, Pickel VM. Organic cation transporter 3 (OCT3) is localized to intracellular and surface membranes in select glial and neuronal cells within the basolateral amygdaloid complex of both rats and mice. Brain Struct Funct. 2017 May;222(4):1913-1928. doi: 10.1007/s00429-016-1315-9.

13: Gilbert R, Widom CS, Browne K, Fergusson D, Webb E, Janson S. Burden and consequences of child maltreatment in high-income countries. Lancet. 2009 Jan 3;373(9657):68-81. doi: 10.1016/S0140-6736(08)61706-7.

14: Gos T, Becker K, Bock J, Malecki U, Bogerts B, Poeggel G, Braun K. Early neonatal and postweaning social emotional deprivation interferes with the maturation of serotonergic and tyrosine hydroxylase-immunoreactive afferent fiber systems in the rodent nucleus accumbens, hippocampus and amygdala. Neuroscience. 2006 Jul 7;140(3):811-21. doi: 10.1016/j.neuroscience.2006.02.078.

15: Gulyás AI, Megías M, Emri Z, Freund TF. Total number and ratio of excitatory and inhibitory synapses converging onto single interneurons of different types in the CA1 area of the rat hippocampus. J Neurosci. 1999 Nov 15;19(22):10082-97. doi: 10.1523/JNEUROSCI.19-22-10082.1999.

16: Guyon N, Zacharias LR, van Lunteren JA, Immenschuh J, Fuzik J, Märtin A, Xuan Y, Zilberter M, Kim H, Meletis K, Lopes-Aguiar C, Carlén M. Adult trkB Signaling in Parvalbumin Interneurons is Essential to Prefrontal Network Dynamics. J Neurosci. 2021 Apr 7;41(14):3120-3141. doi: 10.1523/JNEUROSCI.1848-20.2021.

17: Halász J, Tóth M, Kalló I, Liposits Z, Haller J. The activation of prefrontal cortical neurons in aggression--a double labeling study. Behav Brain Res. 2006 Nov 25;175(1):166-75. doi: 10.1016/j.bbr.2006.08.019.

18: Haller J, Mikics E, Halász J, Tóth M. Mechanisms differentiating normal from abnormal aggression: glucocorticoids and serotonin. Eur J Pharmacol. 2005 Dec 5;526(1-3):89-100. doi: 10.1016/j.ejphar.2005.09.064.

19: Hensch TK. Critical period regulation. Annu Rev Neurosci. 2004;27:549-79. doi: 10.1146/annurev.neuro.27.070203.144327.

20: Kempes M, Matthys W, de Vries H, van Engeland H. Reactive and proactive aggression in children--a review of theory, findings and the relevance for child and adolescent psychiatry. Eur Child Adolesc Psychiatry. 2005 Feb;14(1):11-9. doi: 10.1007/s00787-005-0432-4.

21: Kubota Y. Untangling GABAergic wiring in the cortical microcircuit. Curr Opin Neurobiol. 2014 Jun;26:7-14. doi: 10.1016/j.conb.2013.10.003.

22: Larsen B, Luna B. Adolescence as a neurobiological critical period for the development of higher-order cognition. Neurosci Biobehav Rev. 2018 Nov;94:179-195. doi: 10.1016/j.neubiorev.2018.09.005.

23: Lensjø KK, Christensen AC, Tennøe S, Fyhn M, Hafting T. Differential Expression and Cell-Type Specificity of Perineuronal Nets in Hippocampus, Medial Entorhinal Cortex, and Visual Cortex Examined in the Rat and Mouse. eNeuro. 2017 Jun 7;4(3):ENEURO.0379-16.2017. doi: 10.1523/ENEURO.0379-16.2017.

24: Lesch KP, Araragi N, Waider J, van den Hove D, Gutknecht L. Targeting brain serotonin synthesis: insights into neurodevelopmental disorders with long-term outcomes related to negative emotionality, aggression and antisocial behaviour. Philos Trans R Soc Lond B Biol Sci. 2012 Sep 5;367(1601):2426-43. doi: 10.1098/rstb.2012.0039.

25: Mikics É, Guirado R, Umemori J, Tóth M, Biró L, Miskolczi C, Balázsfi D, Zelena D, Castrén E, Haller J, Karpova NN. Social Learning Requires Plasticity Enhanced by Fluoxetine Through Prefrontal Bdnf-TrkB Signaling to Limit Aggression Induced by Post-Weaning Social Isolation. Neuropsychopharmacology. 2018 Jan;43(2):235-245. doi: 10.1038/npp.2017.142

26: Miskolczi C, Halász J, Mikics É. Changes in neuroplasticity following early-life social adversities: the possible role of brain-derived neurotrophic factor. Pediatr Res. 2019 Jan;85(2):225-233. doi: 10.1038/s41390-018-0205-7.

27: Newman EL, Terunuma M, Wang TL, Hewage N, Bicakci MB, Moss SJ, DeBold JF, Miczek KA. A Role for Prefrontal Cortical NMDA Receptors in Murine Alcohol-Heightened Aggression. Neuropsychopharmacology. 2018 May;43(6):1224-1234. doi: 10.1038/npp.2017.253.

28: Olivier B. Serotonin and aggression. Ann N Y Acad Sci. 2004 Dec;1036:382-92. doi: 10.1196/annals.1330.022.

29: Poeggel G, Nowicki L, Braun K. Early social deprivation alters monoaminergic afferents in the orbital prefrontal cortex of Octodon degus. Neuroscience. 2003;116(3):617-20. doi: 10.1016/s0306-4522(02)00751-0.

30: Provençal N, Booij L, Tremblay RE. The developmental origins of chronic physical aggression: biological pathways triggered by early life adversity. J Exp Biol. 2015 Jan 1;218(Pt 1):123-33. doi: 10.1242/jeb.111401.

31: Rotaru DC, Lewis DA, Gonzalez-Burgos G. The role of glutamatergic inputs onto parvalbumin-positive interneurons: relevance for schizophrenia. Rev Neurosci. 2012 Jan 9;23(1):97-109. doi: 10.1515/revneuro-2011-0059.

32: Scholl JL, Solanki RR, Watt MJ, Renner KJ, Forster GL. Chronic administration of glucocorticoid receptor ligands increases anxiety-like behavior and selectively increase serotonin transporters in the ventral hippocampus. Brain Res. 2022 Nov 30:148189. doi: 10.1016/j.brainres.2022.148189.

33: Siegel A, Bhatt S, Bhatt R, Zalcman SS. The neurobiological bases for development of pharmacological treatments of aggressive disorders. Curr Neuropharmacol. 2007;5(2):135-47. doi: 10.2174/157015907780866929.

34: Spijker S, Koskinen MK, Riga D. Incubation of depression: ECM assembly and parvalbumin interneurons after stress. Neurosci Biobehav Rev. 2020 Nov;118:65-79. doi: 10.1016/j.neubiorev.2020.07.015.

35: Strüber N, Strüber D, Roth G. Impact of early adversity on glucocorticoid regulation and later mental disorders. Neurosci Biobehav Rev. 2014 Jan;38:17-37. doi: 10.1016/j.neubiorev.2013.10.015

36: Takahashi A, Nagayasu K, Nishitani N, Kaneko S, Koide T. Control of intermale aggression by medial prefrontal cortex activation in the mouse. PLoS One. 2014 Apr 16;9(4):e94657. doi: 10.1371/journal.pone.0094657.

37: Tóth M, Halász J, Mikics E, Barsy B, Haller J. Early social deprivation induces disturbed social communication and violent aggression in adulthood. Behav Neurosci. 2008 Aug;122(4):849-54. doi: 10.1037/0735-7044.122.4.849.

38: Toth M, Mikics E, Tulogdi A, Aliczki M, Haller J. Post-weaning social isolation induces abnormal forms of aggression in conjunction with increased glucocorticoid and autonomic stress responses. Horm Behav. 2011 Jun;60(1):28-36. doi: 10.1016/j.yhbeh.2011.02.003.

39: Toth M, Tulogdi A, Biro L, Soros P, Mikics E, Haller J. The neural background of hyper-emotional aggression induced by post-weaning social isolation. Behav Brain Res. 2012 Jul 15;233(1):120-9. doi: 10.1016/j.bbr.2012.04.025.

40: Tremblay RE. Understanding development and prevention of chronic physical aggression: towards experimental epigenetic studies. Philos Trans R Soc Lond B Biol Sci. 2008 Aug 12;363(1503):2613-22. doi: 10.1098/rstb.2008.0030.

41: Tremblay RE, Vitaro F, Côté SM. Developmental Origins of Chronic Physical Aggression: A Bio-Psycho-Social Model for the Next Generation of Preventive Interventions. Annu Rev Psychol. 2018 Jan 4;69:383-407. doi: 10.1146/annurev-psych-010416-044030.

42: Tremblay R, Lee S, Rudy B. GABAergic Interneurons in the Neocortex: From Cellular Properties to Circuits. Neuron. 2016 Jul 20;91(2):260-92. doi: 10.1016/j.neuron.2016.06.033.

43: Tulogdi A, Tóth M, Barsvári B, Biró L, Mikics E, Haller J. Effects of resocialization on post-weaning social isolation-induced abnormal aggression and social deficits in rats. Dev Psychobiol. 2014 Jan;56(1):49-57. doi: 10.1002/dev.21090.

44: Turgay A. Aggression and disruptive behavior disorders in children and adolescents. Expert Rev Neurother. 2004 Jul;4(4):623-32. doi: 10.1586/14737175.4.4.623.

45: Ueno H, Suemitsu S, Murakami S, Kitamura N, Wani K, Okamoto M, Matsumoto Y, Ishihara T. Region-specific impairments in parvalbumin interneurons in social isolation-reared mice. Neuroscience. 2017 Sep 17;359:196-208. doi: 10.1016/j.neuroscience.2017.07.016.

46: van Heukelum S, Mogavero F, van de Wal MAE, Geers FE, França ASC, Buitelaar JK, Beckmann CF, Glennon JC, Havenith MN. Gradient of Parvalbumin- and Somatostatin-Expressing Interneurons Across Cingulate Cortex Is Differentially Linked to Aggression and Sociability in BALB/cJ Mice. Front Psychiatry. 2019 Nov 15;10:809. doi: 10.3389/fpsyt.2019.00809

47: Varga ZK, Zsigmond Á, Pejtsik D, Varga M, Demeter K, Mikics É, Haller J,Aliczki M. The swimming plus-maze test: a novel high-throughput model for assessment of anxiety-related behaviour in larval and juvenile zebrafish (Danio rerio). Sci Rep. 2018 Nov 8;8(1):16590. doi: 10.1038/s41598-018-34989-1

48: Varga ZK, Pejtsik D, Biró L, Zsigmond Á, Varga M, Tóth B, Salamon V, Annus T, Mikics É, Aliczki M. Conserved Serotonergic Background of Experience-Dependent Behavioral Responsiveness in Zebrafish (Danio rerio). J Neurosci. 2020 Jun 3;40(23):4551-4564. doi: 10.1523/JNEUROSCI.2178-19.2020.

49: Veenema AH. Toward understanding how early-life social experiences alter oxytocin- and vasopressin-regulated social behaviors. Horm Behav. 2012 Mar;61(3):304-12. doi: 10.1016/j.yhbeh.2011.12.002.

50: Winkel F, Ryazantseva M, Voigt MB, Didio G, Lilja A, Llach Pou M, Steinzeig A, Harkki J, Englund J, Khirug S, Rivera C, Palva S, Taira T, Lauri SE, Umemori J, Castrén E. Pharmacological and optical activation of TrkB in Parvalbumin interneurons regulate intrinsic states to orchestrate cortical plasticity. Mol Psychiatry. 2021 Dec;26(12):7247-7256. doi: 10.1038/s41380-021-01211-0.

51: Zoratto F, Fiore M, Ali SF, Laviola G, Macrì S. Neonatal tryptophan depletion and corticosterone supplementation modify emotional responses in adult male mice. Psychoneuroendocrinology. 2013 Jan;38(1):24-39. doi: 10.1016/j.psyneuen.2012.04.015.