Patterns of violent aggression-induced brain c-fos expression in male mice selected for aggressiveness

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Abstract

Mice selected for aggressiveness (long and short attack latency mice; LALs and SALs, respectively) constitute a useful tool in studying the neural background of aggressive behavior, especially so as the SAL strain shows violent forms of aggressiveness that appear abnormal in many respects. By using c-Fos staining as a marker of neuronal activation, we show here that agonistic encounters result in different activation patterns in LAL and SAL mice. In LALs, agonistic encounters activated the lateral septum, bed nucleus of stria terminalis, medial amygdala, paraventricular nucleus of the hypothalamus, anterior hypothalamic nucleus and tuber cinereum area (both being analogous with the rat hypothalamic attack area), dorsolateral periaqueductal gray, and locus coeruleus. This pattern is similar with that seen in the territorial aggression of male mice, rats and hamsters, and non-lactating female mice. SALs showed strong fight-induced activations in the central amygdala and lateral/ventrolateral periaqueductal gray. In this strain, no activation was seen in the lateral septum and the dorsolateral periaqueductal gray. This pattern is similar with that seen in other models of violent aggression, e.g., in attacks induced by hypothalamic stimulation in rats, quiet biting in cats, lactating female mice, and hypoarousal-driven abnormal aggression in rats. We suggest here that the excessive activation of the central amygdala and lateral/ventrolateral periaqueductal gray-accompanied by a smaller activation of the septum and dorsolateral periaqueductal gray-underlay the expression of violent attacks under various circumstances.

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1. Introduction

There is a growing interest in abnormal forms of aggressive behavior in laboratory research. The reason is that aggressiveness per se is part of the natural behavioral repertoire of animals and, as such cannot be considered abnormal a priori. In contrast, human research on aggressiveness is often concerned with abnormal manifestations of aggressiveness, e.g., violence. These can be precipitated by brain damage, neurodegenerative disorders, drugs of abuse, hyperarousal, chronic hypoarousal, etc. [1–9]. There were several attempts to model abnormal aggressiveness in laboratory rodents. The electrical stimulation of a specific hypothalamic structure (the hypothalamic attack area) resulted in violent attacks in rats, which were out of context and disregarded species-specific “rules” (e.g., rats attacked females, their dominants, even a dead and frozen rat; [10]). In another model, abnormal attack targeting (i.e. attacks directed towards the head, throat and belly of the opponents) was used to characterize the abnormality of hypoarousal-driven aggressiveness in rats (attacks on vulnerable targets are frequent in defensive and predatory aggression, but occur sporadically in rivalry (territorial) fights between males; [11–14]). Noteworthy, multiple signs of hypoarousal (low plasma cortisol, adrenaline stress reactions, autonomic activation and skin conductance) were shown to correlate with violence in habitually violent offenders, as well as antisocial personality and conduct disorders [1,5,8,9,15–20]. Hyperarousal-driven enhanced/escalated aggressiveness (that is seen, e.g., in intermittent explosive disorder, depression, and post-traumatic stress disorder) was recently modeled by pre-exposing rats or mice to frustration (e.g., omission of a scheduled reward) or “instigation” (sensory but not physical contact with a potential
opponent: social provocation) [21–23]. Rodents that were frustrated or instigated showed escalated levels of aggressiveness in a subsequent encounter. Highly aggressive wild type rats also showed abnormal forms of aggressiveness (they disregarded social signals by opponents) when repeatedly exposed to aggressive encounters [24,25]. Such models offer the chance of understanding the distinctive features and the control of different forms of abnormal aggressiveness, which often model clinically relevant conditions. Data obtained so far demonstrate a differential neural control of abnormal and normal aggressiveness. This was shown by immunocytochemical neuronal activation studies in glucocorticoid deficiency-induced (hyperarousal-driven) abnormal aggression and pharmacological methods in the case of escalated aggressiveness [13,14,22]. Noteworthy, the neural control of abnormal aggression is specific in humans as well (this issue will be addressed in detail in Discussion).

Mice selected for aggressiveness (e.g., the short attack latency mice, SALs) constitute a useful tool in studying the neural background of abnormal attack behavior especially in comparison with its less aggressive counterpart (long attack latency mice, LALs). These mouse strains were produced by artificial selection inbred albino strain (MAS-Gro). Both types of encounters took place in the same experimental room where controls were also housed. Cages were visually separated. The length of encounters was set at 5 min as SAL mice may seriously injure their opponents if allowed to fight longer. We note that aggression tests in general last 5 min with mice. Group assignment was random, and mice were exposed to the above three treatments in a random order. Behavior was video recorded through the transparent front wall of the cage. After 5 min, the intruders were removed, and mice were left undisturbed 1 h for the development of the c-Fos signal. After 1 h, the brains were removed and processed as described below.

2. Methods

2.1. Animals

The subjects of the study were 17 LAL and 17 SAL male mice that were produced by the breeding stock of the University of Groningen. At the time of the experiment, mice were 115–129 days old, and weighted 22±2 g. They were housed together with an intact female from the age of 7–8 weeks in standard cages (17×11×13 cm). Five days before behavioral experiments, male mice were transferred into individual home/testing cages (80×30×30 cm). The behavioral experiments took place on the fifth day of individual housing. All cages were maintained under a controlled environment (22 °C, 60% humidity). Laboratory food (Hope Farms mouse pellets, Woerden, The Netherlands) and tap water were freely available. Mice were maintained at a 12h:12h day/night schedule, with lights on at 0100 h.

2.2. Experimental design

LAL and SAL mice were exposed to three different conditions. Control groups (5 LAL and 5 SAL mice) were left undisturbed in their cage, i.e. they were not exposed to social contacts. Other groups of mice (6 LALs and 6 SALs) were exposed to a 5-min psychosocial encounter with an unfamiliar male (sensory contact model; [38]). These mice were not allowed to have physical contact (they were separated from opponents by a transparent and perforated Plexiglas partition), but were allowed to see, hear and smell the other male. The partition separated the cage in two equal halves. The remaining two groups (6 LALs and 6 SALs) were allowed to interact directly with male opponents for 5 min (agonistic encounters). Oppositions belonged to a non-aggressive inbred albino strain (MAS-Gro). Both types of encounters took place in the same experimental room where controls were also housed. Cages were visually separated. The length of encounters was set at 5 min as SAL mice may seriously injure their opponents if allowed to fight longer. We note that aggression tests in general last 5 min with mice. Group assignment was random, and mice were exposed to the above three treatments in a random order. Behavior was video recorded through the transparent front wall of the cage. After 5 min, the intruders were removed, and mice were left undisturbed 1 h for the development of the c-Fos signal. After 1 h, the brains were removed and processed as described below.

2.3. Behavioral studies

Behaviors were video recorded and later analyzed for attack patterns by an experimenter blind to the treatments. The latency and number of bites, and attack targeting were recorded. As mice moved quickly during fights, video recordings were analyzed at reduced speed, each fighting event being assessed several times. Mice targeted their attacks towards the head (frontal to ears), throat (lower part of the neck), neck (upper part of the neck), back (dorsal regions), flank (lateral parts of the body), and belly (ventral area between the legs). The head, throat and belly were considered vulnerable targets, as these bear vital organs and are not protected by a thick layer of muscles and bones like the neck, back and the
flanks. A total number of 171 bites were recorded, out of which attack targeting could be identified for 165 bites (96.6%).

More conventional behavioral measures were also assessed by means of a commercial behavioral recording and analysis system (the Observer, Noldus Information Technology, Wageningen, The Netherlands). We assessed the duration of: inactivity/resting (no obvious activity), exploration/walking, (walking through the cage or sniffing directed towards the environment), social investigation (sniffing at partner), offense (tail rattling, chasing, wrestling), self care (grooming, scratching), digging/burying. The same behavioral variables were assessed during both psychosocial and agonistic encounters. In the former case, social behaviors were performed by subjects against the transparent partition.

2.4. Brain studies

After the encounters, the intruder animal was removed from the home/test cage, and the subjects were left undisturbed until sacrifice perfusion (i.e. for 1 h). After 1 h, mice were rapidly (within 20 s after picking them up from the cage) and deeply anaesthetized with CO2, and perfused through the ascending aorta using perfusion (i.e. for 1 h). After 1 h, mice were rapidly (within 20 s after picking them up from the cage) and deeply anaesthetized with CO2, and perfused through the ascending aorta with approximately 15 ml ice-cold 0.1 M phosphate-buffered saline followed by approximately 60 ml 4% paraformaldehyde (in 0.1 M phosphate-buffered saline). The brains were removed, post-fixed in the same fixative for 3 h, stored in phosphate-buffered saline with azide. Before sectioning, samples were cryoprotected overnight by 20% sucrose in phosphate-buffered saline at 4 °C. Six series of 30 μm frozen sections were cut in the frontal plane on a sliding microtome.

The details of the immunocytochemical protocol were published earlier [14,39]. Briefly, the c-Fos protein was labeled with a rabbit polyclonal antibody raised against the amino terminus of c-Fos p62 (Santa Cruz Biotechnology, USA, sc-52). This antibody is highly selective, and shows no cross-reactions with other members of the Fos protein family. The primary antibodies (1:2500) were detected by biotinylated anti-rabbit goat serum (1:1000) and streptavidin conjugated HRP (1:1000) (Jackson Laboratories, USA). The peroxidase reaction was developed in the presence of diaminobenzidine tetrahydrochloride (0.2 mg/ml), nickel-ammonium sulphate (0.1%) and hydrogen peroxide (0.003%) dissolved in Tris buffer.

2.5. Quantification of c-Fos immunopositive cells

Section planes were standardized according to the atlas of Franklin and Paxinos [40]. Quantitative analysis was performed in regions where semi-quantitative evaluation revealed significant c-Fos activation. Microscopic images were digitised by a Sony CCD camera and the number of positive profiles was counted by means of the NIH-IMAGE v1.66 software (Power Macintosh 7100). Uniform thresholds were used and the minimum size of positive profiles was set at 5 pixels. An area-specific standard frame was used to outline selected regions. Except the dorsal raphe (where the analysis was performed in the midline), all areas were analyzed bilaterally in two parallel sections. The medial, ventral and lateral orbital cortices were analyzed at Plate 10 (Bregma 2.46 mm) using a rectangular frame (0.135 mm²). The same frame was used for the analysis of the medial prefrontal cortex (infralimbic, prelimbic and cingulate (area 1) cortices) at Plate 16 (Bregma 1.78 mm). The piriform cortex was analyzed at Plate 26 (Bregma 0.62 mm) with a rectangular frame (0.206 mm²). At this level, the intermediate part of the lateral septum was also analyzed (rectangular frame, 0.192 mm²). The medial part of the bed nucleus of stria terminalis was analyzed at Plate 28 (Bregma 0.38 mm) with a rectangular frame (0.173 mm²). At the level of Plate 38 (Bregma −0.82 mm), the parvocellular part of the paraventricular hypothalamic nucleus (triangular frame, 0.066 mm²) and the central part of the anterior hypothalamic area (oval frame, 0.216 mm²) were analyzed. The tuber cinereum area (oval frame, 0.172 mm²), the medial (rectangular frame, 0.165 mm²) and central (oval frame, 0.216 mm²) amygdaloid nuclei, and the different hippocampal regions (rectangular frame, 0.088 mm²) were analyzed at the level of Plate 42 (Bregma −1.34 mm). Different regions of the periaqueductal gray (dorsomedial, dorsolateral, lateral, ventrolateral) were analyzed with a rectangular frame (0.220 mm²) at the level of Plate 70 (Bregma −4.72 mm). The same level was used for the analysis of the dorsal raphe (oval frame, 0.216 mm²), while the locus coeruleus was analyzed at the level of Plate 76 (Bregma −5.4 mm) with an oval frame (0.129 mm²).

2.6. Statistics

Statistical analysis was made via the STATISTICA software (Statistica Inc. Tulsa, USA). Behavioral data were analyzed by the median test. The results of the brain studies were assessed by a two-factor ANOVA, where Factor 1 was the strain (LAL and SAL), and Factor 2 was social contact (no contact/control, psychosocial contact, and social contact/fights). Data underwent square root transformation to fulfill ANOVA requirements. Correlations were assessed by the Spearman test on raw (untransformed) data.

3. Results

3.1. Behavior

During psychosocial encounters, SAL mice were socially more active than LALs. This was shown by a significant increase in social and agonistic contacts (Table 1). We stress again that these behaviors were performed against the transparent partition, but were clearly identifiable, and were directed towards the opponent. The increase in social contact was on the expense of non-social exploration which decreased significantly. Resting and self-care showed non-significant variation. Thus, SALs submitted to psychosocial encounters were more interested in their opponents than LALs.

As expected, the behavior of LAL and SAL mice differed markedly during the agonistic encounters (Table 1). Biting attacks and offensive threats were virtually absent in LALs, whereas these were frequent in SALs. Noteworthy, more than one third of attacks were aimed at vulnerable body parts of the opponents. All intruders during the social confrontations with SAL residents were clearly defeated as indicated by submissive supine postures, fleeing and freezing behaviors. SAL mice also showed reduced social interactions. No significant differences
were noticed in exploration, inactivity (rest), self-care, and digging/burying. The behavior of the two strains was very consistent. For example, SALs showed on average 34.2±2.15 bites, the lowest and highest attack counts being 30 and 41 (the rest of mice showing values very close to the average). Similarly, all SAL mice showed vulnerable attacks, their lowest share being 24.3%, whereas the largest share was 32.9% (the average was 28.8±1.4%).

3.2. Brain studies

Representative photomicrographs of c-Fos activation were shown in Fig. 1. The amygdala was chosen as it showed interesting changes after encounters (see below).

3.2.1. Frontal cortical areas

Within frontal cortical areas, strong c-Fos signals were noticed in the orbital cortex (medial, ventral and lateral sub-

![c-Fos micrographs](image.png)

**Table 1**
The behavior of LAL and SAL mice during the social encounter

<table>
<thead>
<tr>
<th>Strain</th>
<th>Encounter</th>
<th>Bites</th>
<th>%vuln</th>
<th>OFF</th>
<th>EXP</th>
<th>SOC</th>
<th>RES</th>
<th>SC</th>
<th>DIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAL</td>
<td>Psychosocial</td>
<td>–</td>
<td>–</td>
<td>0.0±0.0</td>
<td>18.6±5.2</td>
<td>23.2±8.2</td>
<td>42.9±14.5</td>
<td>15.3±8.2</td>
<td>–</td>
</tr>
<tr>
<td>SAL</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.6±0.8</td>
<td>8.1±0.8</td>
<td>59.9±12.1</td>
<td>26.3±13.6</td>
<td>3.0±1.5</td>
<td>–</td>
</tr>
<tr>
<td>Chi square</td>
<td>–</td>
<td>–</td>
<td>7.54</td>
<td>7.63</td>
<td>4.41</td>
<td>2.39</td>
<td>0.11</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>p&lt;</td>
<td></td>
<td>–</td>
<td>–</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.12</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>LAL</td>
<td>Agonistic</td>
<td>34.2±2.2</td>
<td>28.8±1.4</td>
<td>33.2±2.9</td>
<td>29.6±8.9</td>
<td>6.0±2.0</td>
<td>16.0±6.5</td>
<td>14.9±5.4</td>
<td>1.1±0.5</td>
</tr>
<tr>
<td>SAL</td>
<td>11.0</td>
<td>7.63</td>
<td>0.01</td>
<td>5.0</td>
<td>0.01</td>
<td>0.5</td>
<td>0.78</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Chi square</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.5</td>
<td>0.01</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

Bites, the number of bites delivered; %vuln, the share of vulnerable attacks; OFF, offensive behaviors; EXP, exploration; SOC, social investigation; RES, inactivity/resting; SC, self care; DIG, digging/burying. Data show the duration of behaviors (expressed as % time), except for bites and the share of vulnerable targets, which were shown as counts and %, respectively.

Fig. 1. Photomicrographs depicting c-Fos activation in the medial and central amygdala. The amygdala was chosen as it showed important changes in SAL mice (see text). OT, optic tract; MeA, medial amygdala; CeA, central amygdala; (A) LAL control; (B) LAL psychosocial encounter; (C) LAL social encounter; (D) SAL control; (E) SAL psychosocial encounter; (F) SAL social encounter.
The effect of psychosocial and agonistic encounters on brain c-Fos expression

Table 2

<table>
<thead>
<tr>
<th>Brain area</th>
<th>LAL</th>
<th>SAL</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>PSYCH</td>
<td>AGO</td>
</tr>
<tr>
<td>Medial orbital cx</td>
<td>28.7±12.2</td>
<td>37.1±14.4</td>
<td>30.8±4.3</td>
</tr>
<tr>
<td>Ventral orbital cx</td>
<td>42.0±11.7</td>
<td>60.5±18.2</td>
<td>64.4±9.9</td>
</tr>
<tr>
<td>Lateral orbital cx</td>
<td>43.1±15.3</td>
<td>38.8±13.5</td>
<td>50.2±12.3</td>
</tr>
<tr>
<td>Infraorbital cx</td>
<td>48.7±13.4</td>
<td>80.1±15.0</td>
<td>70.3±8.1</td>
</tr>
<tr>
<td>Preoptic area</td>
<td>37.5±12.7</td>
<td>81.0±19.8a</td>
<td>55.1±9.7</td>
</tr>
<tr>
<td>Cingulate gyrus Cx1</td>
<td>19.7±12.6</td>
<td>29.8±11.7</td>
<td>22.8±7.1</td>
</tr>
<tr>
<td>Piriform cortex</td>
<td>64.2±6.2</td>
<td>105.8±21.8</td>
<td>77.8±7.9</td>
</tr>
<tr>
<td>Anterior hypothalamic n.</td>
<td>39.6±4.6</td>
<td>91.9±17.4a</td>
<td>92.8±16.2a</td>
</tr>
<tr>
<td>Tuber cinereum area</td>
<td>13.2±4.3</td>
<td>27.4±3.4a</td>
<td>37.9±9.6a</td>
</tr>
<tr>
<td>Dorso-medial PAG</td>
<td>64.8±9.7</td>
<td>99.6±17.6</td>
<td>95.3±7.7</td>
</tr>
<tr>
<td>Dorsolateral PAG</td>
<td>51.5±5.8</td>
<td>100.8±17.3a</td>
<td>105.6±20.7a</td>
</tr>
<tr>
<td>Lateral PAG</td>
<td>43.0±7.3</td>
<td>53.2±12.7</td>
<td>51.4±4.1</td>
</tr>
<tr>
<td>Ventrolateral PAG</td>
<td>39.7±8.2</td>
<td>57.9±16.3</td>
<td>58.3±5.6</td>
</tr>
<tr>
<td>Gruiform dentatus</td>
<td>40.2±6.7</td>
<td>27.0±7.9</td>
<td>25.6±9.5</td>
</tr>
<tr>
<td>CA1</td>
<td>9.5±5.5</td>
<td>8.9±4.0</td>
<td>5.5±1.1</td>
</tr>
<tr>
<td>CA2</td>
<td>2.3±0.7</td>
<td>2.0±0.4</td>
<td>2.0±0.5</td>
</tr>
<tr>
<td>CA3</td>
<td>7.8±2.9</td>
<td>10.5±3.3</td>
<td>8.2±2.4</td>
</tr>
</tbody>
</table>

LAL, long attack latency mice; SAL, short attack latency mice; C, undisturbed control; PSYCH, psychosocial encounters (sensory but not physical contact with opponents); AGO, agonistic encounters (physical contact with opponents); cx, cortex; PAG, periaqueductal gray; CA1–3, hippocampal subdivisions.

* Significant difference between fighting LAL and SAL mice (figures printed in bold for easy reading; \(p<0.05\) at least).

a Significant difference compared to undisturbed control (figures printed in italics for easy reading; \(p<0.05\) at least).
This respect; therefore, the location of their “hypothalamic attack area” is unknown. Nevertheless, strong c-Fos activation was seen in two areas corresponding to the rat hypothalamic attack area: the anterior hypothalamic nucleus and the tuber cinereum (Table 2).

The activation of periaqueductal gray (from which attacks can also be induced by stimulation in cats and to a lesser extent in rats) showed significant changes. These changes were, however, sub-region specific (Table 2). The activation of the dorsomedial periaqueductal gray depended on both strain and encounter, with a significant activation in SAL mice only. This was due probably to the lower basal values seen in SALs. Activation in the dorsolateral subdivision was also strain-, and encounter-dependent, and SAL mice showed less activation than LALs. More ventral regions (the lateral and ventrolateral subdivisions) showed a marginal activation due to encounters. In both subdivisions, post-hoc analysis revealed a significant difference from controls in agonistic encounter-exposed SALs only (Table 2).

3.2.4. Stress-responsive structures

The paraventricular nucleus of the hypothalamus showed an agonistic encounter-related c-Fos activation ($F_{\text{encounter}(2,25)} = 9.18$, $p < 0.001$), which depended only marginally on strain ($F_{\text{strain}(1,25)} = 2.92$, $p < 0.09$): SAL mice showed a marginally smaller activation than LALs (Fig. 4). The activation of the locus coeruleus depended on encounter ($F_{\text{encounter}(2,25)} = 4.63$, $p < 0.019$), but not on strain ($F_{\text{strain}(1,25)} = 0.79$, $p < 0.8$). In post hoc comparisons, however, a significant activation was seen in both psychosocial and agonistic encounter-exposed LAL mice, whereas the difference compared with control was significant only for agonistic encounter-exposed SALs (Fig. 4). The activation of the dorsal raphe showed no strain differences ($F_{\text{strain}(1,25)} = 0.01$, $p < 0.9$), and showed no significant encounter-related increase ($F_{\text{encounter}(2,25)} = 2.06$, $p < 0.14$). Nevertheless, agonistic encounter-exposed mice (irrespective to strain) showed a significant increase compared to control mice in post hoc comparisons (Fig. 4).

3.2.5. The hippocampus

No hippocampal region showed an encounter-related increase in activation ($F_{\text{encounter}(2,25)} = 1.98$ ($p < 0.2$), 0.3 ($p < 0.7$), 0.14 ($p < 0.9$) and 0.73 ($p < 0.5$) for the dentate gyrus, CA1, CA2, and CA3 regions, respectively) (Table 2). Nevertheless, all regions but the CA2 showed a significantly higher activation in SAL as compared with the LAL strain ($F_{\text{strain}(1,25)} = 4.53$ ($p < 0.043$), 4.68 ($p < 0.04$), 0.12 ($p < 0.7$) and 7.17 ($p < 0.012$) for the dentate gyrus, CA1, CA2, and CA3 regions, respectively).
3.2.6. Correlations

No significant correlation was found between behavioral measures and brain c-Fos activation in mice exposed to psycho-social encounters. In contrast, behavior and neural activation patterns showed interesting correlations in mice exposed to agonistic encounters. As shown above, the activation of the BNST and the CeA was stronger in SAL mice exposed to agonistic encounters as compared with agonistic encounter-exposed LALs. Interestingly, the activation of both structures showed a negative correlation with the duration of social interactions (central amygdala: \( R = -0.663; p < 0.03 \), Fig. 3; bed nucleus stria terminalis: Spearman \( R = -0.730; p < 0.01 \)) (Fig. 3). A negative correlation was also found between social interactions and the activation of the lateral periaqueductal gray (Spearman \( R = -0.698; p < 0.02 \)). This brain area was significantly activated in agonistic encounter-exposed SALs only. Importantly, no significant correlations were found between c-Fos activation and exploration or resting for either brain region. This shows that the negative correlation between c-Fos counts and social investigation was not secondary to changes in locomotion. We also note that resting and exploration did not correlate significantly with c-Fos activation in any of the brain regions investigated.

4. Discussion

4.1. Main findings

As expected, the aggressiveness of LAL and SAL mice was dramatically different. In addition to the high aggressiveness of SAL mice (repeatedly shown earlier), we noticed that these mice directed about 30% of their attacks towards vulnerable body parts of their opponents. In LALs, agonistic encounters activated a number of brain areas known to play a role in aggressive behavior, including the lateral septum, bed nucleus of stria terminalis, medial amygdala, paraventricular nucleus of the hypothalamus, anterior hypothalamic nucleus and tuber cinereum area (both being analogous with the rat hypothalamic attack area), dorsolateral periaqueductal gray, and locus coeruleus. The activation of the raphe was marginally significant. In SALs, the medial prefrontal cortex (mainly the infralimbic and prelimbic subdivisions), central amygdala and lateral/ventrolateral periaqueductal gray were strongly activated. We mention that these areas were not activated in LALs. In contrast to LALs, no activation was seen in the lateral septum and the dorsolateral periaqueductal gray of SALs. The dorsal raphe was marginally activated in this strain too.

4.2. Comparison with other forms of violent aggression—a hypothesis

Based on the present and earlier data, we hypothesize that violent attacks occur as a consequence of the excessive activation of the central amygdala and lateral/ventrolateral periaqueductal gray, which is accompanied by a smaller activation of the septum and dorsolateral periaqueductal gray (Fig. 5).

The strong activation of the central amygdala in SALs appears especially important for our hypothesis. This brain area showed mild or no activation in the territorial fighting of male rats, male hamsters, and non-lactating female mice [14,39,41,42], and it was shown to inhibit affective aggression in cats (this behavior corresponds to rodent rivalry aggression; [43,44]). This brain area, however, was strongly activated in rats in which attacks were elicited by the electrical stimulation of the hypothalamic attack area [39], in fighting glucocorticoid deficient rats [14], in lactating female mice that fought against male intruders [45]; predatory aggression in cats and rats [43,44,46], and defeated intruders [47]. In all these cases, subjects showed a dramatic increase in attacks aimed at vulnerable body parts of their opponents [10–13,43,44,46,48,49]. SAL mice, which aimed a large share of their attacks towards vulnerable targets, also showed a large fight-induced increase in central amygdala activation. In LAL mice, no central amygdala activation was seen, this being similar to findings obtained in models of territorial aggression (see above). Taken together, these data strongly suggest that the central amygdala is tightly bound to the execution of violent attacks that are aimed at vulnerable targets. The negative correlation between social interactions and central amygdala activation suggests that this brain area not only promotes abnormal forms of attack but also diminishes the “chance” of amiable social interactions, by this further contributing to the occurrence of abnormal social interactions.

Interestingly, the lateral septum showed no activation in violent attacks (when the central amygdala was activated). For example, no activation of the septum was noticed in lactating females fighting with male intruders [45,50] and in cats and rats showing predatory aggression of [44,51]. In contrast, the lateral septum was activated in non-lactating females fighting against male or female intruders [41,45], and in males showing territorial aggression [14]. This brain region was significantly activated by social encounters in LAL but not in SAL mice.

Earlier data show that different subdivisions of the periaqueductal gray are differentially activated in normal and violent forms of aggression. In the cat (best studied in this respect), the dorsolateral subdivision (not activated in SALs) stimulated affective, whereas the ventrolateral subdivision (activated in SALs) stimulated predatory aggression [43,44]. The lateral/ventrolateral periaqueductal gray was also significantly activated in lactating female mice fighting with male intruders [50], in rats...
showing predatory aggression [52], and in defeated rats [53]. As shown above, such subjects attack vulnerable targets. Noteworthy, the dorsal periaqueductal gray was activated in residents submitted to territorial aggression in various species [54,55]. In LALs, the patterns of periaqueductal gray activation were similar with those seen in territorial aggression.

It occurs that SALs show aggression-induced c-Fos activation patterns that are very similar with those seen in other models of violent attack. In contrast, the patterns of c-Fos activation seen in LAL mice are more similar with those seen in normal manifestations of aggressive behavior (e.g., territorial fights in males and non-lactating females). It is especially intriguing that the c-Fos activation patterns seen in SALs appear similar with those seen in predatory aggression. In addition to the similarities outlined above, the activation of the bed nucleus of stria terminals is also consistent in the two situations. It was shown above, such subjects attack vulnerable targets. Noteworthy, the dorsal periaqueductal gray was activated in residents submitted to territorial aggression in various species [54,55]. In LALs, the patterns of periaqueductal gray activation were similar with those seen in predatory aggression.

In conclusion, the violent attacks of SAL mice were accompanied by an excessive activation of the central amygdala and lateral/ventrolateral periaqueductal gray, and by a lack of activation in the septum and dorsolateral periaqueductal gray. Similar c-Fos activation patterns were seen in a number of behavioral models characterized by violent aggressiveness. In LALs, the patterns of c-Fos activation were similar with those seen in normal territorial aggression.

4.3 Prefrontal cortical areas

It is generally believed that abnormal manifestations of aggressive behavior are related to, or caused by, prefrontal deficits in humans. Prefrontal deficits were shown in intermittent explosive disorder (characterized by outbursts of anger; [56]) antisocial aggressive behavior [57], impulsive aggression (characterized by problems in emotion regulation; [58]) etc. In line with these human findings, the destruction of the orbital cortex increased aggressiveness in rats, whereas the stimulation of the prefrontal, piriform, and cingulated cortices reduced feline quiet biting induced by hypothalamic stimulation [43,59]. A critical examination of findings suggested, however, that prefrontal deficits are associated with impulsive but not with other forms of aggression [60]. It was suggested that instrumental aggression (characteristic to hypoarousal-associated aggressiveness in humans) cannot be attributed to prefrontal dysfunctions, whereas these dysfunctions are characteristic to reactive (impulsive) aggression [61]. Similar findings were obtained by Raine et al. [62]; emotional, unplanned impulsive murderers were less able to control aggressive impulses due to deficient prefrontal regulation. In contrast, “predatory murderers” (term by Raine et al. [62]) showed normal prefrontal functioning. Interestingly, the same authors showed prefrontal deficits in hypoarousal-driven aggressiveness in children [63]. The reason of this discrepancy is unknown. In our study, SAL but not LAL mice showed a consistent activation of the medial prefrontal cortex, suggesting that the execution of violent attacks is bound to the activation of this brain area. Other frontal cortical areas showed no consistent agonistic encounter-induced activation, but their level of activation was higher in fighting SAL than in LAL mice (due mainly to strain differences in these areas). These surprising findings can be interpreted in several alternative ways: (1) The prefrontal cortex inhibits aggressive behavior, and its activation reflects a negative feedback triggered by the execution of attacks; (2) The prefrontal cortex shows a rhythmic activity. Increased c-Fos activation may be related to the desynchronization of this rhythm, which might decrease the “power” of inhibitory influences on aggressive behavior; (3) the involvement of this area is different in reactive/impulsive and predatory/instrumental hypoarousal-driven aggression, and finally (4) prefrontal deficits in humans lead to aggression via alterations in moral judgment, that cannot be interpreted easily in rodents. The present findings cannot validate either of these or other alternative hypotheses, but clearly show an increased prefrontal activation in SAL mice.

4.4 Brain activation patterns in psychosocial and agonistic encounters

Interestingly, psychosocial and agonistic encounters often led to a comparable activation of areas involved in aggression control. Typically, the activation was somewhat higher in agonistic as compared with psychosocial encounters, but this difference was statistically significant in a few cases only (often because the change in psychosocially stimulated mice did not reach significance). This finding was not entirely unexpected, as aggression-related brain activation patterns are often similar in different situations. For example, defeat in social encounters activates areas very similar to those activated by winning in resident-intruder conflicts (see, e.g., [48,64,65]). Similarly, social contact-induced brain activation patterns partially overlapped in non-aggressive virgin and aggressive lactating female mice [45]. One can assume that the fight-related activation of many of the investigated brain areas preceded rather than followed fights, because they are involved in “decision making” and not in execution. A similar situation may be valid for LAL mice as well. These mice did not show overt aggression in the present experiment, probably because the latency of their fights is longer than the time frame applied in the present experiment (5 min were chosen because of the high aggressiveness of SALs). However, the brain activation patterns seen in LALs...
were similar with those seen in territorial aggression in a variety of species (see above). Noteworthy, the anterior hypothalamic and tuber cinereum areas were activated in these mice, and these appear analogous with the hypothalamic attack area of rats.

4.5. Concluding remarks

As repeatedly shown earlier (and confirmed by the present study), short attack latency mice show excessive aggression that can be considered abnormal in many respects. We studied here the neural background of agonistic encounters in this, and the long-attack latency strain, to identify brain regions that might be responsible for such violent attacks. SAL mice showed an excessive activation of the central amygdala and lateral/ventrolateral periaqueductal gray, whereas the septum, and the dorsolateral periaqueductal gray were not activated. This pattern of changes resembles in many respects those seen in other forms of violent attacks (predatory aggression in cats and rats, attacks by latching females towards male intruders, attacks by rats that were electrically stimulated in the hypothalamic attack area, and glucocorticoid deficiency-induced aggression in rats that shows many similarities with human hyporaousal-driven (antisocial) aggressiveness). We suggest that the excessive activation of the central amygdala, and lateral/ventral subdivisions of the periaqueductal gray—accompanied by a reduced activation of the septum and dorsolateral periaqueductal gray—may underlay the execution of violent attacks. Interestingly, most of the above-mentioned violent forms of aggressiveness are associated with deficits in glucocorticoid production. Attacks on vulnerable targets were induced in rats by chronically inhibiting glucocorticoid production [11–14]. SAL mice were shown to be stress hyporesponsive compared to LALs [32,34–37]. Lactating female mice are also stress hyporesponsive [66], and show smaller glucocorticoid responses to intruding males than virgin females [67]. Predatory aggression (quiet biting) induces no glucocorticoid responses in cats, whereas affective aggression (similar to rodent rivalry aggression) induces a strong response [68]. Taken together, these data suggest that the specific neural background of violent aggressiveness is associated with low glucocorticoid production. Importantly, this endocrine condition was bound to aggressiveness in antisocial personality disorder and its “childhood form”, conduct disorder [1,5,9,17].

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References


