Final report of the project entitled

THE ROLE OF THE PREFRONTAL CORTEX IN AGGRESSION: EMOTIONAL/COGNITIVE PROCESSING AND THE ACTIVATION OF SUBCORTICAL EXECUTIVE FUNCTIONS

(NKFI 112907)

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Introduction

Theories regarding the role of the prefrontal cortex in aggression were earlier dominated by the concept of inhibitory control, which states that aggressiveness is inhibited by this brain area mainly by its connections to various subdivisions of the amygdala. Although widely accepted, this theory was questioned by a series of experimental studies performed in both animals and humans. The starting point of our reasoning were these conflicting findings partly coming from our laboratory. We hypothesized that the medial prefrontal cortex does not affect aggression as a whole but by a series of neural subpopulations, the impact of which on aggression may be even opposite, by this enabling the brain area to fine-tune aggressive and social behavior in a more complex manner than generally thought. We proposed to study separately the role of such neuron populations mainly by axonal optogenetics.

The medial prefrontal cortex-hypothalamus connection

Our hypothesis was confirmed best when we studied those prefrontal neuron populations, which target two hyppothalamic areas involved in the execution of attacks, particularly the mediobasal and lateral hypothalamus (Biro et al., J. Neurosci, 2018). In these studies we established in male rats that dominantly distinct medial prefrontal neuron populations project to and produce dense fiber networks with glutamate release sites in the mediobasal hypothalamus (MBH) and lateral hypothalamus (LH; i.e., two executory centers of species-specific and violent bites, respectively). Optogenetic stimulation of medial prefrontal terminals in MBH distinctively increased bite counts in resident/intruder conflicts, whereas the stimulation of similar terminals in LH specifically resulted in violent bites. No other behaviors were affected by stimulations. These findings show that the medial prefrontal cortex controls aggressiveness by behaviorally dedicated neuron populations and pathways, the roles of which may be opposite to those observed in experiments where the role of the whole medial prefrontal cortex (or of its major parts) has been investigated. Overall, our findings suggest that the medial prefrontal cortex organizes into working units that fulfill specific aspects of its wide-ranging roles.

An excerpt of these findings can be found in Fig. 1 and 2.

The mediobasal prefrontal cortex-amygdala connection

The next step was to investigate the aggression-related roles of those prefrontal neuron populations that target the amygdala. To this end

(1) We evaluated whether neurons projecting to the above-mentioned two hypothalamic sites are different or similar to those projecting to various amygdala sites (medial, central, and basolateral amygdala). These studies were performed by a series of triple retrograde stainings.

(2) We studied the role of prefrontal cortex-amygdala connections by axonal optogenetics as with hypothalamic projections.

The results of these studies are under analysis. The studies were completed late because the execution of triple retrograde staining was more difficult and time-consuming than foreseen. Our findings obtained so far suggest that prefrontal neurons targeting the amygdala are overwhelmingly different from those that project to the hypothalamus, and their ultimate role is inhibitory, but this also depends on the amygdala subdivision targeted by optogenetics. We estimate that the evaluation of the findings and the writing of the manuscript may take another year.

The location of prefrontal neurons involved in abnormal aggression

We showed previously, that early stressors result in adulthood in abnormal aggression characterized primarily by attacks delivered on vulnerable body parts of opponents (head, throat, belly), and the diminishment of social signals that precede such attacks. The study shortly presented above suggested that such behaviors may be controlled by the prefrontal cortex – lateral hypothalamus pathway. We investigated the role of the prefrontal cortex in aggression, and the location of aggression-controlling neurons mainly by voxel-based neural activation mapping (Biro et al., Brain Struct Funct, 2017). We made use of the post-weaning social isolation paradigm (PWSI), an established laboratory model of abnormal aggression. When studied in the resident-intruder test as adults, rats submitted to PWSI showed increased attack counts, increased share of bites directed towards vulnerable body parts of opponents (head, throat, and belly) and reduced social signaling of attacks. These deviations from species-typical behavioral characteristics were associated with a specific reduction in the thickness of the right medial PFC (mPFC), a bilateral decrease in dendritic and glial density, and reduced vascularization on the right-hand side of the mPFC. Thus, the early stressor interfered with mPFC development. Despite these structural deficits, aggressive encounters

enhanced the activation of the mPFC in PWSI rats as compared to controls. A voxel-like functional analysis revealed that overactivation was restricted to a circumscribed sub-region, which contributed to the activation of hypothalamic centers involved in the initiation of biting attacks as shown by structural equation modeling. These findings demonstrate that structural alterations and functional hyperactivity can coexist in the mPFC of rats exposed to early stressors, and suggest that the role of the mPFC in aggression control is more complex than suggested by the inhibitory control theory.

Noteworthy, the location of prefrontal neurons involved in the control of abnormal aggression was largely similar to those neurons that project to the lateral hypothalamus. An excerpt of the main findings was represented in Fig. 3.

Prefrontal neuronal plasticity and abnormal aggression

Our earlier studies suggested that abnormal aggression elicited by post-weaning social isolation paradigm is resistant to social learning to which rats were subjected in adulthood. We hypothesized that this inability of social learning to "correct" abnormal behavior is explained by the adulthood-specific decrease in neural plasticity. We studied this issue by studying the effects of social learning and the plasticity enhancer fluoxetine in rats submitted to the post-.weaning social isolation model of abnormal aggression (Mikics ezt al., Neuropsychopharmacology, 2017). We found that synergistic interactions between psychosocial and biological factors specifically ameliorate escalated aggression induced by early adverse experiences. Rats reared in isolation from weaning until early adulthood showed abnormal forms of aggression and social deficits that were temporarily ameliorated by re-socialization, but aggression again escalated in a novel environment. We demonstrate that when re-socialization was combined with the antidepressant fluoxetine, which has been shown to reactivate juvenile-like state of plasticity, escalated aggression was greatly attenuated, while neither treatment alone was effective. Early isolation induced a permanent, re-socialization-resistant reduction in Bdnf expression in the amygdala and the infralimbic cortex. Only the combined treatment of fluoxetine and re-socialization was able to recover Bdnf expression via epigenetic regulation. Moreover, the behavior improvement after the combined treatment was dependent on TrkB activity. Combined treatment specifically strengthened the input from the ventral hippocampus to the medial prefrontal cortex, suggesting that this pathway is an important mediator of the beneficial behavioral effects of the combined psychosocial and pharmacological treatment of abnormal aggression. Our findings suggest that synergy between pharmacological induction of plasticity and

psychosocial rehabilitation could enhance the efficacy of therapies for pathological aggression, particularly in the medial prefrontal cortex.

Our findings were exemplified in Fig. 4 and 5.

The role of serotonergic nuclei in abnormal aggression

Serotonergic mechanisms hosted by raphe nuclei have important roles in affiliative and agonistic behaviors but the separate roles of the two nuclei are poorly understood. Therefore, we studied the roles of the dorsal (DR) and median raphe region (MRR) in aggression by optogenetically stimulating the two nuclei, with special reference to the neural phenomena observable in the prefrontal cortex (Balázsfi et al., Behav Neurosci, 2018). Mice received three 3 min-long stimulations, which were separated by non-stimulation periods of 3 min. The stimulation of the MRR decreased aggression in a phasic-like manner. Effects were rapidly expressed during stimulations, and vanished similarly fast when stimulations were halted. No carryover effects were observed in the subsequent three trials performed at 2-day intervals. No effects on social behaviors were observed. By contrast, DR stimulation rapidly and tonically promoted social behaviors: effects were present during both the stimulation and non-stimulation periods of intermittent stimulations. Aggressive behaviors were marginally diminished by acute DR stimulations, but repeated stimulations administered over 8 days considerably decreased aggression even in the absence of concurrent stimulations, indicating the emergence of carryover effects. No such effects were observed in the case of social behaviors. We also investigated stimulation-induced neurotransmitter release in the prefrontal cortex, a major site of aggression control. MRR stimulation rapidly but transiently increased serotonin release, and induced a lasting increase in glutamate levels. DR stimulation had no effect on glutamate, but elicited a lasting increase of serotonin release. Prefrontal serotonin levels remained elevated for at least 2 h subsequent to DR stimulations. The stimulation of both nuclei increased GABA release rapidly and transiently. Thus, differential behavioral effects of the two raphe nuclei were associated with differences in their neurotransmission profiles. These findings reveal a surprisingly strong behavioral task division between the two raphe nuclei, which was associated with a nucleus-specific neurotransmitter release in the prefrontal cortex.

Excerpts of the main findings were shown in Fig. 6.

Other studies

We performed in addition to those mentioned above several studies, which

- prepared the field for subsequent studies e.g. by investigating the brain areas involved in the control of abnormal aggression (Tulogdi et al., Behav Brain Res, 2015)

- studied the neurochemical mechanisms underlying aggression control (Aliczki et al., Psychopharmacology, 2015)

- studied aggression and related behaviors in VGLuT3-KO mice (Balázsfi et al., Stress, 2018). The rationale of these studies was that the neurochemical mechanisms of aggression revealed so far were mainly linked to glutamatergic neurotransmission.

- investigated the possibility of expanding our findings to human subjects (Haller et al., Front Behav Neurosci. 2015).

Review articles

Overall, our studies aimed at investigating novel mechanisms of aggression control and identifying novel approaches to the treatment of aggression-related psychopathologies. As ideas are difficult to explain in full in research articles, we wrote 5 review articles to substantiate our novel approaches. One of these reviews was published in Nature Reviews Neuroscience, whereas other three in Neuroscience and Biobehavioral Reviews.

Fig. 1. *Photostimulation of mPFC afferents in the LH selectively increased the share of abnormal attacks*. ***A***, The schematic of the study. ***B–E***, Variables that characterize biting attacks (for explanations, see legends below). ***F***, Behaviors recorded during resident/intruder tests. Trials were shown here as contiguous columns. ***G***, The temporal distribution of biting attacks. The presence of stimulation was indicated by the color code. Each row of vertical lines depicts bites delivered by individual rats; distance from the left-hand *y*-axis shows their timing. Curves are second-order polynomial fits of total bite frequencies over the encounter (see right-hand *y*-axis for the scale). ***H***, Changes in the share of vulnerable bites over trials in two representative rats in which stimulation was associated with the first and third or the second and fourth trials, respectively. Please see Figure 3-1 for the location of the tips of optic fibers within the LH. Abnormal attacks, Bites not preceded by social signals or aimed at vulnerable targets (e.g., the head, throat or belly); DOM, dominant posture; EXP, exploration; GRO, grooming; highly abnormal: bites targeting vulnerable body parts of opponents without social signaling; OFF, offensive behaviors; RES, resting; SOC, social interactions. *Significant difference between stimulated and nonstimulated R/I tests ($p < 0.01$).

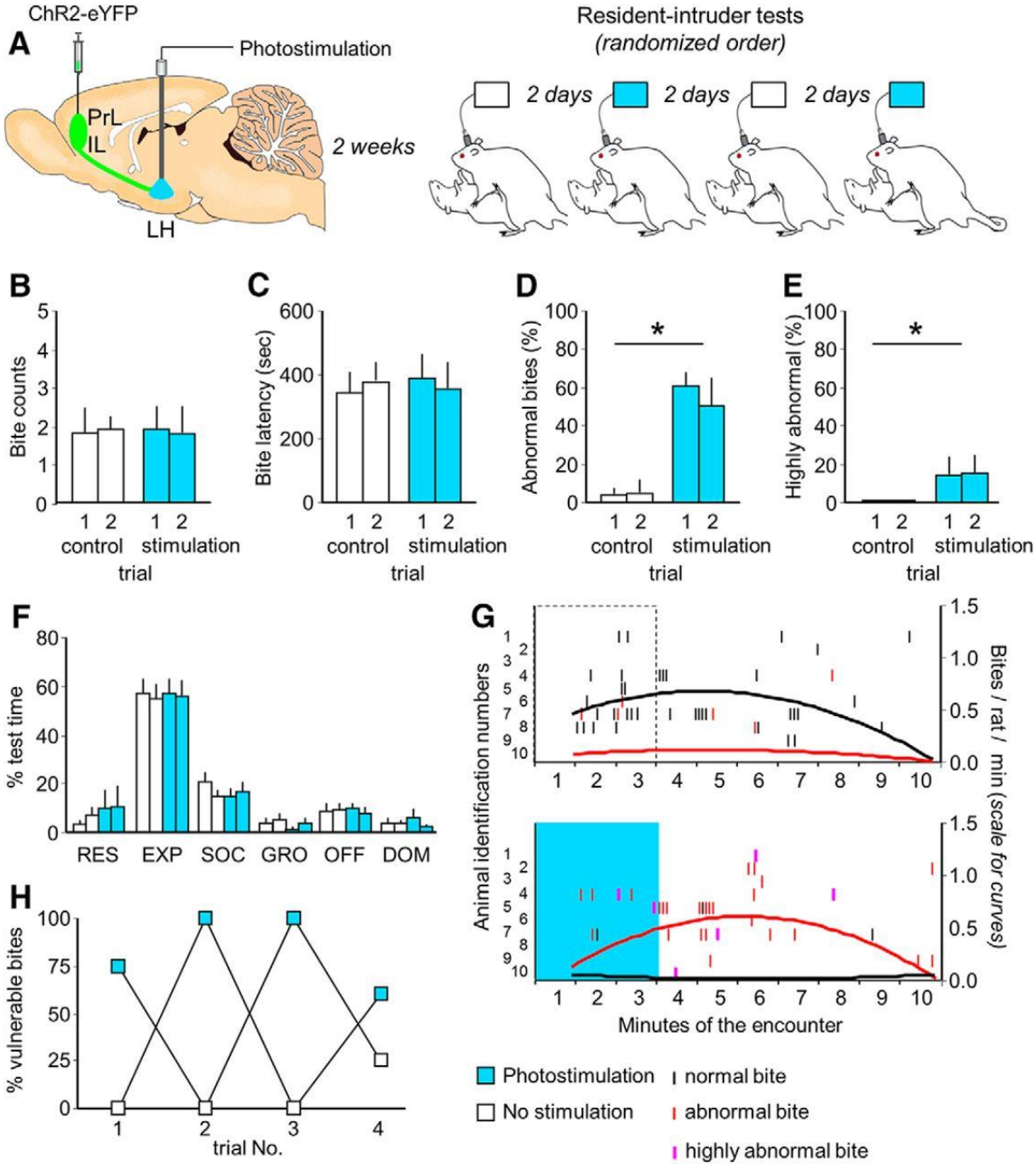


Fig. 2. *Photostimulation of mPFC afferents in the MBH selectively increased attack counts.* ***A***, The schematic of the study. ***B–E***, Variables that characterize biting attacks. ***F***, Behaviors recorded during resident/intruder tests. Subsequent trials were shown here as contiguous columns. ***G***, The temporal distribution of biting attacks. ***H***, Changes in bite counts over trials in two representative rats in which stimulation was associated with the first and third or the second and fourth trials, respectively. Please see Figure 4-1 for the location of the tips of optic fibers within the MBH. For explanations and abbreviations, see Figure 3. *Significant difference between stimulated and non-stimulated R/I tests ($p < 0.01$).

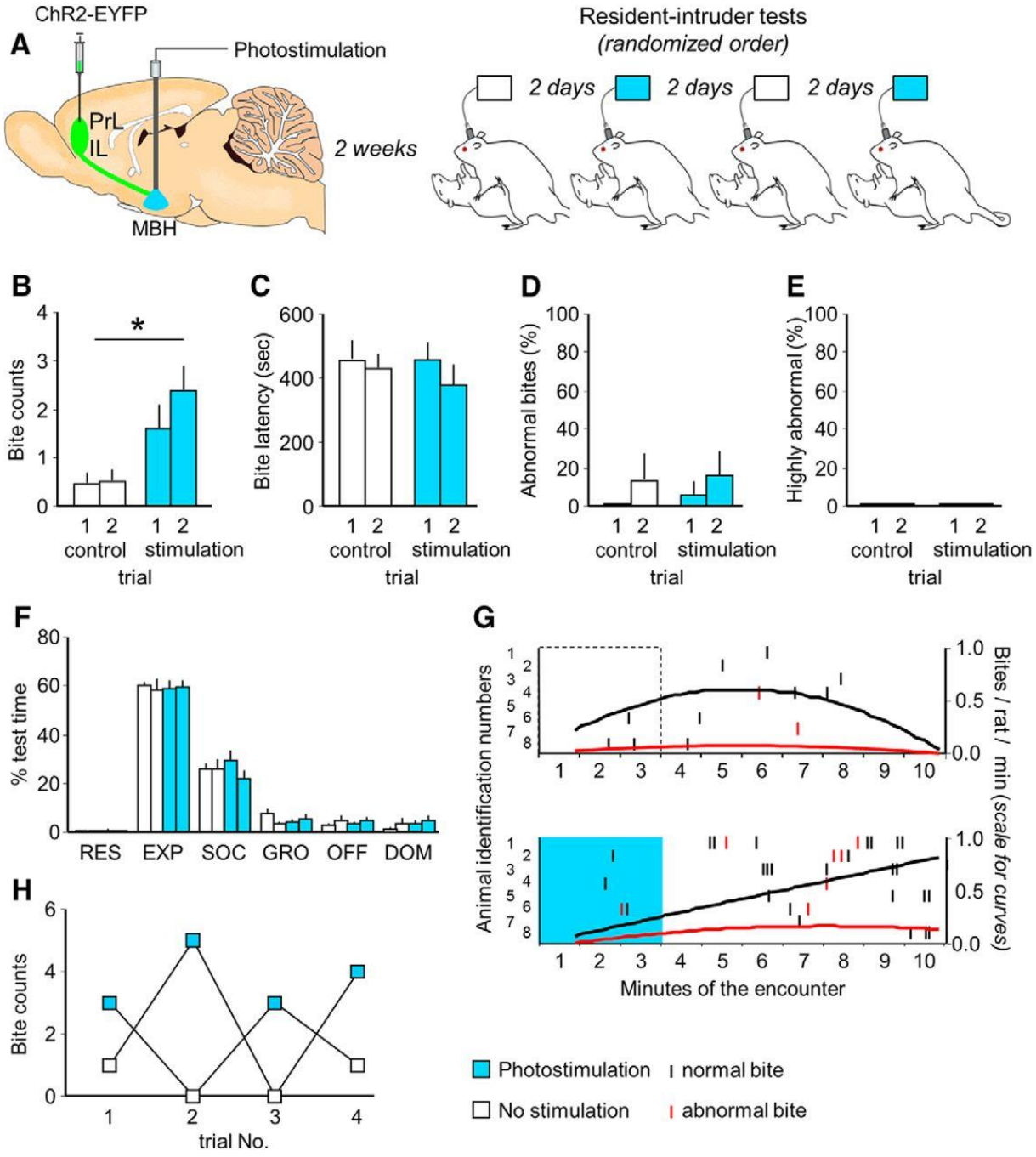


Fig. 3. *Voxel-like representation of c-Fos activation within the mPFC area investigated (Bregma 3.20-2.70)*. The grids shown in this figure are identical with the one outlined in Fig. 3a. a Differences between rats exposed and not exposed to the resident–intruder test in the control group. Row 7 was outlined by a thick frame to show the region chosen for illustration in panel b. b Graph illustrating the difference between fighting and not fighting control rats at row 7 of the grid. c Neuron numbers in control rats within the cells of the grid as shown by NeuN staining. d Differences between rats exposed and not exposed to the resident–intruder test in the PWSI group. Row 7 was outlined by a thick frame to show the region chosen for illustration in panel e. e Graph illustrating the difference between fighting and not fighting PWSI rats at row 7 of the grid. f Neuron numbers in PWSI rats within the cells of the grid as shown by NeuN staining. g Graph illustrating group differences in neuron counts at row 7 of the grid (this row was outlined in graphs c and f). Control socially reared rats, Nf not exposed to RIT (not fighting), PWSI post-weaning social isolation, RIT exposed to the resident–intruder test, and asterisk significant effect of fighting ($p < 0.05$ at least)

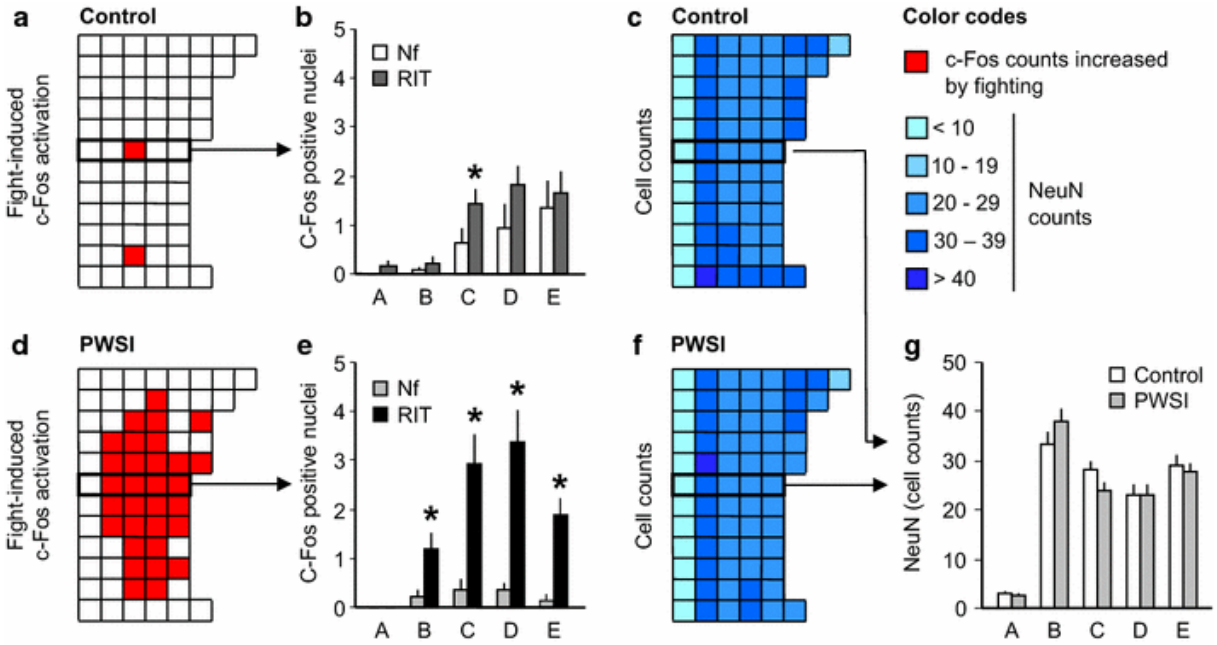


Fig. 4. *Combined social behavioral and plasticity-enhancing pharmacological treatment alters Bdnf and TrkB expression in the infralimbic cortex of isolation-reared rats*. (a) The experimental protocol for tissue dissection. (b) Post-weaning social isolation reduced the expression of both BDNF variants in the infralimbic (IL) but not in the prelimbic (PrL) cortex. The effect was abolished specifically by the combined treatment. The rearing in isolation also decreased Bdnf 1 in the lateral hypothalamus (LH), as well as Bdnf 1 and 4 levels in the medial amygdala (MeA). Fluoxetine upregulated Bdnf 1 and 4 levels independently of re-socialization (see the text) in the LH, MeA, and central amygdala (CeA). HAA, hypothalamic attack area. (c) Fluoxetine treatment induced a significant correlation between Bdnf 4 levels and the % methylated cytosine (mC) at Bdnf p4-111/-66 in the IL. (d) In the infralimbic cortex, post-weaning social isolation induced the expression of the dominant-negative isoform TrkB.T1 of Bdnf receptor TrkB, while the combined treatment restored the levels of TrkB.T1 and the ratio TrkB.FL/T1 to the levels found in socially reared rats. Data are present as mean±SEM. +p<0.05 between the treatment and socially reared control groups (two-tailed t-test); #p<0.05 compared to the Isolation treatment group (post hoc analysis). N=6–7 rats/group.

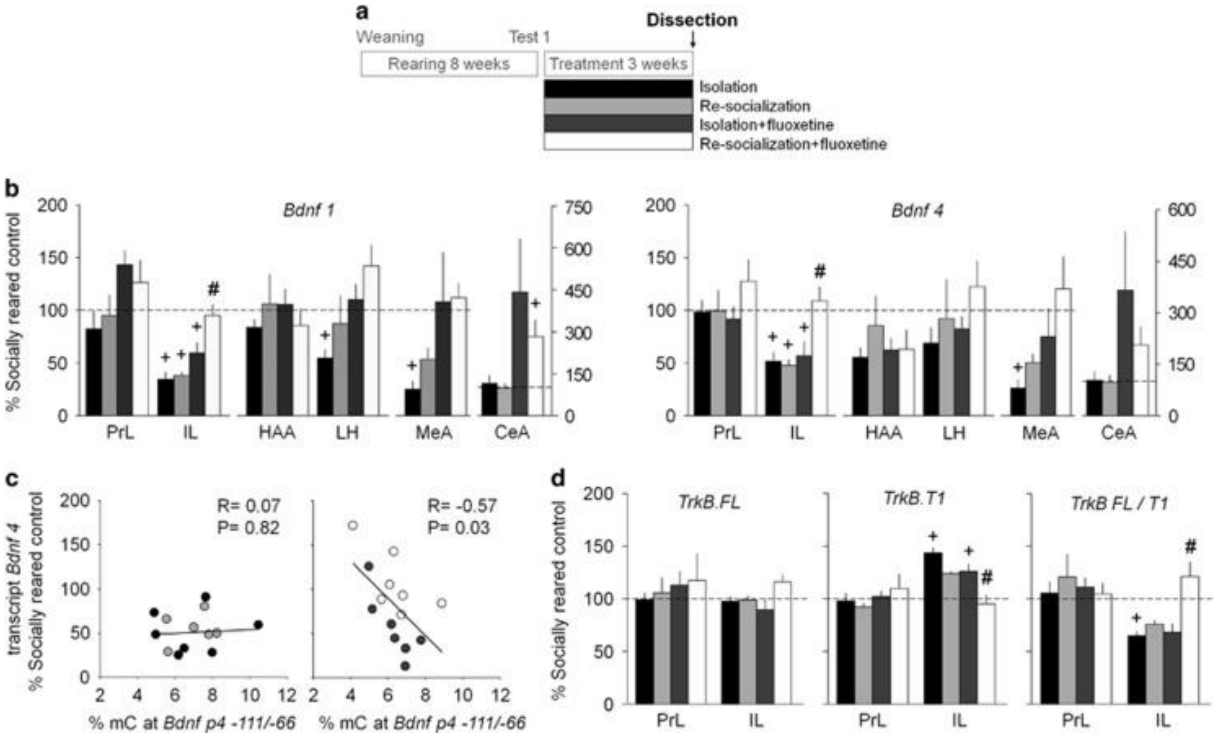
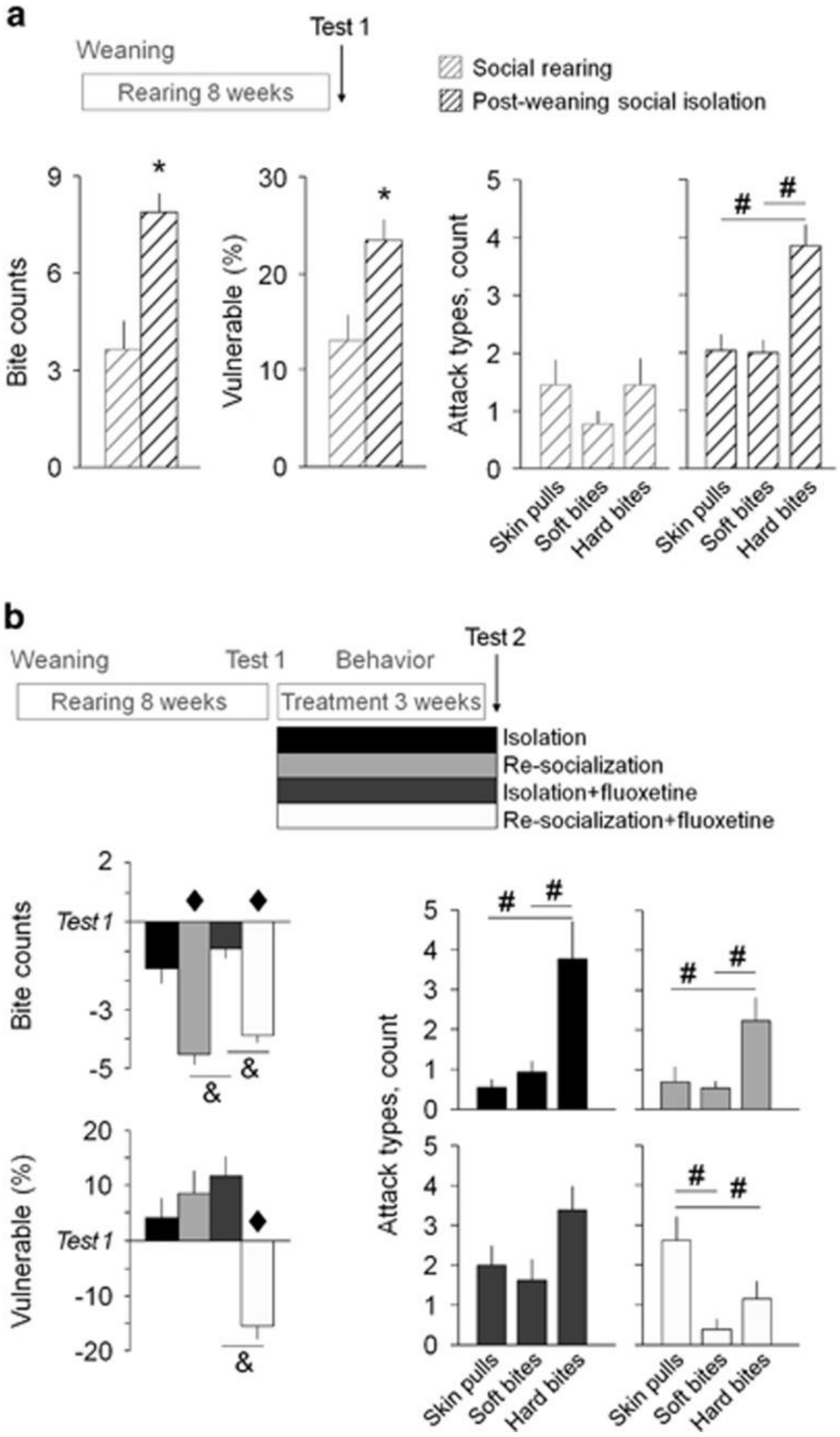


Fig. 5. Behavioral effects of post-weaning social isolation and re-socialization with or without fluoxetine treatment. (a) In the first resident-intruder test (Test 1), isolation-reared rats bit opponents more frequently, and delivered more bites to vulnerable body parts of opponents (head, throat, and belly). Hard bites were more dominant over soft bites and skin pulls in isolation-reared rats as compared to socially reared rats. N=40–80 rats/group. (b) In the second resident-intruder Test 2, the combined treatment reduced the number of bites delivered, and the share of bites aimed at vulnerable targets as compared with the Test 1. The dominance of hard bites over soft bites and skin pulls was reversed by the combined treatment Re-socialization+fluoxetine. More minor effects were observed with single treatments. N=12–13 rats/group. Data are present as mean±SEM. *$p<0.05$ between socially reared and isolation-reared groups; #$p<0.05$ between the attack types; ♦$p<0.05$ between the resident-intruder Test 1 and Test 2; &$p<0.05$ between the treatment groups.

See figure on next page.


a
Weaning
Rearing 8 weeks
Test 1
Social rearing
Post-weaning social isolation
Bite counts
Vulnerable (%)
Attack types, count
Skin pulls
Soft bites
Hard bites
b
Weaning
Test 1
Behavior
Test 2
Rearing 8 weeks
Treatment 3 weeks
Isolation
Re-socialization
Isolation+fluoxetine
Re-socialization+fluoxetine
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Bite counts
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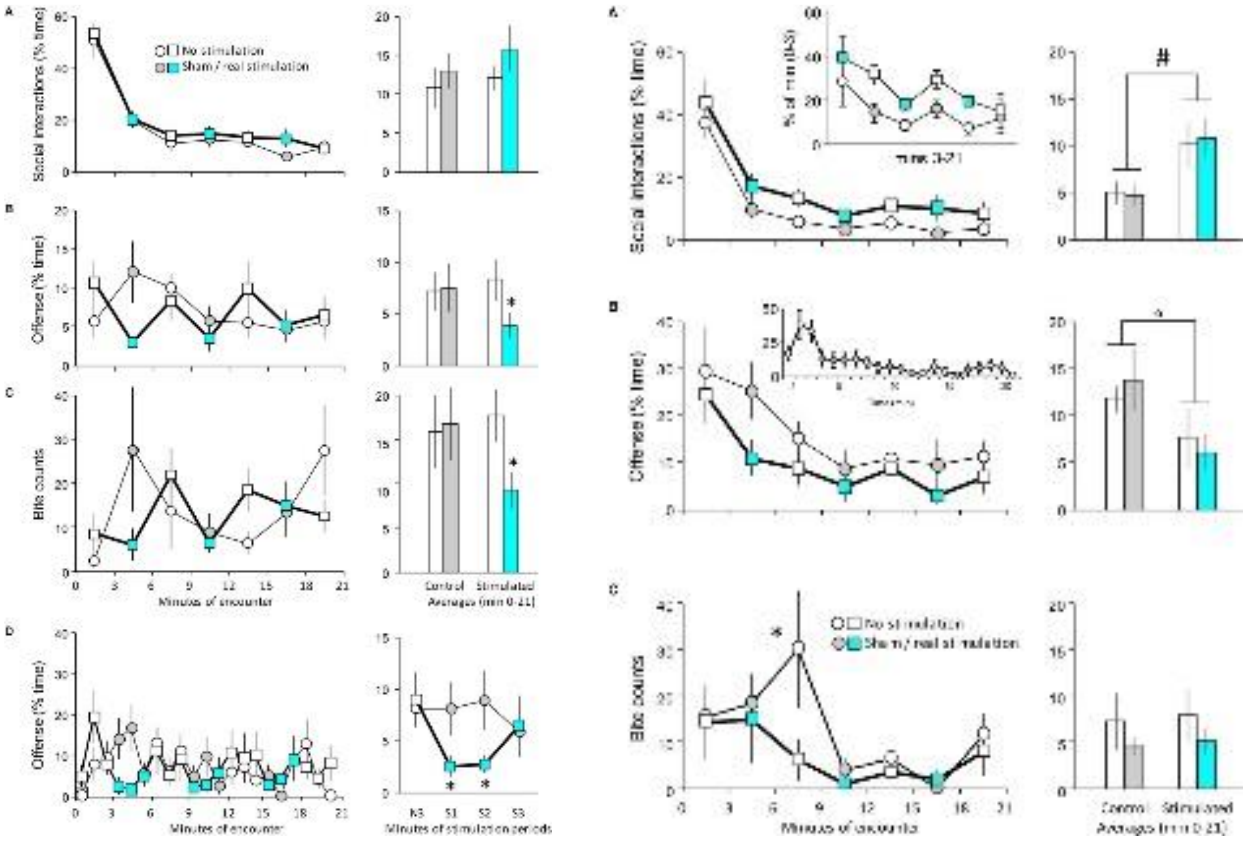
Attack types, count
Skin pulls
Soft bites
Hard bites



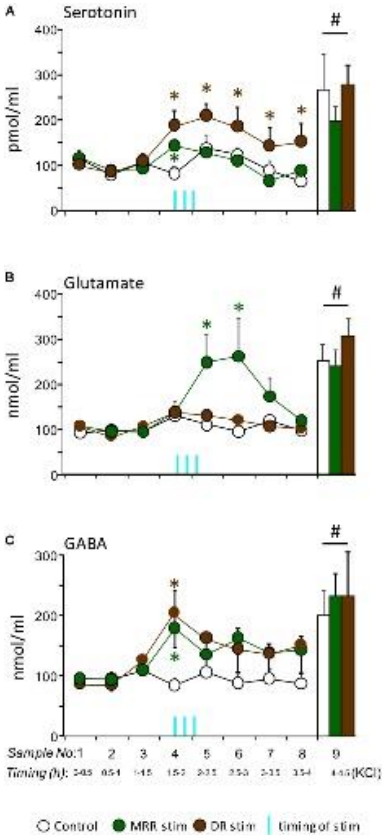


Fig. 6. *The behavioral effects of the optogenetic stimulation of the median raphe region (upper left) and dorsal raphe (upper right) as well as prefrontal neurotransmitter release in the medial prefrontal cortex (left to legends).* Left-hand panels in behavioral graphs: the duration of behaviors in 3-min bins; Right-hand panels: the average duration of behaviors (0–21 min). The timing of stimulation was indicated by the color code; circles indicate controls, squares and bold lines indicate stimulated mice. Neurochemical findings: In vivo release of serotonin (A), glutamate (B), and GABA (C) in the prefrontal cortex of mice stimulated optogenetically in their raphes (median raphe region, MRR; dorsal raphe, DR). The stimulation protocol was identical with that employed for behavioral studies. Vertical blue lines, the timing of stimulations. Note that the first stimulation was started 90 min after the last basal sampling and 15 min before the fourth sampling, whereas the third stimulations started right at the beginning of the fifth fraction. Sample sizes: control n = 6; MRR stimulation n = 9; DR stimulation n = 5. Vertical columns at the right-hand side of graphs, neurotransmitter responses to the infusion of KCl into the raphes. DR, dorsal raphe; MRR, median raphe region; ∗significant effect of stimulations compared to control levels, same time-point; #significant effect of KCl infusion as compared to baseline levels (the first three time points of each curve).