Behavioral changes across novelty habituation: Contextual modulation of self-grooming after a stress event

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Introduction

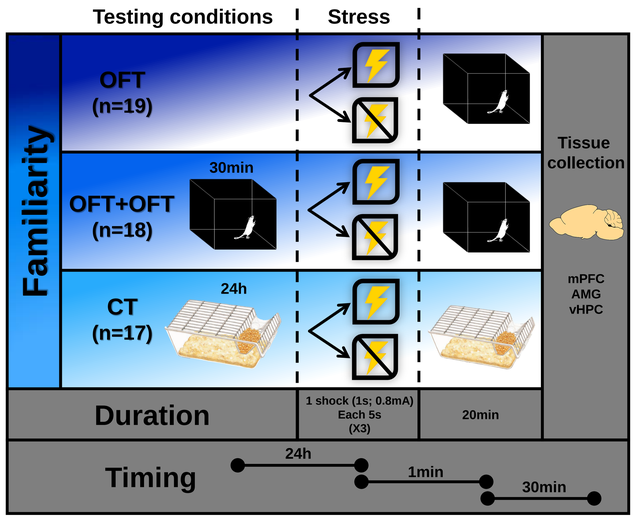
- Grooming is a very complex behavior that is widely used by different models in neuroscience research. However, there is still an open discussion about its ethological relevance related with the stress response[1,2,3].
- Nowadays, grooming is rather considered as an indicator of stress (i.e., ongoing stress state). Yet, a growing body of evidence suggests that some forms of grooming could favor emotional de-arousal, acting on its own as a negative feedback of some stress responses[4,5,6,7].
- By inducing stress, and testing the animals in contexts with different gradients of familiarity, we aimed to assess the association between grooming behavior, stress, and emotional de-arousal.

Materials and Methods

Subjects: Fifty-four male Wistar (~220g) rats were behaviorally screened in a spontaneous activity test. Then, they were assigned to the following groups in a counterbalanced manner based on their locomotion, rearing, and grooming behavior.

Groups: Animals were assessed on a novel open field test (OF; **OFT**), an OF after a previous exposure to the same test (**OFT+OFT**), or on an individual housing cage where the animals were housed during the last 24h (**CT**). Half of the animals in those groups were acutely stressed by a footshock (named Stressed animals), and the rest of the animals were exposed to the shock chamber but received no footshock (Non-stressed animals).

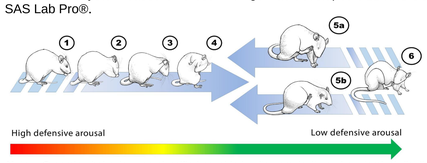
General procedure: Animals were individually transported to the shock-chamber room. After placed in the chamber, stressed animals received three 1s-footshocks (0.8mA) 5s apart. Once the shock series finished, the animals remained in the shock chamber for 2min. Afterward, rats were placed in a transport cage for a cool-down period of 1min. Finally, rats were behaviorally assessed either on the OFT or the CT for 20min. Thirty minutes later, animals were beheaded and their brain removed for a rapid dissection of their bilateral medial prefrontal cortex (**mPFC**), amygdala (**AMG**), and ventral hippocampus (**vHPC**). Monoamines quantification was performed in those regions using high precision liquid chromatography (HPLC).

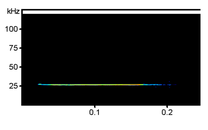


Notes: OFT: Open field test; dimensions: 70x70x40cm; **CT:** Cage test (animals tested in individual home cages): dimensions: 22x37.5x18cm). All the animals were group housed (5 per cage), and had free access to food and water. The animals tested in the CT were individually housed in a new cage during the 24h previous to the test. During the testing phase, the animals in that group were assessed in the same cage they remained the last 24h.

Behavioral analysis:

OFT: Locomotion: Distance traveled (m) registered by Any-Maze®. **Rearing**: Biped postures (free-standing or against the walls) elevated ≥ 45° from the floor were manually scored by trained observers. **Grooming:** Manually scored by trained observers. We have previously observed that short bouts in the head area are prompted to appear during the initial phases of exploration (i.e., 1-2-3), whereas long and complex sequences are displayed later on when exploratory activity has started to decrease (e.g., 1 → 2 → 3 ↔ 4 ↔5a/b). Therefore, a classification system based on those findings was developed[6]. **22-kHz USV**: Automatically registered by AviSoft SAS Lab Pro®.



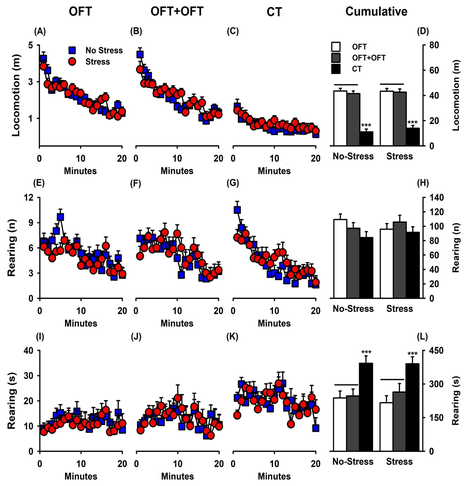


Notes: Grooming behavior was classified considering its complexity and anatomical distribution. The use of hind paws into the grooming sequences requires the animals to engage in very intricate body positions. That compromises the rat capacity to promptly respond to a threat. For those reasons, we consider the grooming bouts that include the hind paws as more complex than the grooming sequences that do not. We named those sequences as **Variations.** We also considered if the grooming was directed to the head and fore paws (**Cephalic**), to the body (**Caudal**), or if it appeared as a chain that includes both cephalic and caudal regions of the body (**Sequential**).

Monoamine quantification: Each brain filtrate sample was analyzed for their contents of **dopamine (DA)**, **norepinephrine (NE)**, **serotonin (5HT)** and some of their metabolites **(3,4-dihydroxyphenylacetic acid (DOPAC)**, **5-hydroxyindoleacetic acid (5-HIAA)** and **homovanillic acid (HVA)** using reverse phase HPLC, with an Eclipse Plus-C18 column (150 × 4.6 mm, 5µm, Agilent Technologies, USA), coupled with electrochemical detection (HPLC-EC). The column eluate was monitored by a pulsed electrochemical detector (464 Waters Corporation, MA, USA) operated at a potential of 700 mV. Data was acquired and integrated using Data Apex software (CSW32-Chromatography Station for Windows, Hungary). The substrate concentration was expressed as nanograms per milligram of wet tissue weight (for details see 5).

Results – Stress reduced total grooming but increased cephalic grooming, causing no changes in locomotion or rearing behavior

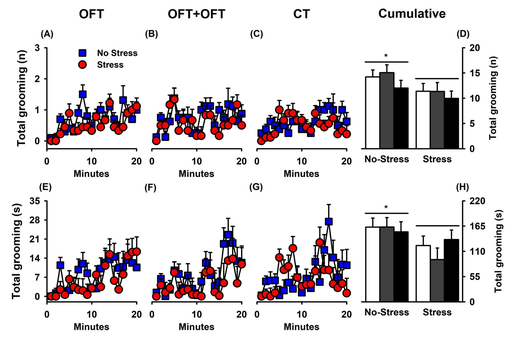
(Fig.1) Locomotion and Rearing Behavior



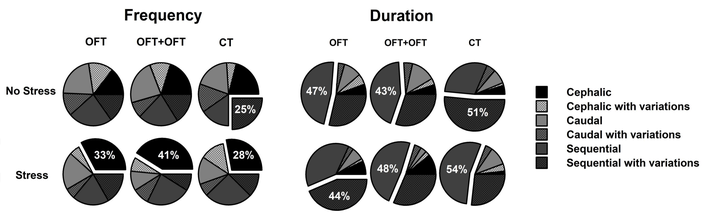
Main results:

- Locomotion (*p*<.001, η2=.43) and rearing frequency (*p*<.001, η2=.25) progressively reduced within the testing session (Fig.1.A-C, E-G).
- Animals displayed less locomotion (*p*<.001, η2=.11) and more rearing duration (*p*<.001, η2=.41) in the CT (Fig.1.D,L).
- Testing conditions did not affect rearing frequency (Fig.1.H).
- Stress did not affect locomotion and rearing (Fig.1).

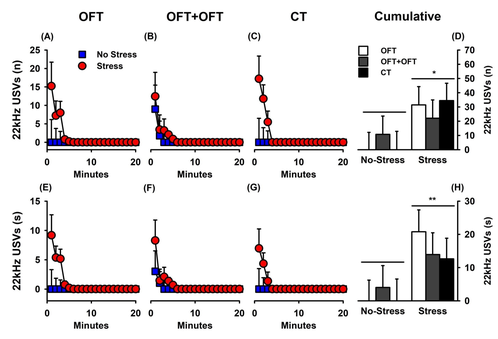
(Fig.2) Total grooming



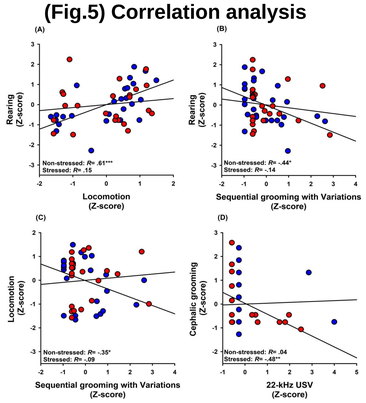
(Fig.3) Grooming by sub-types



(Fig.4) 22-kHz USVs



(Fig.5) Correlation analysis



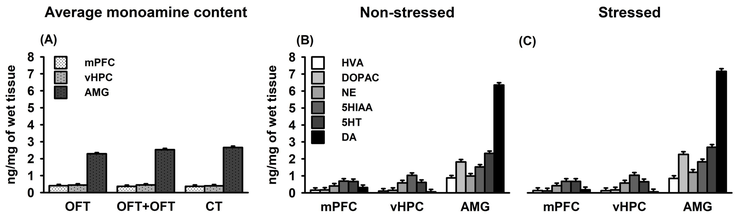
Main results:

- The frequency (*p*<.001, η2=.07) and duration of grooming (*p*<.001, η2=.08) increased within the testing session (Fig.2.A-C,E-G).
- The frequency (*p*<.05, η2=.12) and duration of grooming (*p*<.05, η2=.12) was diminished by stress (Fig.2.D,H).
- Stress altered the grooming distribution (*p*<.05, η2=.04) by selectively increasing cephalic grooming (CG; *p*<.001, η2=.20), whereas in the non-stressed condition grooming subtypes distributed evenly (Fig.3).
- Stressed rats emitted more (*p*<.05, η2=.12) and longer (*p*<.01, η2=.13) 22-kHz USVs, which showed a slower decay than that in non-stressed animals (*p*<.05; η2=.10).
- Locomotion and rearing frequency were positively associated to each other, but only in non-stressed rats. Likewise, only non-stressed rats showed a negative association between sequential grooming with variations (SGV) and locomotion, and SGV and rearing frequency. Only in stressed rats 22-kHz USV and CG were negatively associated (Fig.5).

Notes: *: *p*<.05. **: *p*<.01.***: *p*<.001. Horizontal lines over the bar graphs represent main effects between groups.

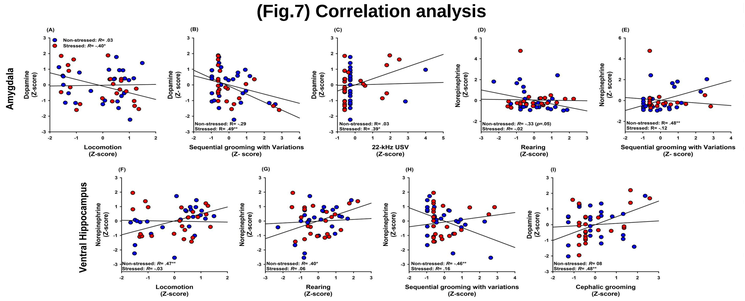
Results – Stress increased the overall monoaminergic content and change the association between neurochemistry and behavior

(Fig.6) Monoamine content by groups and brain regions



Notes: The average monoamine content included all of the studied monoamines.

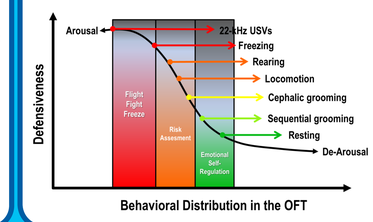
(Fig.7) Correlation analysis



Main results:

- Stress increased monoaminergic contents in all brain regions (Fig.6; *p*<.05, η2=.01).
- The overall monoaminergic content was larger in the amygdala than in the other regions (*p*<.001, η2=.64).
- DA was the most abundant monoamine (*p*<.001, η2=.69), especially in the amygdala (Region*Monoamine: *p*<.001, η2=.64).
- In stressed rats, amygdalic DA content was associated negatively with locomotion and SGV and positively with 22-kHz USV (Fig.7).
- In non-stressed rats, amygdalic NE content was associated negatively with rearing (Fig.7.D) and positively with SGV (Fig.7.E).
- In non-stressed rats, ventro-hippocampal NE content was associated positively with locomotion, rearing and CG (Fig.7.F-H), and negatively with SGV (Fig.7.I).

Summary



- A single stress event reduces total grooming, but increases cephalic grooming regardless of the testing conditions.
- One single footshock experience is not enough to affect exploratory and risk-assessment behavior either in a familiar or unfamiliar context.
- Grooming subtypes related with de-arousal and novelty habituation were inversely associated with behavioral indicators of emotional distress (e.g., 22-KHz calls).
- Footshock experience robustly increases the dopamine concentration, especially in the amygdala, and irrespective of the testing conditions.

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