- FINAL RESEARCH REPORT -

SOCIAL DEPRIVATION AND ABNORMAL AGGRESSION (OTKA PD 76283)

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1. Overall outcome

We have successfully accomplished all aims of the research plan. During the study period we carried out all major planned experiments and revealed important, novel aspects of brain mechanisms underlying abnormal aggression that results from early life social adversities. Our studies resulted in 4 peer-reviewed international articles, whereas three further manuscripts are under preparation (see below).

2. Aims of the study

Aggression and violence create enormous burden both to the individual and to the society, which underlines the importance of research conducted in this field. At the moment, no reliable treatment method is described for antisocial individuals with serious violent behavior. It is most likely that the poor efficacy in the treatment of abnormal aggressive behavior is due to a deficient understanding of the underlying pathomechanisms. Pathological aggressive behavior can only be understood via relevant laboratory models.

We hypothetized that continuous social deprivation after weaning in a laboratory model results in severe behavioral alterations in emotional behavior and might be used as a model of human abnormal aggressive behavior. We assumed that behavioral alterations in socially deprived animals are accompanied by profound changes in autonomic arousal and stress reactivity during social encounters, and alterations in the functioning of two major neurotransmitter systems (5-HT and substance P), which are the first line candidates of pharmacological interventions in these disorders. Specific aims of the present project were (1) to characterize aggression and related behavioral changes associated with early social deprivation, (2) to evaluate autonomic and HPA axis reactivity during social encounters in the model, (3) to describe brain mechanisms underlying behavioral abnormalities associated with social deprivation, and (4) to assess pharmacological profile of 5-HT and substance P system in aggression induced by social deprivation. Below we briefly report results of our studies.

3. Results

3.1. Behavioral characterization of rats after post-weaning social isolation

3.1.1. Aggression

In all experiments during the study period we were able reproduce all aggression-related changes after post-weaning social isolation, upon which our hypotheses were based (Toth et al., 2008): in rats socially isolated from weaning a marked increase was present both in the number of attacks and the share of attacks targeting vulnerable body parts of the opponent (head, throat, belly) in the resident/intruder test. The increase in bite attacks was accompanied by an increase in both offense and defense, indicating disturbed social communication. The attack/threat ratio was shifted towards attacks and a significant portion of attacks were not preceded by offence, suggesting a decrease in intention signaling. (**Fig. 1.**, Tóth et al.2012). During aggressive encounters, socially deprived rats rapidly switched from one behavior to another, i.e. showed an increased number of behavioral transitions as compared to controls. We tentatively termed this behavioral feature "behavioral fragmentation" and considered it a form of behavioral arousal. ***Thus, early social deprivation induces abnormal forms of aggression that resembles aggression-related problems in humans with a history of childhood neglect.***

3.1.2. Other behaviors

Similar phenomena were observed in the social interaction test, a test commonly used to measure social anxiety: rats socially isolated from weaning spent significantly more time with social sniffing as well as agonsitic behaviors (offense, defense) than socially reared controls. These behavioral changes were accompanied by a siginficant increase in behavioral transitions („behavioral fragmentation") (**Fig. 2A.**, Mikics et al., manuscript in preparation). In the social avoidance test, isolated rats spent more time in the opponent's compartment (in this test direct physical contact with the opponent is prohibited), and showed more transitions between chambers and increased locomotor activity compared to controls .In non-social novelty-related tests, the elevated plus-maze and open-field tests, isolated rats showed a moderate but significant increase in anxiety. Also, they expressed increased locomotor activity in the open-field test in the second 5 minutes of testing, suggesting reduced habituation to the novel situation. **(Fig. 2B** and **2C**). In the forced swim test, isolated rats spent significantly less time with immobility and showed an increase in the frequency of swimming movements that we considered as a sign of increased behavioral arousal. Moreover, our recent preliminary analysis revealed multiple correlations between certain behavioral parameters and glucocorticoid responses displayed in different test situations (i.e. aggression in the social interaction test and exploratory behavior on the elevated plus-maze; Mikics et al., manuscript in preparation). This suggests that correlated sets of individual behavioral and physiological characteristics exist and underlines the significance of individual vulnerability to early social adversities.

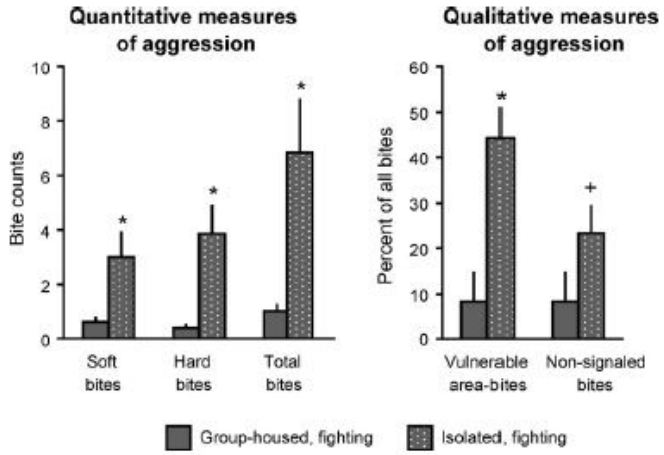

Fig.1. Quantitative and qualitative changes of aggressive behavior resulting from post-weaning social isolation. Isolated animals showed increased attack counts and abnormal attack patterns. *, p < 0.017; +, p = 0.079.

Thus, rats socially deprived from weaning showed a wide array of behavioral changes including marked social deficits, mild anxiety, hyperactivity and increased behavioral arousal. Although some of these effects were already published in the literature (e.g. locomotor activity, anxiety), these behavioral changes and their correlations have not yet been investigated in such detail before.

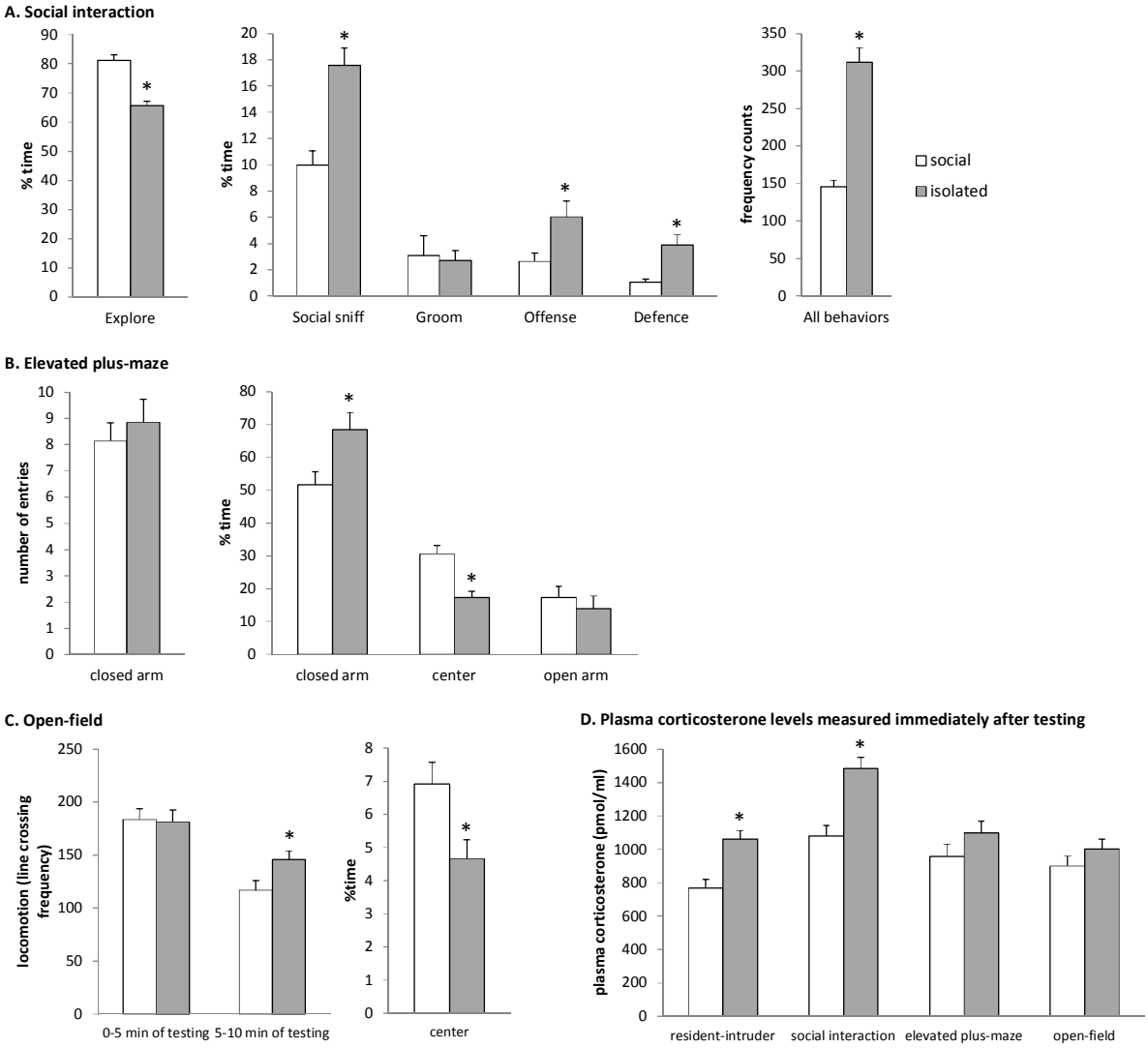

Fig. 2. Effects of early social deprivation on behavior in the social interaction test (A), elevated plus-maze (B), open-field (C) and glucocorticoid reactivity in different situations (D). *, significant difference from socially reared controls; *p<0,02.

3.1.3. Behavioral effects of resocialization in adulthood

In these experiements, we studied whether violent aggression resulted from post-weaning social deprivation could be reversed by resocialization in adulthood. During the first weak of resocialization, isolation-reared rats showed multiple social deficits including increased defensiveness and decreased huddling during sleep. Deficits were markedly attenuated in the second and third weeks. Despite improved social functioning in groups, isolated rats readily showed abnormal features of aggression in a resident-intruder test performed after the 3-week-long resocialization. ***Thus, early social deprivation-induced deficits in prosocial behavior were eliminated by resocialization during adulthood, but abnormal aggression was resilient to this treatment.*** (**Fig. 3.** and **4**., Tulogdi et al., 2012)

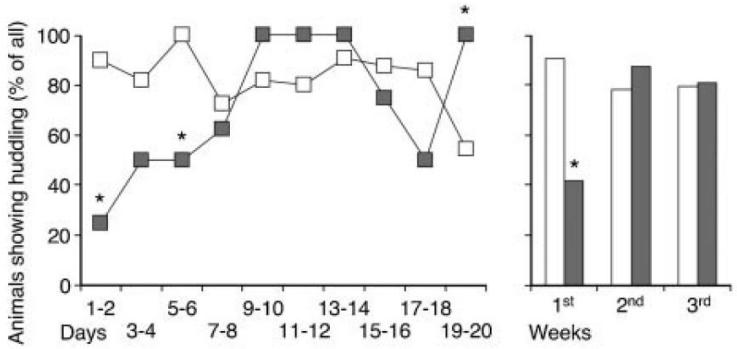

Fig. 3. Decreased sleep-related huddling in isolated rats during the inactive period of the day. Percentage of rats showing huddling (rats which slept in direct physical contact with at least one cage mate are shown. In the left-hand panel, 2 consecutive days were considered together, in the middle panel, weekly averages are shown.White boxes: socially reared rats; grey boxes: isolation-reared rats resocialized for 3 weeks. *,significant difference from socially reared rats (p <0 .05).

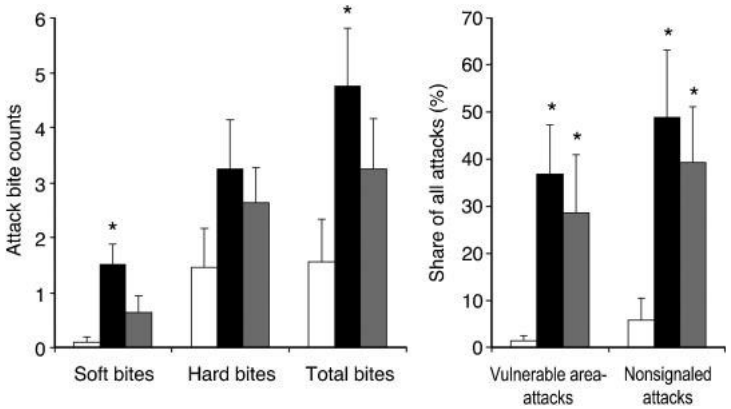

Fig. 4. Attack counts in the second resident-intruder test, which followed the 3-week-long resocialization period. White columns: socially reared rats; black columns: isolation-reared rats that were left isolated throughout; grey columns: isolation-reared rats resocialized with other isolation-reared rats for 3 weeks. *,significant difference from socially reared rats (p<0.05).

3.2. Arousal –related description of aggressive behavior of socially deprived animals

Basal levels of plasma corticosterone regularly assessed by radioimmunoassay between 27 and 78 days of age were not affected by social isolation. In contrast, aggression-induced glucocorticoid responses were approximately doubled by early socially deprivation (**Fig 5.**, Toth et al,.2011). We have recently also shown that increased glucocrticoid responses were only present after social challenges i.e. in the resident-intruder or social interaction tests, whereas plasma corticosterone levels did not differ between groups in non-social, novelty-related situations such as the elevated plus-maze and open-field tests (**Fig. 2D.**, Mikics et al., manuscript in preparation) suggesting enhanced endocrine arousal in conjunction with social challenges only.

Diurnal oscillations in heart rate assessed by in vivo biotelemetry were not affected by social isolation. In contrast, the aggression-induced increase in heart rate was higher in socially isolated than in socially housed rats (**Fig. 6.,** Toth et al., 2011). This difference was not due to differences in workload as locomotor activity during aggressive interactions did not differ between groups. Autonomic hyperarousal seemed to be stable over time as it was present during repeated aggressive interacions aswell (data not shown here).

Thus, post-weaning social isolation induced abnormal forms of aggression that developed on the background of increased behavioral, endocrine and autonomic arousal.

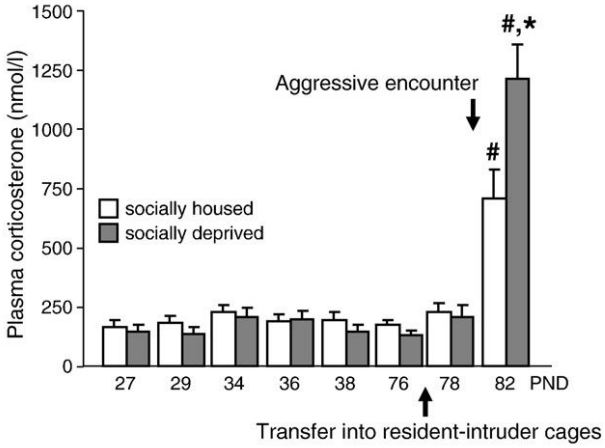

Fig. 5. Basal corticosterone levels during development and the response to aggression in the resident-intruder test. *, significant increase compared to socially housed subjects (p<0.01); #, significant increase compared to basal levels (p<0.01).

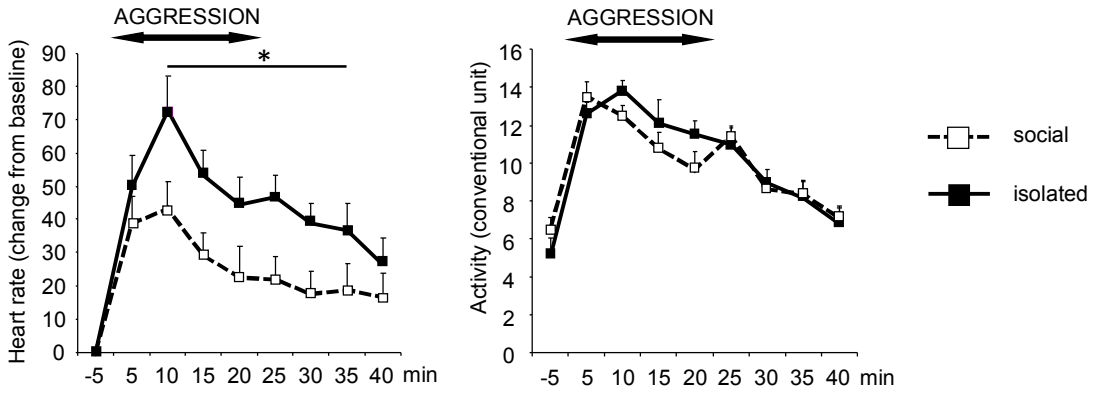

Fig.6. Heart rate changes and locomotor activity during the 20-min-long aggressive encounter. The duration of the interaction is indicated. *, significant difference from socially reared controls.

3.3. Brain mechanisms underlying behavioral abnormalities associated with social deprivation

3.3.1. Neural activation patterns during aggressive interaction

We studied the neural background of this type of aggression by assessing the expression of the activation marker c-Fos in 22 brain areas of male Wistar rats submitted to resident–intruder conflicts. Post-weaning social isolation readily produced the behavioral alterations noticed earlier. Social isolation significantly increased the activation of brain areas that are known to directly or indirectly control inter-male aggression. Particularly, the medial and lateral orbitofrontal cortices, anterior cingulate cortex, medial prefrontal cortex (mPFC, infralimbic and prelimbic nuclei), bed nucleus of the stria terminalis, medial and basolateral amygdala, hypothalamic attack area, hypothalamic paraventricular nucleus and locus coeruleus showed increased activations (**Fig. 7.**, for exact data see Toth et al., 2012) compared to socially reared, normally aggressive controls. Using immunohistochemical methods, we have recently found a significant decrease in mPFC volume and glial density of isolated rats. Using high accuracy analysis methods we also demostrated that the over-activation of mPFC of isolated animals is restricted to those subregions of the prelimbic and infralimbic cortices that were previously shown to directly innervate hypothalamic attack area, the brain area that directly controls aggressive attcks (unpublished data).

Brain activation patterns of isolated rats contrast our earlier findings obtained in rats with experimentally induced hypoarousal, where abnormal attack patterns were associated with over-activated central amygdala, lateral hypothalamus, and ventrolateral periaqueductal gray that are believed to control predatory attacks (**Fig 7.**, Tulogdi et al., 2010). We have observed no similar activation patterns in rats socially isolated from weaning.

In summary, these findings suggest that despite some phenotypic similarities, the neuronal background of hypo- and hyperarousal-associated abnormal forms of aggression are markedly different. While the neuronal activation patterns induced by normal rivalry and hypoarousal-driven aggression are qualitative different, hyperarousal-associated aggression resulting from early social deprivation appears to be an exaggerated form of rivalry aggression.

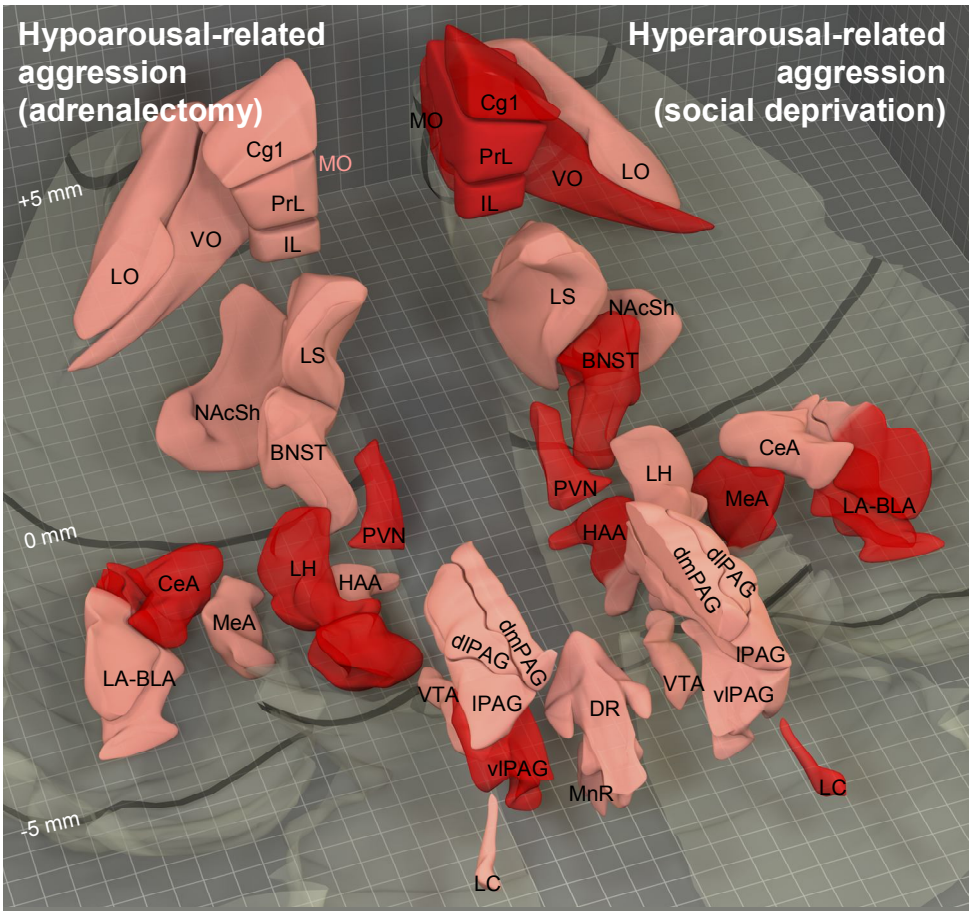

Fig.7. Aggressive interaction-induced neural activation patterns in two models of abnormal aggression. Three dimensional representation of the brain by the Blender software, based on Paxinos and Watson (1998). The left-hand side of the brain shows activation during hypoarousal-related aggression, the right-hand side shows activation during hyperarousal-related aggression. Pink brain areas show normal activaation by fighting, red brain areas are over-activated compared to normally aggressive controls. For exact results see original articles (Tulogdi et al., 2010; Toth et al., 2012).

3.3.2. Pharmacological characterization of abnormal aggression: serotonergic and substance P system

Our recent pharmacological experiments demonstrated that both the serotonergic anxiolytic 5-HT1a partial agonist buspirone and neurokinin-1 receptor antagonist L-703,606 significantly and dose-dependently reduce aggressiveness of socially deprived animals, and in particular, both compounds selectively decrease abnormal forms of aggression aswell (attacks targeted at vulnerable body parts of opponents and non-signaled attacks) (**Fig. 8 and 9.**, Mikics et al., unpublished data) ***suggesting an important regulatory role of 5-HT1A and NK-1 receptors (the main brain receptors of substance P) in hyperarousal-related abnormal aggression that results from early life adversities***.

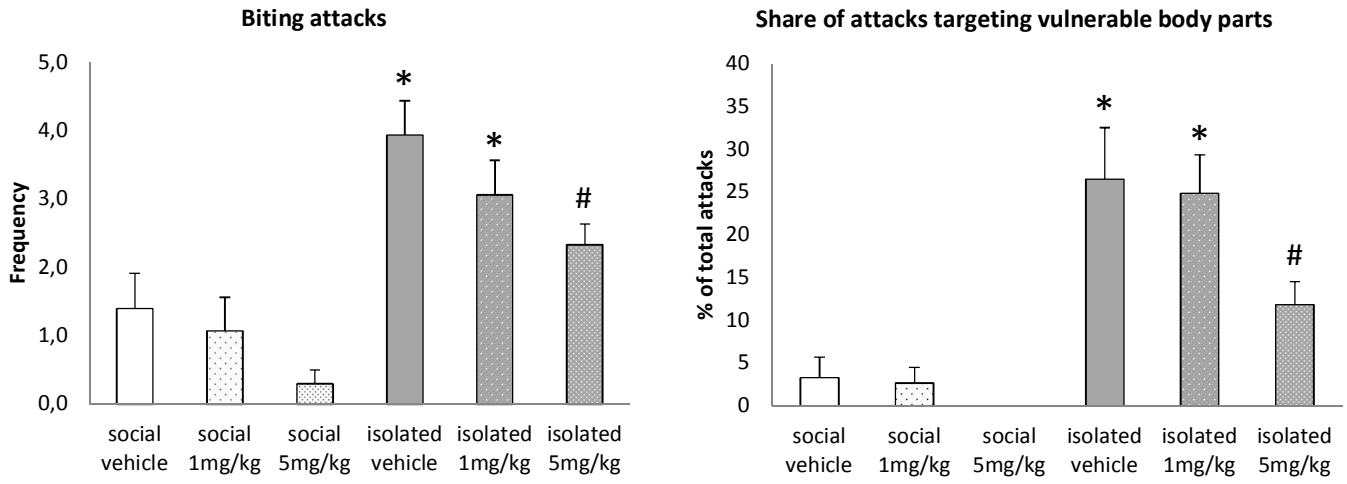

Fig.8. Effects of 5-HT1a receptor partial agonist buspirone on post-weaning social isolation induced abnormal aggression. Buspirone was administered 60min before resident-intruder test in doses of 0 (vehicle), 1 and 5 mg/kg. *:significant difference from 'social vehicle' group, *p<0,02; #:significant difference from 'isolated-vehicle' group, #p<0,03

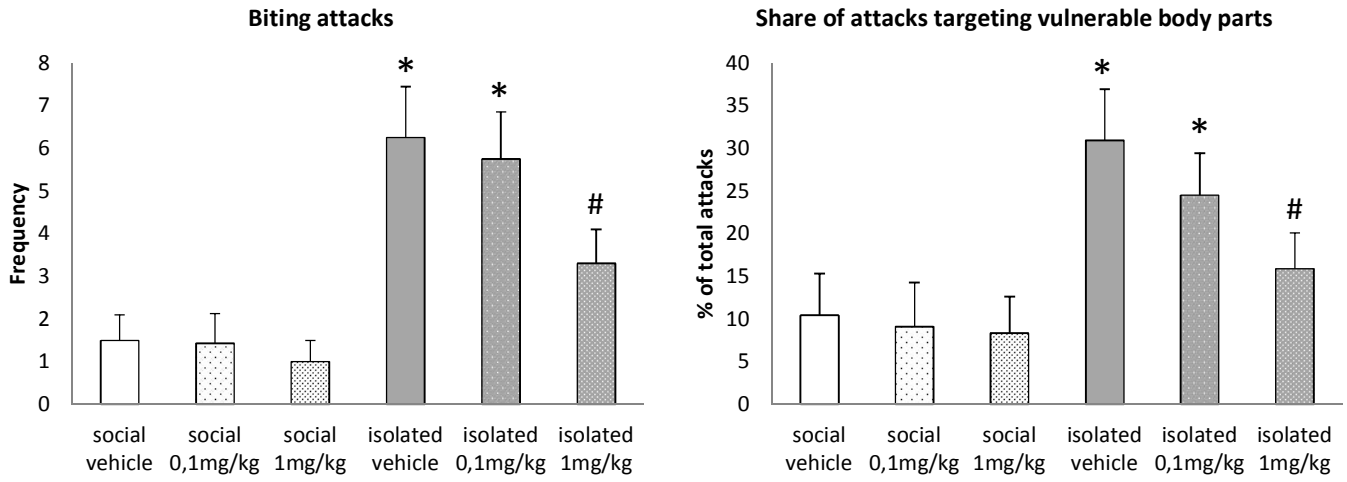

Fig.9. Effects of NK-1 receptor antagonist L-703,606 on post-weaning social isolation induced abnormal aggression. L-703,606 was administered 60min before resident-intruder test in doses of 0 (vehicle), 0,1 and 1 mg/kg. *:significant difference from 'social vehicle' group, *p<0,03; #:significant difference from 'isolated-vehicle' group, #p<0,03

3.3.3. Serotonergic neurotransmission-related gene expression levels in brain areas relevant for aggression control

Using real-time reverse transcription quantitative polymerase chain reaction (qRT-PCR) technique, we measured mRNA expression levels of major components of the serotonergic system in two key brain regions of aggression control, the medial prefrontal cortex and the medial amygdala. The reason of focusing on these brain areas was that they showed profound agression-induced over-activation in socially deprived rats (see above) and their is evidence that they play crucial roles in human abnormal aggression aswell.

We found changes in mRNA expression levels of the serotonin metabolizing enzyme monoamine oxydase A (MAOA) in the medial prefrontal cortex (mPFC) and the medial amygdala: while MAOA mRNA levels were significantly higher in socially deprived rats under resting conditions (no fight), aggressive intraction-induced MAOA increase of socially reared rats was reversed in isolated rats in both brain regions (**Fig 10.,** Mikics et al., manuscript in preparation). These findings may help to explain the complex interaction between MAOa variants, childhood neglect and violent behavior in adulthood in humans.

There were no differences in 5-HT1a receptor mRNA levels between groups, however, there was a significant negative correlation between 5-HT1a mRNA levels in the mPFC and medial amygdala that was accompanied by inverse correlations between plasma corticosterone levels and 5-HT1a mRNA levels in the two brain regions in non-fighting isolated rats (data not shown). As 5-HT1a receptor partial agonist buspirone dose-dependently increases plasma glucocorticoid levels and decreases abnormal aggression of isolated rats (see above), these results reflect the possible key role of this receptor in the control of hyperarousal-related abnormal aggression and underline the importance of further studies in this direction (Mikics et al., manuscipt in preparation).

In summary, post-weaning social isolation induces specific changes in key components of serotonergic function in the medial prefrontal cortex and medial amgdala, that may explain complex human findings.

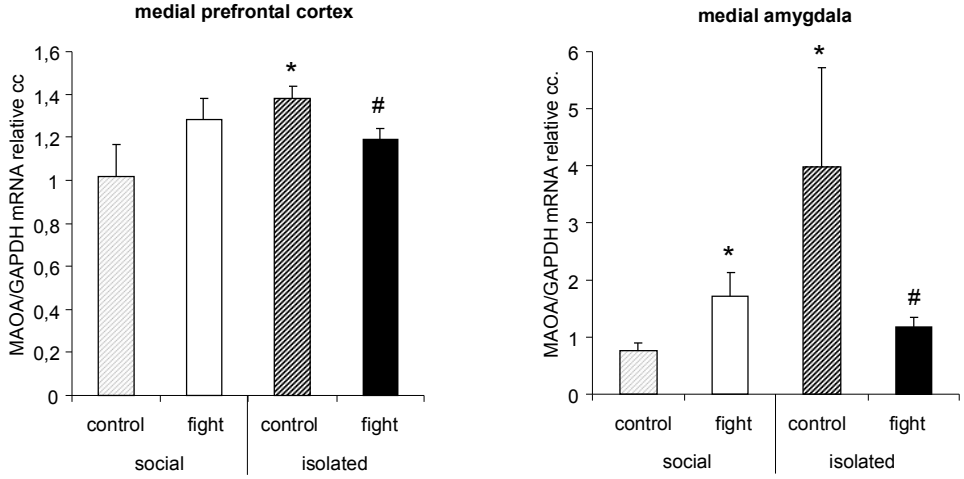

Fig. 10. Changes in MAOA mRNA expression levels in the medial prefrontal cortex and medial amygdala of socially reared or isolated animals under basal conditions (control) and 60min after fighting (fight). Relative changes to the housekeeping gene GAPDH are shown. *, significant difference from „control-social", *p<0.04; #significant difference from „fight-isolated" #p<0.05

3.3.4. Epigenetic changes in brain areas relevant for aggression control

It was recently suggested that a specific focus on epigenetic mechanisms will most likely advance our understanding of the biopsychosocial mechanisms that lead to abnormal aggression and this approach has a great promise for identifying effective intervention strategies. Here we investigated changes in mRNA levels of key epigenetic enzymes in the medial prefrontal cortex and the medial amygdala using qRT-PCR methods. According to our preliminary findings, there is a significant decrease in the epigenetic enzyme DNA methyltransferase 1 (DNMT1) expression in the medial prefrontal cortex of adult rats after post-weaning social isolation (**Fig 11**., Mikics et al., unpublished data). mRNA expression levels of another epigenetic enzyme DNMT3a showed no changes among groups, however, there was a significant positive correlation between DNMTA3a mRNA levels (measured 60min after after aggressive interaction) in the medial amygdala and biting attacks, and in particular with the frequency of abnormal attacks tageting vulnerable body parts of the opponent (**Fig 12.**, Mikics et al., unpublished data). ***This suggests that post-weaning social isolation results in chronic epigenetic changes, and abnormal aggression induces acute epigenetic changes in the brain. Thus, epigenetic changes in the brain may directly mediate the aggression-related effects of early life adversities.***

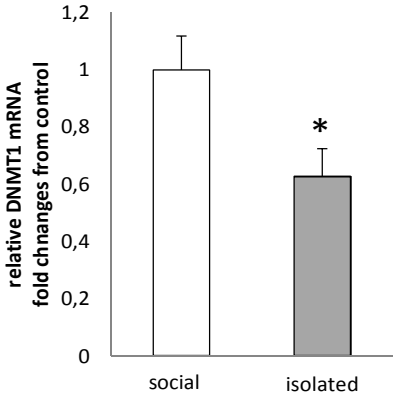

Fig.11. Effects of post-weaning social isolation on DNA methyl transferase 1 (DNMT1) gene expression levels in the medial prefrontal cortex. DNMT1 levels were normalized to housekeeping gene GAPDH. *p: significant difference from social group, *p<0,02

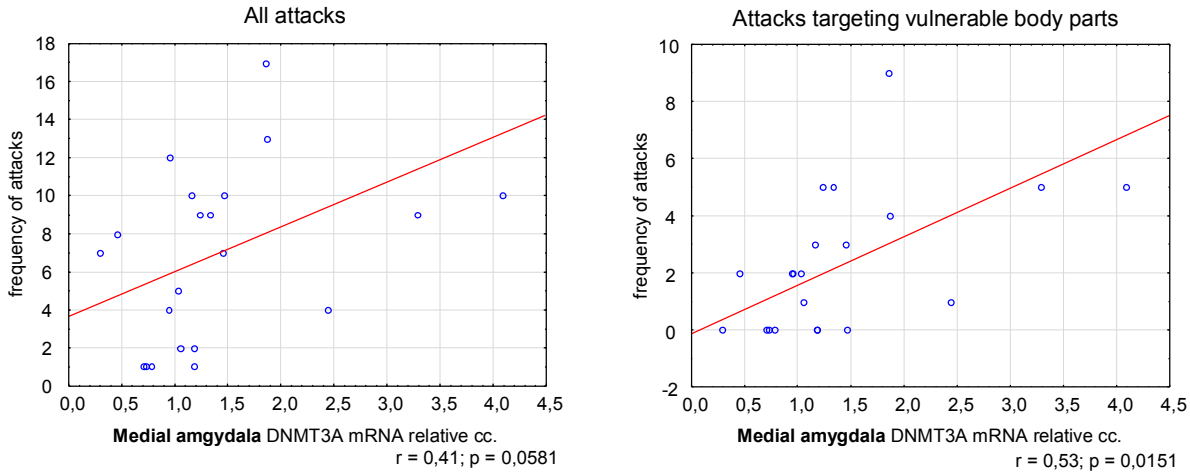

Fig. 12. Correlation between frequency of attacks (left) and abnormal attacks (right) with DNMT3a mRNA expression levels in the medial amygdala. Brain punches were collected 60min after fight. DNMT3a levels were normalized to housekeeping gene GAPDH.

4. Summary

Abnormal aggression is a relatively frequent problem that is an enormous burden to the individual and the society. The lack of understanding the underlying pathomechanisms hampers its therapy. We have successfully characterized a highly relevant model of abnormal aggressive behavior based on altered psychosocial development. We demonstrated that rats socially deprived from weaning showed a wide array of correlated behavioral changes including marked social deficits, mild anxiety, hyperactivity and increased behavioral arousal. Early social deprivation-induced deficits in prosocial behavior were eliminated by resocialization during adulthood, but abnormal aggression was resilient to this treatment. Abnormal aggression of isolated rats was accompanied by marked automonic and endocrine arousal, and elicited overactivation of several brain regions relevant for aggression control. Pharmacological and molecular

studies highlighted the specific and complex role of the serotonergic and substance P systems in abnormal aggression resulting from early social adversities. Finally we showed that social isolation results in chronic epigenetic changes, and in turn, abnormal aggression induces acute epigenetic alterations in the brain. Thus, epigenetic mechanisms in the brain may directly mediate the aggression-related effects of early life adversities. Our results may lead to the identification of effective treatment strategies for human abnormal aggression. We thank OTKA for funding the research project.

5. Published articles of this research

Tulogdi A, Toth M, Halasz J, Mikics E, Fuzesi T, Haller J.: Brain mechanisms involved in predatory aggression are activated in a laboratory model of violent intra-specific aggression., Eur J Neurosci. 32(10): 1744-53., 2010.

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. 60(1):28-36, 2011.

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. 233(1):120-9, 2012.

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., in press DOI 10.1002/dev.21090, 2012.