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Dynamics in brain activation and behaviour in acute and repeated social defensive motivated behaviour

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ABSTRACT

In nature, confrontations between conspecifics are recurrent and related, in general, to the lack of resources such as food and territory. In this sense, adequate defence against a conspecific aggressor is essential for the individual's survival and the group integrity. However, repeated social defeat is a significant stressor, promoting several behavioural changes, including on social defence per se. But what would be the neural basis of these behavioural changes? To explore some hypotheses about this, we investigated the effects of repeated social stress on neural circuits underlying the motivated behaviour social defence in male mice. The hypothalamus is an essential centre of these circuits. Different hypothalamic structures receive information about the conspecific from the medial amygdala and the bed nucleus of the terminal stria. Furthermore, the hypothalamus can receive environmental information via the septo-hippocampal-hypothalamic circuit. Both information is processed by the dorsal preammillary nucleus (PMD) and the ventrolateral portion of the ventromedial nucleus of the hypothalamus, which communicate with the periaqueductal grey, an important downstream site for behavioural emission. During our analysis, we observed that animals re-exposed three times to the aggressor spent more time in passive defence during their last exposure than in their first one. These animals also present a smaller mobilization of areas related to the processing of conspecific cues. In contrast, we did not observe changes in the PMD mobilization. Therefore, our data indicate that the balance between the activity of circuits related to conspecific processing and the PMD determines the pattern of social defence behaviour. Changes in this balance may be the basis of the adaptations in social defence after repeated social defeat.

1 Introduction

2 Conflicts between animals of the same species are frequent during the life of social animals¹⁻³. In general, these conflicts
3 occur due to a lack of resources (water, food, territory) or sexual partners, and their occurrence divides animals between
4 dominants - those who were often the winners - and subordinates¹⁻³. Submission is a relevant stressor in groups of social
5 animals. Subordinate animals have a shorter life expectancy, a lower success rate in mating and greater weight loss than their
6 dominant ones^{1,2}. Furthermore, these animals show a series of neuroendocrine and autonomic responses associated with
7 stress, such as changes in blood corticosterone levels and in heart rate^{1,2,4}. In the laboratory, the exposure - acute or chronic -
8 of an animal to a conspecific aggressor is one of the most used protocols for the study of coping and psychopathology-like
9 behaviours⁴⁻⁷.

10 Chronic exposure to social stress promotes a series of changes in the subordinate animal behaviour⁶. In short, animals begin
11 to express what is known as anxious- and depressive-like behaviours, which is related to changes in the pattern of locomotion
12 and exploration in paradigms such as the open field and the elevated plus-maze^{4,6,8}. Coping strategies are also changed. Butler
13 et al. (2018)⁷ have shown that cichlid fishes re-exposed to social stress initially perform a proactive coping strategy, which
14 changes for a reactive strategy after the third exposure. Although these coping strategies are related to social defence, we have
15 little information about the effect of chronic social stress on social defence itself. This behaviour is divided between active
16 defence, which occurs during the attack of dominant animals, and passive defence, which includes freezing or on-the-back
17 posture⁹. Social defence is essential for individual survival and the maintenance of group integrity since it enables the division
18 of winners and losers without deaths^{10,11}. Therefore, social defence is a goal-oriented behaviour essential to the survival of
19 social animal species⁹.

20 Goal-oriented behaviours involve a range of behaviours essential to the survival of the individual and the species, such
21 as ingestive (eating and drinking), defensive (fight or flight), and reproductive (sexual and parental) behaviours^{12,13}. The
22 execution of these behaviours depends on different neural circuits, whose neuronal populations and connections are genetically
23 determined, which makes it possible for animals to be born knowing how to perform them⁹. An essential brain structure for

24 goal-oriented behaviours is the hypothalamus^{12,13}. This structure receives information both from the internal environment
25 (e.g. energy status, extracellular fluid volume, temperature) and from the external environment (e.g. presence of a predator,
26 characteristics of a co-specific, distance to threats)^{12,13}. Furthermore, the hypothalamus projects to regions associated with
27 the organization of behaviour, in addition to controlling endocrine and autonomic systems^{12,13}. Therefore, the hypothalamus
28 acts as an integrator centre that regulates goal-oriented behaviours, including social defence. Concerning repeated exposure to
29 social stress (in the laboratory or nature), even though changes in social defence have been described, the neural bases of these
30 changes are still unclear^{1,2,7,14}. Given the importance of the hypothalamus for the execution of these behaviours, behavioural
31 changes are expected to be associated with changes in the activity of hypothalamic nuclei and associated areas. But, how does
32 repeated social stress impact the activity of these structures?

33 During acute exposure to social stress, social defence mobilizes regions related to the processing of social and environmental
34 information^{9,15}. Social cues are relevant for defeated animals since they could give them information about health, sex and
35 the hierarchy ranking of the aggressor¹⁶⁻¹⁸. In rodents, this information is mainly related to olfactory cues^{3,18}. The social
36 information is processed by the medial amygdala (MeA) and bed nucleus of stria terminalis (BST)¹⁶⁻²⁰, which project
37 for regions of the hypothalamus that regulate social behaviours - the hypothalamic conspecific responsive circuit^{9,21}. The
38 environmental context related to social conflict is another significant variable to social defence²². Works with rats suggest
39 that a septo-hippocampal-hypothalamic pathway participates in the processing of environmental cues related to physical or
40 psychological limits^{15,23}. An important area in this system is the juxtadorsomedial part of the lateral hypothalamic area
41 (LHA_{jd}), which may receive spatial information from the septo-hippocampal system and transmit this information to brain
42 regions that organize defensive behaviours, as the dorsal premammillary nucleus of the hypothalamus (PMD)^{9,15,23}.

43 Interestingly, in rats, the exposure to restraint stress also promotes mobilization of the septo-hippocampal-hypothalamic
44 circuit and PMD¹⁵. Restraint stress is a typical protocol used to study the effect of stress on behavioural and physiological
45 variables²⁴. The protocol is characterized by the limitation of animal movement and an absence of escape possibility²⁴. Since
46 the aversive context of restraint stress is the limit imposed by the walls of the apparatus, it is believed that the septo-hippocampal-
47 hypothalamic circuit works in processing this environmental information¹⁵. This circuit is mobilized in other aversive contexts,
48 as predatory and social stress, which would be expected since the environmental context is relevant to coping with general
49 threats^{15,23,25,26}.

50 To study how reexposure to social stress impacts social defence as a goal-oriented behaviour, and to evaluate the possible
51 neural bases of these changes, we have submitted C57Bl/6 male mice to one or three sessions of social stress. We also have
52 investigated if, like rats, a septo-hippocampal-hypothalamic circuit is responsive to two different stressful contexts: social and
53 restraint stress. Our analysis suggests that animals submitted to three social defeat sessions spent more time in passive defence
54 during the last exposure. This is followed by a decrease in the mobilization of brain areas that process social cues. Also, there
55 is no evidence of a difference in the mobilization of areas related to the septo-hippocampal-hypothalamic circuit during the first
56 and last exposure. Our study suggests a broad mobilization of this circuit in aversive contexts since it is also mobilized during
57 restraint stress. Our work provides insightful data for future studies about the neural bases of social defence and changes in
58 defensive behaviour under repeated defeated male mice.

59 Results

60 Behavioural effects of reexposure to the resident-intruder paradigm in social defence

61 *Intruders and residents behavioural pattern during the resident-intruder paradigm*

62 Two groups of C57Bl/6 mice were submitted to the resident-intruder paradigm, one group to only one exposure (n = 7; 1
63 Exp. group) and another group to three exposures (n = 11; 3 Exp. group), for three days, once a day, and with different
64 residents. During the sessions, the resident's attacks began almost immediately after the intruder was placed and, after the
65 first attack, intruders were left for 5 min with the dominant male. Most of the time, the intruder animals expressed defensive
66 behaviours, which were classified as active defence - when the animal is under attack by the resident - or passive defence -
67 when the resident leaves the intruder alone (Fig. 1; Supplementary Information Table S1). In this case, the most common
68 posture performed, classified as passive defence, was freezing, followed by checking (turning to maintain intruder's orientation
69 toward the resident) (Supplementary Information Table S1). As regards the active defence, intruders generally performed a
70 defensive upright position (standing under the hind limbs and towards the resident) (Supplementary Information Table S1).
71 Flight and jumping were also very common during the paradigm (Supplementary Information Table S1). In general, intruders
72 spent almost 20% of the time in active defence, which in all cases, was lower than the time spent in passive defence (Fig. 1;
73 Supplementary Information Table S1). During the first exposures to the paradigm, mice intruders spent nearly 25% of the time
74 in exploring the resident's home cage (Fig. 1; Supplementary Information Table S1). As to resident's behaviours, lateral threat
75 (moving laterally to the intruders, via an arc-like path) was the most common performed behaviour (Supplementary Information
76 Table S3). Move towards (moving in a straight line toward the intruder) and clinch attack (direct attacks against the intruder)
77 was also very frequent (Supplementary Information Table S3).

78 **Intruder animals spend more time in passive defence and less time in exploration during last exposure**

79 The behavioural pattern of the intruder animal changes dramatically between the first and third exposure. On average, intruders
80 spent 64.65 s less on exploratory behaviour during the last exposure in relation to the first exposure (although residents are
81 different; M_{diff} - 95% CI [32.65, 96.66], $d_{\text{unb}} = 2.01$ [1.01, 3.46]; Supplementary Information Table S2). Also, for the same
82 intruders, the time spent on passive defence has increased in 82.70 s during the last exposure (M_{diff} - 95% CI [-113.64, -51.75],
83 $d_{\text{unb}} = -2.24$ [-3.60, -1.38]; Supplementary Information Table S2). The short confidence intervals (CIs), and mostly restricted
84 to high standardized effect size values, suggest that there is a large and robust effect of reexposure under the behavioural
85 pattern of the intruder (Supplementary Information Table S2). During the first exposure, the intruder starts the session with
86 a pattern of exploratory behaviour interspersed with confrontational events between intruder and resident, whose frequency
87 decreases throughout the session, while in the last exposure intruder behaviour already starts predominantly defensive (Fig. 2).
88 On the other hand, the behavioural pattern of residents has not substantially changed between the first and third sessions of
89 the paradigm, which has also occurred with the intruder's time spent on active defence (Fig. 1, Supplementary Information
90 Fig. S1; Table S2, S4). This is consistent with the CIs of the standardized effect sizes, which are long and include zero. During
91 all the sessions, resident and intruder spent a minimal portion of time in social and other behaviours (Fig. 1; Supplementary
92 Information Fig. S1 and Tables S1, S3).

93 Overall, despite variations in the behaviour pattern of intruders, exploratory analyses do not provide clear evidence of
94 a variation in the spatio-temporal measurements between the first and third exposure to the paradigm, which also occurred
95 with residents (Fig. 1, Supplementary Information Table S2, S4). The CIs are long and, for the most part, are consistent with
96 anywhere from large reduction, no change up to a large increase in times or distances evaluated. The only exception is when
97 comparing the distance covered by the intruders submitted to 3 exposures of the paradigm during the first and last exposures of
98 them (Fig. 1, Supplementary Information Table S2). On average, these mice covered 275.96 cm less on total distance during
99 the last exposure (M_{diff} - 95% CI [-14.52, 566.45], $d_{\text{unb}} = 0.72$ [0.05, 1.55]; Supplementary Information Table S2). In this
100 case, the standardized effect sizes suggest a moderate difference between these groups, although the CIs are consistent with no
101 difference up to a large reduction of the covered distance in the third day of exposure. In short, intruder and resident animals
102 spent most of the time at the border of the resident's home cage and, in general, the resident had a greater distance travelled
103 than the intruder during the sessions (Fig. 1, Supplementary Information Fig. S1 and Tables S1, S3).

104 **Effects of reexposure to the resident-intruder paradigm in brain activation pattern**

105 We analysed the activation of some areas of the brain in control animals ($n = 12$), animals exposed once to the resident-intruder
106 paradigm ($n = 7$) and animals exposed to this paradigm three times ($n = 11$). It was quantified the density of Fos-labelled cells
107 in 31 brain regions, which includes septal, amygdalar, hypothalamic and periaqueductal grey sites. Only the caudodorsal part
108 of the lateral septum (LSc) and the dorsomedial portion of the ventromedial hypothalamic nucleus (rVMHdm) did not show
109 differences in the Fos-labelled cell density between the three groups (Fig. 5; Supplementary Information Table S5, S6). In
110 general, these CIs are large and include moderate positive and negative values of the standardized effect size and does not
111 provide clear evidence of differences between these groups. In all other analysed sites, exploratory analyses provide clear
112 evidence that animals exposed one or three times to social stress showed considerably greater activation compared to the control
113 group (Fig. 5; Supplementary Information Table S5, S6).

114 **Circuits that process social information are less mobilized during last exposure**

115 When comparing animals that have gone through 1 or 3 exposures of the resident-intruder paradigm, the more robust and
116 clear differences in the brain activation pattern between these groups are in regions related to the processing of conspecific
117 information (Fig. 5; Supplementary Information Table S5, S6). For example, it is possible to observe a moderate reduction in
118 activity in most of the analysed medial amygdalar nuclei (MeA), especially the anterodorsal (MeAad), posterodorsal (MeApd)
119 and posteroventral (MeApv) portions throughout the exposures (Fig. 3, 5; Supplementary Information Table S5, S6). In these
120 cases, the CIs are mostly restricted to moderate up to high positive standardized effect size values, which supports the conclusion
121 that these regions are less mobilized during the last exposure. Other important structures in the processing of conspecific
122 information are the nuclei of the sexually dimorphic circuit (medial preoptic nucleus - MPN, ventral premammillary nucleus -
123 PMV, tuberal nucleus - TU, and rostral and caudal portion of ventrolateral part of the ventromedial nucleus - rVMHvl and
124 cVMHvl), which in general are also less mobilized during the last exposure (Fig. 3, 5; Supplementary Information Table S5, S6).
125 About the rVMHvl, cVMHvl and PMV, the CIs are large but restricted to moderate up to high standardized effect size values,
126 indicating that the effect of reexposure on activation of these areas is probably no less than moderate in size. The standardized
127 effect size of the difference in TU is moderate, but the CI is long and consistent with a small negative effect size value up to a
128 large positive effect size value, suggesting that the effect is, most likely, no more than moderate in size. Regarding the MPN,
129 the standardized effect size is small and the CI is consistent with anywhere from a decrease, no change, up to an increase in the
130 activation of this area through the reexposure, which does not provide clear evidence of a difference between intruder submitted
131 to 1 or 3 exposures of social stress (Fig. 3, 5; Supplementary Information Table S5, S6).

The mobilization of septo-hippocampal-hypothalamic circuit between first and third exposure is not different

Concerning the analysed nuclei of the septo-hippocampal-hypothalamic circuit (rostromedial part of the lateral septum - LSr, the juxtaparaventricular part of the lateral hypothalamic area - LHA_{jp}, and LHA_{jd}), effect size analyses do not suggest clear evidence of a difference in the mobilization of these nuclei between animals submitted to a one or three exposure of social defeat (Fig. 3, 5; Supplementary Information Table S5, S6). The standardized effect size values are small and the CIs are large and include negative and positive values (Fig. 3, 5; Supplementary Information Table S5, S6). Similarly, we were unable to observe robust differences in the neural activation of the PMD and of hypothalamic nuclei that participate in neuroendocrine and autonomic responses to stress, between animals in the 1 Exp and 3 Exp group (Fig. 3, 5; Supplementary Information Table S5, S6). Finally, the small standardized effect size and the large CI suggest that there is no difference in the Fos expression of the rVMHdm between these two groups (Fig. 3, 5; Supplementary Information Table S5, S6). It is important to note that the rVMHdm does not participate in the response to the social cues, but the predatory cues²⁷. Thus, the absence of difference in neural activation between these two groups suggests that there is no baseline reduction in Fos expression due to reexposure to the resident-intruder paradigm.

Periaqueductal grey matter mobilization during first and third exposure

About the analysed periaqueductal grey matter (PAG) columns, in general, exploratory analyses do not provide evidence of a difference in the activation pattern between animals submitted to 1 or 3 exposures to the resident-intruder paradigm (Fig. 5; Supplementary Information Table S5, S6). Overall, the standardized effect sizes are moderate or small and the CIs are short, but in moderate sizes and varying between negative and positive values, which is consistent with an absence of difference between the groups. The only exception is the caudal portion of the ventrolateral part (cPAG_{vl}), whose standardized effect size is high and suggests that this area was more active during the last day of exposure to the paradigm. However, although the high standardized effect size, the confidence interval is wide and consistent with anywhere from an absence of difference up to a large difference between intruders submitted to 1 or 3 exposures of resident-intruder paradigm (Fig. 5; Supplementary Information Table S5, S6).

Correlations

To explore the relationship between different evaluated regions and analysed behaviours, we calculated Pearson's *r* and its 95% CI among these variables (Supplementary Data 1 and 2). We performed these analyses with the data from animals submitted to one or three exposures of the resident-intruder paradigm, separately. Interestingly, the correlation pattern changes between the two groups.

Correlations between intruders and residents behaviours

During first exposure, flight behaviour is both related to attack and threat behaviours of the resident (flight vs. attack: $r = 0.72$, 95% CI [-0.07, 0.96]; flight vs. lateral threat: $r = 0.87$, 95% CI [0.35, 0.98]). Concerning flight vs. attack comparison, there is a moderate positive correlation, but the 95% CI is large and consistent with anywhere from an absence of correlation to a very strong positive correlation. About flight vs. lateral threat, the correlation is strong and the 95% CI is restricted to moderate and strong positive correlation values. Interestingly, during the last exposure, the correlation between flight and threat disappears, while flight vs. attack correlation was maintained (flight vs. attack: $r = 0.80$, 95% CI [0.38, 0.95]; flight vs. lateral threat: $r = 0.08$, 95% CI [-0.55, 0.65]). The 95% CI of the coefficient related to flight vs. attack comparison suggests, at least, a moderate correlation between these behaviours. Another interesting difference between first and third exposure is the correlation between intruder bites and flight behaviours. Regarding first exposure, the positive correlation is strong and the 95% CI is large, but restricted to moderate and very positive strong correlation ($r = 0.82$, 95% CI [0.17, 0.97]). The correlation intensity decreases in the third exposure when the coefficient is moderate and the 95% CI is consistent with anywhere between an absence of correlation to a strong positive correlation ($r = 0.62$, 95% CI [0.03, 0.89]).

In both exposures, there is a very strong negative correlation between exploratory and defensive behaviours (1 Exp.: $r = -0.98$, 95% CI [-0.99, -0.89]; 3 Exp.: $r = -0.98$, 95% CI [-0.99, -0.91]). Their 95% CI are short and restricted to a very strong correlation. Also, the correlation between passive and active defence is very strong and their 95% CI was a little larger, but restricted to moderate and very strong negative coefficients (1 Exp.: $r = -0.88$, 95% CI [-0.98, -0.40]; 3 Exp.: $r = -0.87$, 95% CI [-0.97, -0.56]). Another similarity between the two groups is the correlation between flight behaviour and intruder travelled distance (1 Exp.: $r = 0.92$, 95% CI [0.57, 0.99]; 3 Exp.: $r = 0.90$, 95% CI [0.64, 0.97]). The coefficients suggest a very strong correlation and both 95% CI are consistent with, at least, a moderate positive correlation between flight behaviour and travelled distance.

Correlations between brain activation and behaviour

Regarding the portions of the MeA, when we analysed data from animals submitted to only one exposure to the resident-intruder paradigm, we were not able to observe a clear correlation with any behavioural variable or other brain areas mobilization (Supplementary Data 1). This absence of clear correlations changes when we analyse data from animals that have submitted to

3 exposures. In this case, the correlations suggest a positive relationship between MeAav, MeApd and MeApv with behaviours involved with active defence (Supplementary Data 2). However, the calculated coefficients are generally moderate, and the 95% CI is large and consistent with an absence of correlation to a strong positive correlation. The exception is the correlation between MeApd and MeApv with boxing, which has a strong correlation and a small confidence interval restricted to moderate to strong Pearson's coefficient values (MeApd vs. boxing: $r = 0.80$, 95% CI [0.40, 0.95]; MeApv vs. boxing: $r = 0.90$, 95% CI [0.65, 0.97]).

On the sexually dimorphic circuit, the correlation pattern is also different between the two groups. In 1 Exp. group, as the MeA, there is not a clear correlation between sexually dimorphic circuit mobilization and other areas or behaviours (Supplementary Data 1). Some exceptions are the correlation between PMV and rVMHvl mobilization, as well as the correlation between these areas and defensive upright behaviour (Supplementary Data 1). Remarkably, PMV and rVMHvl are strongly negatively correlated ($r = -0.93$, 95% CI [-0.99, -0.57]). The 95% CI is short and restricted to moderate up to very strong negative coefficient values. The rVMHvl is also moderately negatively correlated with LHAsf. However, the 95% CI is large and corroborates with an absence of correlation up to a very strong negative correlation ($r = -0.73$, 95% CI [-0.96, 0.05]). The defensive upright behaviour is negatively correlated with rVMHvl, while is positively correlated with PMV. About the defensive upright behaviour vs. rVMHvl, the Pearson correlation coefficient is moderate and its 95% CI are large and consistent with an absence of correlation up to a strong negative correlation ($r = -0.73$, 95% CI [-0.96, 0.05]). Regarding defensive upright behaviour vs. PMV, the coefficient is moderate and its 95% CI are large and consistent with moderate up to a strong positive correlation ($r = 0.86$, 95% CI [0.29, 0.98]). Interestingly, these correlations disappear to intruders submitted three times to the resident-intruder paradigm (Supplementary Data 2).

Concerning PMD, in animals subjected to 1 exposure to the resident-intruder paradigm, we observed a strong positive correlation between their expression of Fos and time spent in checking ($r = 0.86$, 95% CI [0.31, 0.98]). In this case, the 95% CI is short and restricted to moderate to strong positive Pearson's coefficient values. In animals submitted to three exposures, we no longer observe this correlation. However, there is a moderate correlation between PMD Fos expression with turning to face, whose Pearson's coefficient is moderate and has a 95% CI restricted to weak up to strong positive correlation values ($r = 0.69$, 95% CI [0.16, 0.91]). Also, the PMD is negatively correlated with rVMHvl mobilization. The Pearson correlation coefficient is moderate and the 95% CI is consistent with anywhere from a very strong negative correlation up to a weak positive correlation ($r = -0.67$, 95% CI [-0.95, 0.17]).

About the PAG, in animals submitted to only one exposure to the resident-intruder paradigm, there is not a clear correlation between the PAG columns and others areas or different analysed behaviours. Overall, the correlations are weak, and the 95% CI is broad and consistent with moderate negative correlation to moderate positive correlation (Supplementary Data 1). Regarding the group of animals submitted to three exposures, there is a moderate correlation between the pattern of activity of the dorsomedial (rPAGdm and cPAGdm) and lateral (rPAGl and cPAGl) columns with active defence behaviours, namely flight and boxing (Supplementary Data 2). In this case, the 95% CI are broad and restricted to positive values of Pearson's coefficients. Furthermore, there is a strong negative correlation between time spent in passive defence and cPAGdm activity, which has a 95% short CI and restricted to moderate to strong Pearson's coefficient values ($r = -0.84$, 95% CI [-0.96, -0.48]). We also observed a negative correlation between passive defence and cPAGvl activity, however with a moderate coefficient and a large 95% CI consistent with a lack of correlation with a strong negative correlation ($r = -0.62$, 95% CI [-0.89, -0.03]). About the cPAGl, there is a moderate positive correlation between this column and the rVMHvl and LHAjd mobilization. In both cases, the 95% CI are large and consistent with a weak positive correlation up to a strong correlation (cPAGl vs. LHAjd: $r = 0.69$, 95% CI [0.15, 0.91]; cPAGl vs. rVMHvl: $r = 0.79$, 95% CI [0.36, 0.94]).

Social defeat and restraint stress

To investigate if the neural circuits mobilized during social defeat are also mobilized by other aversive situations, we compared the activation pattern between a group of animals submitted to one exposure of the resident-intruder paradigm ($n = 7$) and one group of animals submitted to restraint stress ($n = 7$). As the restraint stress is characterized by the limitation of the animal movement, the comparison between these types of stress can suggest the circuits that would be behind the signal of entrapment imposed by the aggressor during the social defeat. As before, most of the time, intruder animals expressed defensive behaviours and a small portion of the time was also spent in exploring the resident's home cage. During the restraint stress, animals presented clear signs of stress and, sometimes, vocalization.

Differently from social defeat, circuits that process social information are not mobilized during restraint stress

Regarding the regions associated with the processing of social information, most of them presented high standardized effect sizes and large CIs restricted to moderate up to high effect size values, which suggests a greater activation in animals submitted to the social stress than restraint stress (Fig 4, 6; Supplementary Information Table S7, S8). The only exception is the MPN, which presents a moderate negative standardized effect size with a CI consistent with negative up to positive effect sizes, which does not provide clear evidence of a difference between the groups.

239 ***The septo-hippocampal-hypothalamic circuit is also mobilized during restraint stress***

240 About the nuclei of the septo-hippocampal-hypothalamic circuit, exploratory analyses do not suggest a clear difference in the
241 mobilization of LHAjp and LHAjd between animals submitted to social stress or restraint stress (Fig 4, 6; Supplementary
242 Information Table S7, S8). In these cases, the standardized effect sizes are small and the CIs are consistent with anywhere from
243 negative difference, no difference up to positive difference between the groups. The same was observed with the PMD, the
244 rVMHdm and hypothalamic nuclei that participate in neuroendocrine and autonomic responses to stress (Fig 4, 6; Supplementary
245 Information Table S7, S8). The only exception is the anterior part of the dorsomedial nucleus of the hypothalamus (DMHa),
246 whose standardized effect size is high and the CI is restricted to high up to small negative effect size values, which suggest
247 that the neural activation of this area is greater in restrained animals than social subjugated animals (Fig 4, 6; Supplementary
248 Information Table S7, S8).

249 ***Periaqueductal grey matter mobilization during social defeat and restraint stress***

250 In the periaqueductal grey matter, except the rostral and caudal portion of the dorsolateral part (rPAGdl and cPAGdl), social
251 defeat mice showed, at least moderately, a higher neural activity than restrained animals in all others analysed columns (Fig 6;
252 Supplementary Information Table S7, S8). In general, the standardized effect sizes are moderate and the CIs are large and
253 consistent with no difference up to a high difference between these groups. The exception is the cPAGl that has a high
254 standardized effect size and a CI covering only high effect size values. About rPAGdl and cPAGdl, exploratory analyses do not
255 provide clear evidence of difference between animals submitted to restraint and social stress (Fig 6; Supplementary Information
256 Table S7, S8). The effect size related to rPAGdl is small and the CI is consistent with anywhere from negative difference, no
257 difference up to positive difference between these groups. Concerning cPAGdl, the effect size is moderate and suggests that this
258 area is slightly more mobilized in social stress than restraint stress. However, the CI is large and consistent with small negative
259 effect size values up to high positive effect size values.

260 **Discussion**

261 Many works have studied the effects of repeated social stress with different aims, such as in the neuroendocrine and autonomic
262 response, memory, psychopathology-like behaviour and coping strategy. In the present work, our goal was to investigate the
263 effects of repeated social stress on neural circuits underlying the motivated behaviour social defence and to explore some
264 hypotheses about the mechanisms that lead to adaptations in this behaviour after reexposures. We observed that, during last
265 exposure to social stress, there is a lower mobilization of circuits associated with the processing of conspecific information,
266 while the septo-hippocampal-hypothalamic circuit and the PMD present an activity pattern similar to the first day of social
267 stress. The septo-hippocampal-hypothalamic circuit is also mobilized during the restraint stress, which suggests broader
268 participation in defensive responses. With all this in view, our data suggest that the balance between the activity of circuits
269 related to conspecific processing and the PMD may determine the pattern of expressed defensive behaviour. The reexposure to
270 the conspecific impacts the mobilization of these structures, which may be the basis of the adaptations in social defence.

271 During acute social stress, the mobilized structures in mice are similar to those observed in rats, although the behavioural
272 pattern is different between these animals^{9,15,23}. While rats rarely perform exploratory behaviours, mice, during their first
273 exposure, spend a substantial part of their time exploring the environment^{9,15}. After reexposure to the paradigm, the intruder's
274 time spent in exploration decreases and the relation between exploratory and defensive behaviour becomes more similar to the
275 rat's relation^{9,15}. Interestingly, during acute social stress, the PMD is relatively more mobilized than the VMHvl in rats^{9,15},
276 while in mice it is the opposite. Since the PMD is essential in passive defence and the VMHvl is associated with active defence
277 and social exploration^{9,28,29}, this brought us to the idea that, in mice, the behavioural variation after reexposure to social stress
278 (balance between exploration and passive and active defence) would be related to the balance in the mobilization of these neural
279 structures. Our data corroborate this idea since, during the last exposure to social stress, the intensity of mobilization of the
280 circuits underlying the social defence becomes closer to what is observed in rats.

281 As expected, the nuclei of the sexually dimorphic circuit are mobilized during a social confrontation. Different works
282 with rats and mice suggest the participation of this circuit in many types of social behaviour as fighting, parenting and mating
283 behaviour^{9,20,21,30-36}. These nuclei are also very important for social memory and social recognition^{37,38}. In the present work,
284 VMHvl and PMV were the most mobilized regions during social stress. Concerning VMHvl, some works indicate its role in
285 defensive behaviour, especially active defence²⁸. This region is also related to social fear and the VMHvl inhibition impairs
286 fear response to a social aversive stimulus^{39,40}. The PMV is activated by male and female olfactory cues and their inhibition
287 impairs both maternal and inter-male attacks^{20,21,41}. Their activity during the social confrontation can be due mainly to the
288 social representation of the resident and the execution of active defence behaviours^{20,28,38,42}. Our data suggest a reduction in
289 the mobilization of these areas after the reexposure to the resident-intruder paradigm. This reduction in the sexually dimorphic
290 circuit mobilization was not associated with a reduction in time spent on active defence. Also, there was not a clear correlation
291 between the Fos expression of these regions and some behaviour associated with active defence. Different phenomena can

292 be behind this. First, there is a large diversity of neurons in nuclei of the sexually dimorphic circuit, especially the VMHvl²⁹.
293 While some neurons are related to social exploration, other neurons participate mainly in active defence behaviour^{3,29,42}.
294 Therefore, the mobilization of VMHvl may depend not only on active defence. Another possibility – which may be occurring
295 concurrently – is that the active defence can be defined not only on the number of neurons mobilized in the sexually dimorphic
296 circuit but also on the variation in the frequency or intensity of neural activation of these areas.

297 One relevant region upstream of the sexually dimorphic circuit is the MeA^{12,43}. Its nuclei receive many direct and indirect
298 projections from main and accessory olfactory systems and process the social information from olfactory cues^{16,44}. The MeA
299 participates in the neural representation of social conspecific and its activity is related to sexual, defensive and aggressive
300 behaviours both in mice and rats^{9,16,17,45,46}. The activation of gabaergic MeA neurons promotes aggressive and sexual
301 behaviours, depending on the intensity of activation⁴⁷. As the activity of the sexually dimorphic circuit is also necessary for
302 these behaviours, it has been proposed that the role of MeA occurs by a disinhibitory circuit^{42,47}. This idea agrees with our
303 data since we observed an increase in Fos-labelled neuron density in MeA and sexually dimorphic circuit during social stress.
304 After reexposure to social stress, there was a reduction of mobilization of MeA nuclei, which may be a factor that leads to a
305 reduction of activity of the sexually dimorphic circuit. However, there is not a clear correlation between the mobilization of
306 this circuit and MeA mobilization. These data suggest a more complex relationship between these regions, which also may be
307 related to direct and indirect pathways between MeA and sexually dimorphic circuit^{12,43}.

308 Another region that can participate in the processing of social information is the BST^{19,20,45,48,49,52}. Its activity is
309 related to different social behaviours such as social defence, inter-male aggression, maternal aggression and parental be-
310 haviours^{19,20,45,48–50,52}. Neuroanatomic evidence suggests that the BST participates in the disinhibitory circuit between MeA
311 and sexually dimorphic circuit^{12,43,48}. If the BST activity was just dependent on this disinhibitory circuit, it would be expected
312 an increase in BST mobilization in animals of the reexposure group, since in these animals there was a decrease in MeA
313 activation. However, our data do not suggest a clear difference in BST mobilization between animals submitted to one or
314 three sessions of the resident-intruder paradigm. As the MeA, BST neurons also receive direct and indirect projections from
315 main and accessory olfactory systems, which also can influence the BST activity during social defeat^{19,51}. The BST activity is
316 important to the expression of conditioned defeat, which is related to defensive behaviours⁵². So, the BST may be relevant to
317 the increase in time spent in passive defence during the last exposure.

318 In addition to social information, spatial information is essential to the organization of defensive strategy not only for social
319 confrontation but also in many aversive situations^{22,53}. The distance of the threat and the existence of an escape route or safe
320 place can impact the behaviour to be developed^{22,53}. In this sense, we believe that the LHAjd is important for the processing of
321 environmental cues. The LHAjd is mobilized during the first and the last exposure to social stress and is also mobilized during
322 restraint stress, both in mice, as we observed in the present work, and rats¹⁵. Since the restraint stress is characterized only
323 by the presence of a physical limit that prevents animal movement and, in social stress, the conspecific represents a physical
324 and psychological limit, the LHAjd may be processing the entrapment component associated with both contexts^{15,23}. Some
325 functional studies also highlight the role of the LHAjd in defensive behaviour. Rats with lesioned LHAjd perform less risk
326 assessment during post-encounter context associated to a conspecific²³. Also, the inhibition of LHA promotes a deficit in escape
327 behaviour in response to predatory and physical threats^{54–57}. Thus, a septo-hippocampal-hypothalamic circuit, that comprises
328 the LHAjd, is expected to participate in the processing of spatial information during many aversive contexts^{15,23,25,26,58}. This
329 circuit also comprises the two major sources of afferent to LHAjd: the subiculum and the lateral septum⁵⁹. The subiculum
330 detains many border-vector cells, which participate in the processing of environmental limits⁶⁰. Neuroanatomic evidence also
331 suggests that this structure participates as an intermediate of information present in the hippocampal system to cortical and
332 subcortical structures^{61,62}. The lateral septum is the structure that most receives projections from CA1 and CA3 hippocampal
333 regions⁶³. Recent works show the role of the lateral septum as a decoder of the cognitive map from the hippocampus to
334 downstream regions⁶⁴. These data suggest a broader role for LHAjd in defence by participating in the spatial information
335 process.

336 The integration of spatial and social information is relevant to social defence. The PMD seems to be an important region
337 for the integration of threat cues (social, predatory and physical) and spatial information^{9,15,25,26,65–68}. As LHAjd, the PMD
338 is mobilized during social and restraint stress. This nucleus receives strong projections from LHAjd and, therefore, may be
339 receiving environmental-related information from the septo-hippocampal-hypothalamic circuit during aversive situations^{15,59}.
340 Furthermore, the PMD activity is essential to the passive defence behaviour⁹. However, there is not a clear difference between
341 the PMD mobilization during the first and last exposure to social stress, which would be expected since the time spent in passive
342 defence is greater during the last exposure. This suggests that the mobilization of PMD neurons is not the only determinant of
343 time spent in this passive defence, which is corroborated by the absence of a clear correlation between PMD mobilization and
344 this behaviour. Thus, the ratio between active and passive defence may be determined by the relation of mobilized neurons in
345 PMD and VMHvl. During the last exposure to social stress, the decrease in the VMHvl mobilization and the maintenance
346 of PMD mobilization can lead to an increase in time spent on passive defence. Interestingly, the LHA_{sf} mobilization is also

347 decreased during the last exposure to social stress, which agrees with a presumed role of LHAsf in the communication between
348 sexually dimorphic nuclei and PMD^{59,65,69}.

349 For the development of this defensive strategy, the PAG is an important effector region that receives projections directly from
350 hypothalamic nuclei, like PMD, VMHvl and LHA^{9,28,55,68}. The PAG location suggests its role in mediating the information
351 output from prosencephalic structures to the brainstem and spinal cord regions⁷⁰. Also, PAG participates in the regulation of
352 primary emotional responses by projecting information to prosencephalic structures⁷⁰. PAG columns (especially rPAGdm
353 and rPAGl) are mobilized during both social and restraint stress. These columns receive projections from PMD and LHAjd
354 and may be the effector sites from the septo-hippocampal-hypothalamic circuit since they are also mobilized during other
355 aversive situations as predatory encounter and foot shock^{15,23,25,26,58}. Also, the mobilization of PAGdm,l was similar between
356 the first and last exposure to social stress. These columns also receive projections from VMHvl and have subpopulations
357 associated with escape or freezing behaviours^{55,68,71-73}. Therefore, these PAG columns may be important to the variation in
358 social defence strategy, which could be related to the relationship between the activity of PMD and VMHvl. PAG columns also
359 receive projections from the medial prefrontal cortex (mPFC) and the inhibition of mPFC-PAGd projections mimics behavioural
360 alterations after a social defeat protocol⁷⁴. So, this circuit may also be relevant for the variation in the social defence strategy
361 during reexposure to the resident intruder paradigm.

362 The behavioural response to an aversive context is accompanied by neuroendocrine and autonomic responses. The
363 mobilization of paraventricular and dorsomedial nuclei of the hypothalamus (PVH and DMH), both during the first and third
364 exposure, as well during restraint stress, agrees with their role in neuroendocrine and autonomic responses to stressors^{14,75-78}.
365 Previous works show the participation of these regions as an integrator centre for regulation of the hypothalamic-pituitary-
366 adrenal axis^{75,79}. This pathway regulates corticosterone production, a hormone that mediates the endocrine response to stress⁷⁹.
367 The increase in DMH activity is also related to an increase in cardiac frequency, which is common in aversive situations,
368 including social defeat and restraint stress^{78,80-82}.

369 Taken together, our data indicate a change in the mobilization pattern of neural circuits underlying social defence during
370 the last exposure. This change is characterized by a decrease in the mobilization of structures that process social information,
371 namely MeA, VMHvl and PMV. We speculate that some mechanism, which involves memory recovery about the social stress
372 experienced, is mobilized during the last day of exposure, similarly to what happens during contextual fear. This mechanism
373 may promote less mobilization of the conspecific responsive circuit and a pattern of behaviour that avoids the attack of the
374 resident. A structure that could participate in this process is the BST. The BST receives different corticolimbic projections
375 - such as from the mPFC and the basolateral amygdala (BA) - and projects to different hypothalamic structures associated
376 with the regulation of defensive behaviour^{48,83,84}. Different evidence suggests the importance of BST for the expression of
377 behavioural response associated with contextual fear^{52,85-87}. BST injuries reduce the time spent on freezing during the context
378 day after foot shock⁸⁵ and BST inhibition with muscimol reduces the expression of fear behaviours associated with social
379 defeat⁵². Furthermore, acute and chronic stressors can alter the plasticity of BST⁸⁴. In this sense, at the beginning of the last
380 exposure to the resident-intruder paradigm, the BST could be participating in an anticipation system for the social stress that
381 will be suffered by the animal, based on past experiences. Thus, BST could interfere with the balance between the conspecific
382 responsive circuit and the PMD activity, which promotes a pattern of social defence predominantly passive. Indeed, more
383 studies are needed to elucidate this anticipation system that can act on neural circuits underlying social defence and orchestrate
384 this behaviour.

385 In summary, the reexposure to social stress promotes changes in the social defence behavioural pattern of C57Bl/6 male
386 mice, which is accompanied by changes in the neural mobilization of circuits related to the expression of this goal-oriented
387 behaviour. Our data are following previous works that have shown that social defence behaviour depends on the mobilization
388 of neural circuits that process conspecific cues and environmental cues. Furthermore, our data suggest that the pattern of
389 social defence (e.g. more passive or more active) is determined by the balance between the mobilization of circuits related
390 to the processing of the conspecific information (in particular the VMHvl nucleus) and the PMD activity (which can receive
391 environmental information from the septo-hippocampal-hypothalamic circuit). Further studies are necessary to investigate the
392 mechanisms by which repeated social stress affects the mobilization of these circuits and impacts social defence behaviour.

393 **Methods**

394 **Animals**

395 Fifty-three adult male C57Bl/6 mice (~9 weeks old) obtained from the local breeding facilities were used in the present
396 study. Animals were housed, at least one week before behaviour tests, in individual cages (30 x 20 x 13 cm; Beiramar Ind. e
397 Com. Ltda., SP, Brazil) under controlled illumination (12 h light/dark cycle – 7 am to 7 pm light), humidity and temperature
398 (22 ± 1°C), and with free access to food and water. For resident-intruder paradigm experiments, sexually experienced Swiss
399 Webster males (3-12 months old, housed with Swiss Webster females) were used as residents. All the mice received humane
400 care in compliance with the ARRIVE guidelines and were handled according to the Brazilian National Law (11.794/2008). The

401 experiments were approved by the Committee on Care and Use of Laboratory Animals of the Institute of Biomedical Sciences,
402 University of São Paulo, Brazil (Instituto de Ciências Biomédicas, Universidade de São Paulo; Protocol number 58/2016 and
403 6417260520).

404 **Behavioural tests**

405 ***Resident-intruder paradigm***

406 Animals submitted to the resident-intruder paradigm were separated into two groups. One group was submitted to only
407 one session of the resident-intruder paradigm (n = 16). The other group was submitted to three sessions of the resident-
408 intruder paradigm, performed for three days, once a day, with different residents (n = 11). During each session, as previously
409 described^{9,15}, the Swiss female was removed and a C57Bl/6 mouse was placed in the dominant home cage. Animals were
410 separated 5 min after the first resident attack and the intruders were returned to their home cage. For a control group (n = 12),
411 the mice were left undisturbed in an individual cage.

412 ***Restraint***

413 After 24 h isolation, animals (n = 14) were restrained for 30 min in an acrylic tube (ø 3 cm X 10 cm length, volume = 70.69 ml;
414 Beiramar Ind. e Com. Ltda., SP, Brazil). After this period, the animals were returned to their cages.

415 **Behaviour analysis**

416 The resident-intruder paradigm sessions were recorded with horizontally and vertically mounted video cameras (Sony Handycam
417 DCR-SR68; Sony, TKY, Japan). For the behaviours score, the videos were analysed by a trained observer using the BORIS v7.97
418 software⁸⁸. To preserve the blindness of the observer, a random identification, unknown to the observer, was labelled for each
419 video before analyses. The DORIS v20.3.16 software was used for movement tracking⁸⁹. For tracking, the body centre was
420 used to estimate the centre of mass of the analysed animals. The accounted behaviours were based on Blanchard, 1979¹¹
421 and Motta, 2009⁹. About the intruder, the duration of the following behaviours was observed: non-social exploration, social
422 exploration, defensive behaviours (separated into active defence – that occurs when the animal is under attack, like upright
423 position, boxing, turning to face, flight and dashing away from the resident - and passive defence – that occurs when the resident
424 leaves the intruder alone, such as freezing and checking) and other behaviours (like grooming and rearing)^{9,11}. Concerning
425 the resident, we observed: non-social exploration, social exploration, aggressive behaviours (separated into threat – lateral
426 threat and move towards -, attack – keep down, lateral and clinch attack - and chase) and other behaviours^{9,11}. The number of
427 bites delivered by the resident to the intruder and the number of retaliation bites from the intruder were also counted. With the
428 movement tracking, the following spatio-temporal variables were measured: time spent in the centre and the border of the
429 dominant home cage, total distance travelled and distance travelled in the centre and the border, for the intruder and resident.

430 **Fos immunostaining**

431 Ninety minutes after the behavioural tests, the animals were anaesthetized with isoflurane (1 ml/ml; Cristália Laboratories, SP,
432 Brazil) and transcardially perfused with 0.9% saline followed by 4% (mass/volume) paraformaldehyde (PFA) in 0.1 M sodium
433 phosphate buffer (NaPBS) at pH 7.4. The fixed brains were removed and left overnight in a solution of 20% sucrose in 0.02 M
434 potassium phosphate buffer (PBS) at 4°C. The brains were then frozen and sectioned on a sliding microtome in the coronal
435 plane into four stepwise collated series (30 µm thickness). The series were maintained in an antifreeze solution (15% sucrose,
436 30% Ethylene glycol in 0.05 M NaPBS) at 20°C. One series was processed for immunohistochemistry using a rabbit anti-Fos
437 antiserum (Ab-5; Oncogene Research Products, CA, USA) for 72 h at 4°C at a dilution of 1:40000 (2% goat serum (NGS),
438 0.3% Triton X-100 in 0.02 M PBS), under constant shaking. For the detection of the primary anti-serum, the sections were
439 incubated at room temperature for 90 min in a solution of biotinylated goat anti-rabbit IgG (Vector Laboratories) at a dilution of
440 1:200 (2% NGS, 0.3% Triton X-100 in 0.02 M PBS). Then, the sections were placed in the avidin-biotin-peroxidase (HRP)
441 complex solution (ABC Elite Kit; Vector Laboratories, CA, USA) for 90 min. To visualize the complex, the sections were
442 exposed for about 20 min to a solution containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich, MO,
443 USA), 0.0396% nickel sulfate, 0.04% ammonia chloride, glucose oxidase (1:1000) and 0.2% β-D glucose (after five minutes
444 of incubation), in 0.1 M NaPBS. The reaction was stopped by extensive washing in potassium phosphate buffer. Sections were
445 mounted on gelatin-coated slides and then dehydrated and coverslipped with DPX neutral mounting medium (Sigma-Aldrich,
446 MO, USA). One adjacent series was stained with thionin to serve as a reference series for cytoarchitectonic purposes.

447 ***Exclusion criteria***

448 Part of the material was excluded from the analysis due to problems involving perfusion and fixation steps that impaired the
449 immune reaction for labelling Fos-expressing cells. Therefore, the number of animals analysed was: fourteen C57Bl/6 mice
450 submitted to only one session (n = 14, n = 7 for the study of the effects of reexposure to the resident-intruder paradigm, and
451 n = 7 for comparisons between animals submitted to social and restraint stress), eleven animals submitted to three sessions

452 (n = 11) of the resident-intruder paradigm, seven mice submitted to the restraint stress (n = 7) and twelve control animals
453 (n = 12).

454 **Quantification of Fos-labeled cells**

455 For the density of Fos-labeled cell quantification, a random identification was assigned to each animal to avoid the observer's
456 knowledge of the animal's experimental treatment during the analysis. Images were generated using the 10X objective of a
457 Nikon Eclipse 80i microscope (Nikon Corporation, TKY, Japan) equipped with a Nikon digital camera (Nikon Corporation,
458 TKY, Japan). During the analyses, the regions of interest were defined with the help of adjacent Nissl-stained sections, and
459 Fos-labeled cells were counted therein. Only darkly labelled oval nuclei that fell within the borders of a region of interest were
460 counted. The density of Fos-labeled cells was determined by dividing the number of Fos-immunoreactive cells by the area of
461 the region of interest. Cell densities were obtained on both sides of the brain and averaged for each individual. These analyses
462 were performed with the aid of Fiji, a distribution of the ImageJ v1.48 software⁹⁰.

463 We quantified the density of Fos-labeled cells in 31 brain regions that followed the Allen Mouse Brain Atlas and The
464 Mouse Brain in Stereotaxic Coordinates^{91,92}. In the hypothalamus, we analysed nuclei involved with the response to
465 conspecifics, namely, the medial preoptic nucleus (MPN), the ventrolateral part of the ventromedial nucleus (rostral and
466 dorsal portion) (rVMHvl and cVMHvl), tuberal nucleus (TU) and the ventral premammillary nucleus (PMV)^{9,21,28,39,58};
467 the dorsal premammillary nucleus (PMD), that is involved with information integration of threats cues^{9,15,45,59}; nuclei
468 related with the neuroendocrine and autonomic response to stressors, such as paraventricular nucleus (both parvicellular and
469 magnocellular parts) (PVHparv and PVHmag) and dorsomedial nucleus of the hypothalamus (rostral and caudal portion)
470 (DMHa and DMHc)^{27,76,78,93}; and the juxtaparaventricular, juxtadorsomedial, juxtaventromedial (dorsal and ventral portion)
471 and subforminal regions of the lateral hypothalamus (LHAjp, LHAjd, LHAjvd, LHAjvv and LHA sf), which are related with
472 the integration between the septo-hippocampal system and defensive behaviour system components^{45,59,69,94}. Furthermore,
473 we also analysed regions involved with the processing of social and contextual cues as the medial amygdala (anteroventral,
474 anterodorsal, posteroventral and posterodorsal portion) (MeAav, MeAad, MeApv and MeApd), bed nuclei of stria terminalis
475 (principal and transversal/interfascicular portion) (BSTpr and BSTtr,if) and lateral septum (caudodorsal and rostroventral
476 part) (LSc and L Sr)^{17,95,96}. At last, in the periaqueductal grey matter - an important region involved with fear and defensive
477 responses - we analysed the dorsomedial (rostral and caudal), dorsolateral (rostral and caudal), lateral (rostral and caudal) and
478 ventrolateral portion (rPAGdm, cPAGdm, rPAGdl, cPAGdl, rPAGl, cPAGl, rPAGvl and cPAGvl)^{45,70,97}.

479 **Statistical analysis**

480 The objective of the analysis was to explore differences in neural activation and behavioural pattern between mice submitted
481 once or three times to the resident-intruder paradigm and to compare neural activation between mice submitted to social and
482 restraint stress. We expected to generate new hypotheses of how different neural circuits are involved with the expression of
483 social defence and how these circuits are mobilized in response to different types of stress.

484 All data are expressed as the mean \pm sample standard deviation ("s"), and "n" is the sample size. We also estimated the
485 95% confidence interval (95% CI) of the mean. To compare different experimental groups, we calculated the difference
486 between means (M_{diff}) and estimated the 95% CI for each M_{diff} . We also calculated the unbiased Cohen's d (d_{unb}) and its
487 respective 95% CI for each comparison^{98,99}. To measure the correlation between variables, we calculated Pearson's r and its
488 95% CI⁹⁸. These analyses were conducted using the esci v0.9.1 library from jamovi v1.2 and the pingouin v0.3.8 library from
489 python v3.8.3. For plotting graphs, we used the seaborn v0.11.0 and matplotlib v3.3.2 libraries from python v3.8.3.

490 **Data availability**

491 All raw data is available in the University of São Paulo data repository, in the link: [https://uspdigital.usp.br/
492 repositorio/](https://uspdigital.usp.br/repositorio/).

References

1. Caroline Blanchard, D. *et al.* Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. *Psychoneuroendocrinology* **20**, 117–134 (1995).
2. Blanchard, D. C. & Blanchard, R. J. Behavioral correlates of chronic dominance-subordination relationships of male rats in a seminatural situation. *Neurosci. Biobehav. Rev.* **14**, 455–462 (1990).
3. Diaz, V. & Lin, D. Neural circuits for coping with social defeat. *Curr. Opin. Neurobiol.* **60**, 99–107 (2020).
4. Martinez, M., Calvo-Torrent, A. & Pico-Alfonso, M. A. Social defeat and subordination as models of social stress in laboratory rodents: A review. *Aggress. Behav.* **24**, 241–256 (1998).

5. Miczek, K. A. A new test for aggression in rats without aversive stimulation: Differential effects of d-amphetamine and cocaine. *Psychopharmacol. (Berl)*. **60**, 253–259 (1979).
6. Golden, S. A., Covington, H. E., Berton, O., Russo, S. J. & Russo, S. J. A standardized protocol for repeated social defeat stress in mice. *Nat. Protoc.* **6**, 1183–91 (2011).
7. Butler, J. M., Whitlow, S. M., Roberts, D. A. & Maruska, K. P. Neural and behavioural correlates of repeated social defeat. *Sci. Rep.* **8**, 6818 (2018).
8. Macedo, G. C. *et al.* Consequences of continuous social defeat stress on anxiety- and depressive-like behaviors and ethanol reward in mice. *Horm. Behav.* **97**, 154–161 (2018).
9. Motta, S. C. *et al.* Dissecting the brain's fear system reveals the hypothalamus is critical for responding in subordinate conspecific intruders. *Proc. Natl. Acad. Sci.* **106**, 4870–4875 (2009).
10. Blanchard, R. J., Blanchard, D., Takahashi, T. & Kelley, M. J. Attack and defensive behaviour in the albino rat. *Anim. Behav.* **25**, 622–634 (1977).
11. Blanchard, R. J., O'Donnell, V. & Caroline Blanchard, D. Attack and defensive behaviors in the albino mouse. *Aggress. Behav.* **5**, 341–352 (1979).
12. Swanson, L. W. Cerebral hemisphere regulation of motivated behavior. *Brain Res.* **886**, 113–164 (2000).
13. Watson, C., Paxinos, G. & Puelles, L. The Mouse Nervous System. In *Mouse Nerv. Syst.*, chap. 20, 795 (Elsevier, 2012), 1 edn.
14. Martinez, M., Phillips, P. J. & Herbert, J. Adaptation in patterns of c-fos expression in the brain associated with exposure to either single or repeated social stress in male rats. *Eur. J. Neurosci.* **10**, 20–33 (1998).
15. Motta, S. C. & Canteras, N. S. Restraint stress and social defeat: What they have in common. *Physiol. Behav.* **146**, 105–110 (2015).
16. Bergan, J. F., Ben-Shaul, Y. & Dulac, C. Sex-specific processing of social cues in the medial amygdala. *eLife* **3**, e02743 (2014).
17. Li, Y. *et al.* Neuronal representation of social information in the medial amygdala of awake behaving mice. *Cell* **171**, 1176–1190 (2017).
18. Li, Y. & Dulac, C. Neural coding of sex-specific social information in the mouse brain. *Curr. Opin. Neurobiol.* **53**, 120–130, (2018).
19. Bayless, D. W. *et al.* Limbic neurons shape sex recognition and social behavior in sexually naive males. *Cell* **176**, 1190–1205 (2019).
20. Chen, A.-X. *et al.* Specific hypothalamic neurons required for sensing conspecific male cues relevant to inter-male aggression. *Neuron* **108**, 1–12 (2020).
21. Motta, S. C. *et al.* Ventral premammillary nucleus as a critical sensory relay to the maternal aggression network. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 14438–43 (2013).
22. Mobbs, D., Headley, D. B., Ding, W. & Dayan, P. Space, time, and fear: Survival computations along defensive circuits. *Trends Cogn. Sci.* **24**, 228–241 (2020).
23. Rangel, M. J. *et al.* Evidence of a role for the lateral hypothalamic area juxtadorsomedial region (LHAjd) in defensive behaviors associated with social defeat. *Front. Syst. Neurosci.* **10**, 92 (2016).
24. Buynitsky, T. & Mostofsky, D. I. Restraint stress in biobehavioral research: Recent developments. *Neurosci. Biobehav. Rev.* **33**, 1089–1098 (2009).
25. Viellard, J., Baldo, M. V. C. & Canteras, N. S. Testing conditions in shock-based contextual fear conditioning influence both the behavioral responses and the activation of circuits potentially involved in contextual avoidance. *Behav. Brain Res.* **315**, 123–129 (2016).
26. Mendes-Gomes, J. *et al.* Defensive behaviors and brain regional activation changes in rats confronting a snake. *Behav. Brain Res.* **381**, 112469 (2020).
27. Canteras, N. S. The medial hypothalamic defensive system: Hodological organization and functional implications. *Pharmacol. Biochem. Behav.* **71**, 481–491 (2002).
28. Wang, L. *et al.* Hypothalamic control of conspecific self-defense. *Cell Rep.* **26**, 1747–1758 (2019).

29. Kim, D.-W. *et al.* Multimodal analysis of cell types in a hypothalamic node controlling social behavior. *Cell* **179**, 713–728 (2019).
30. Lin, D. *et al.* Functional identification of an aggression locus in the mouse hypothalamus. *Nature* **470**, 221–227 (2011).
31. Lee, H. *et al.* Scalable control of mounting and attack by Esr1+ neurons in the ventromedial hypothalamus. *Nature* **509**, 627–632 (2014).
32. Wu, Z., Autry, A. E., Bergan, J. F., Watabe-Uchida, M. & Dulac, C. G. Galanin neurons in the medial preoptic area govern parental behaviour. *Nature* **509**, 325–330 (2014).
33. Wang, L., Chen, I. Z. & Lin, D. Collateral pathways from the ventromedial hypothalamus mediate defensive behaviors. *Neuron* **85**, 1344–1358 (2015).
34. Wong, L. *et al.* Effective modulation of male aggression through lateral septum to medial hypothalamus projection. *Curr. Biol.* **26**, 593–604 (2016).
35. Hashikawa, K. *et al.* Esr1+ cells in the ventromedial hypothalamus control female aggression. *Nat. Neurosci.* **20**, 1580–1590 (2017).
36. Karigo, T. *et al.* Distinct hypothalamic control of same- and opposite-sex mounting behaviour in mice. *Nature* **589**, 258–263 (2020).
37. Spiteri, T. *et al.* The role of the estrogen receptor α in the medial amygdala and ventromedial nucleus of the hypothalamus in social recognition, anxiety and aggression. *Behav. Brain Res.* **210**, 211–220 (2010).
38. Remedios, R. *et al.* Social behaviour shapes hypothalamic neural ensemble representations of conspecific sex. *Nature* **550**, 388–392 (2017).
39. Silva, B. A. *et al.* Independent hypothalamic circuits for social and predator fear. *Nat. Neurosci.* **16**, 1731–1733 (2013).
40. Sakurai, K. *et al.* Capturing and manipulating activated neuronal ensembles with CANE delineates a hypothalamic social-fear circuit. *Neuron* **92**, 739–753 (2016).
41. Yokosuka, M. *et al.* Female-soiled bedding induced Fos immunoreactivity in the ventral part of the preammillary nucleus (PMv) of the male mouse. *Physiol. Behav.* **68**, 257–261 (1999).
42. Wei, D., Talwar, V. & Lin, D. Neural circuits of social behaviors: innate yet flexible. *Neuron* **109** (2021).
43. Canteras, N. S., Simerly, R. B. & Swanson, L. W. Organization of projections from the medial nucleus of the amygdala: A PHAL study in the rat. *J. Comp. Neurol.* **360**, 213–245 (1995).
44. Dulac, C. & Wagner, S. Genetic Analysis of Brain Circuits Underlying Pheromone Signaling. *Annu. Rev. Genet.* **40**, 449–467 (2006).
45. Faturi, C. B. *et al.* Functional mapping of the circuits involved in the expression of contextual fear responses in socially defeated animals. *Brain Struct. Funct.* **219**, 931–946 (2014).
46. Hu, R. K. *et al.* An amygdala-to-hypothalamus circuit for social reward. *Nat. Neurosci.* 1–12 (2021).
47. Hong, W., Kim, D.-W. & Anderson, D. Antagonistic Control of Social versus Repetitive Self-Grooming Behaviors by Separable Amygdala Neuronal Subsets. *Cell* **158**, 1348–1361 (2014).
48. Dong, H.-W. & Swanson, L. W. Projections from bed nuclei of the stria terminalis, posterior division: Implications for cerebral hemisphere regulation of defensive and reproductive behaviors. *J. Comp. Neurol.* **471**, 396–433 (2004).
49. Lei, K., Cushing, B. S., Musatov, S., Ogawa, S. & Kramer, K. M. Estrogen receptor- α in the bed nucleus of the stria terminalis regulates social affiliation in male prairie voles (*Microtus ochrogaster*). *PLoS One* **5**, e8931 (2010).
50. Tsuneoka, Y. *et al.* Distinct preoptic-BST nuclei dissociate paternal and infanticidal behavior in mice. *EMBO J.* **34**, 2652–2670 (2015).
51. Von Campenhausen, H. & Mori, K. Convergence of segregated pheromonal pathways from the accessory olfactory bulb to the cortex in the mouse. *Eur. J. Neurosci.* **12**, 33–46 (2000).
52. Markham, C. M., Norvelle, A. & Huhman, K. L. Role of the bed nucleus of the stria terminalis in the acquisition and expression of conditioned defeat in Syrian hamsters. *Behav. Brain Res.* **198**, 69–73 (2009).
53. Evans, D. A., Stempel, A. V., Vale, R. & Branco, T. Cognitive control of escape behaviour. *Trends Cogn. Sci.* **23**, 334–348 (2019).
54. Schwartzbaum, J. S. & Leventhal, T. O. Neural substrates of behavioral aversion in lateral hypothalamus of rabbits. *Brain Res.* **507**, 85–91 (1990).

55. Li, Y. *et al.* Hypothalamic circuits for predation and evasion. *Neuron* **97**, 911–924 (2018).
56. Barbano, M. F. *et al.* VTA glutamatergic neurons mediate innate defensive behaviors. *Neuron* **107**, 1–15 (2020).
57. Chen, S. *et al.* A hypothalamic novelty signal modulates hippocampal memory. *Nature* **586**, 270–274 (2020).
58. Canteras, N. S. Hypothalamic survival circuits related to social and predatory defenses and their interactions with metabolic control, reproductive behaviors and memory systems. *Curr. Opin. Behav. Sci.* **24**, 7–13 (2018).
59. Hahn, J. D. & Swanson, L. W. Connections of the lateral hypothalamic area juxtadorsomedial region in the male rat. *J. Comp. Neurol.* **520**, 1831–1890 (2012).
60. Stewart, S. *et al.* Boundary coding in the rat subiculum. *Philos. Trans. R. Soc. B Biol. Sci.* **369**, 20120514 (2014).
61. O'Mara, S. The subiculum: what it does, what it might do, and what neuroanatomy has yet to tell us. *J. Anat.* **207**, 271–282 (2005).
62. O'Mara, S. Controlling hippocampal output: The central role of subiculum in hippocampal information processing. *Behav. Brain Res.* **174**, 304–312 (2006).
63. Risold, P. Y. & Swanson, L. W. Connections of the rat lateral septal complex. *Brain Res. Rev.* **24**, 115–195 (1997).
64. Tingley, D. & Buzsáki, G. Transformation of a spatial map across the hippocampal-lateral septal circuit. *Neuron* **98**, 1229–1242 (2018).
65. Canteras, N. S. & Swanson, L. W. The dorsal preammillary nucleus: An unusual component of the mammillary body. *Proc. Natl. Acad. Sci. U. S. A.* **89**, 10089–10093 (1992).
66. Canteras, N., Chiavegatto, S., Ribeiro do Valle, L. & Swanson, L. Severe reduction of rat defensive behavior to a predator by discrete hypothalamic chemical lesions. *Brain Res. Bull.* **44**, 297–305 (1997).
67. Cezario, A. F., Ribeiro-Barbosa, E. R., Baldo, M. V. C. & Canteras, N. S. Hypothalamic sites responding to predator threats - the role of the dorsal preammillary nucleus in unconditioned and conditioned antipredatory defensive behavior. *Eur. J. Neurosci.* **28**, 1003–1015 (2008).
68. Wang, W. *et al.* Coordination of escape and spatial navigation circuits orchestrates versatile flight from threats. *Neuron* **109**, 1–13 (2021).
69. Goto, M., Canteras, N. S., Burns, G. & Swanson, L. W. Projections from the subfornical region of the lateral hypothalamic area. *J. Comp. Neurol.* **493**, 412–438 (2005).
70. Motta, S. C., Carobrez, A. P. & Canteras, N. S. The periaqueductal gray and primal emotional processing critical to influence complex defensive responses, fear learning and reward seeking. *Neurosci. Biobehav. Rev.* **76**, 39–47 (2017).
71. Deng, H., Xiao, X. & Wang, Z. Periaqueductal gray neuronal activities underlie different aspects of defensive behaviors. *J. Neurosci.* **36**, 7580–7588 (2016).
72. Assareh, N., Sarrami, M., Carrive, P. & McNally, G. P. The organization of defensive behavior elicited by optogenetic excitation of rat lateral or ventrolateral periaqueductal gray. *Behav. Neurosci.* **130**, 406–414 (2016).
73. Javier Marín-Blasco, I. *et al.* The lateral periaqueductal gray and its role in controlling the opposite behavioral choices of predatory hunting and social defense. *bioRxiv* (2020).
74. Franklin, T. B. *et al.* Prefrontal cortical control of a brainstem social behavior circuit. *Nat. Neurosci.* **20**, 260–270 (2017).
75. Pacák, K. & Palkovits, M. Stressor Specificity of Central Neuroendocrine Responses: Implications for Stress-Related Disorders. *Endocr. Rev.* **22**, 502–548 (2001).
76. Reyes, T. M., Walker, J. R., DeCino, C., Hogenesch, J. B. & Sawchenko, P. E. Categorically distinct acute stressors elicit dissimilar transcriptional profiles in the paraventricular nucleus of the hypothalamus. *J. Neurosci.* **23**, 5607–16 (2003).
77. Sévoz-Couche, C. *et al.* Involvement of the dorsomedial hypothalamus and the nucleus tractus solitarius in chronic cardiovascular changes associated with anxiety in rats. *J. Physiol.* **591**, 1871–1887 (2013).
78. Brasil, T. F. S. *et al.* The dorsomedial hypothalamus is involved in the mediation of autonomic and neuroendocrine responses to restraint stress. *Front. Pharmacol.* **10**, 1547 (2019).
79. Kovács, K. J. CRH: The link between hormonal-, metabolic- and behavioral responses to stress. *J. Chem. Neuroanat.* **54**, 25–33 (2013).
80. Fokkema, D. S., Koolhaas, J. M., van der Meulen, J. & Schoemaker, R. Social stress induced pressure breathing and consequent blood pressure oscillation. *Life Sci.* **38**, 569–575, (1986).

81. Bohus, B. *et al.* Neuroendocrine states and behavioral and physiological stress responses. *Prog. Brain Res.* **72**, 57–70 (1987).
82. Xavier, C. H., Beig, M. I., Ianzer, D., Fontes, M. A. P. & Nalivaiko, E. Asymmetry in the control of cardiac performance by dorsomedial hypothalamus. *Am. J. Physiol. Integr. Comp. Physiol.* **304**, 664–674 (2013).
83. Herman, J. P. *et al.* Central mechanisms of stress integration: Hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front. Neuroendocr.* **24**, 151–180 (2003).
84. Conrad, K. L., Louderback, K. M., Gessner, C. P. & Winder, D. G. Stress-induced alterations in anxiety-like behavior and adaptations in plasticity in the bed nucleus of the stria terminalis. *Physiol. Behav.* **104**, 248–256 (2011).
85. Sullivan, G. M. *et al.* Lesions in the bed nucleus of the stria terminalis disrupt corticosterone and freezing responses elicited by a contextual but not by a specific cue-conditioned fear stimulus. *Neuroscience* **128**, 7–14 (2004).
86. Meloni, E. G., Jackson, A., Gerety, L. P., Cohen, B. M. & Carlezon, W. A. Role of the bed nucleus of the stria terminalis (BST) in the expression of conditioned fear. *Annals New York Acad. Sci.* **1071**, 538–541 (2006).
87. Kim, S. Y. *et al.* Diverging neural pathways assemble a behavioural state from separable features in anxiety. *Nature* **496**, 219–223 (2013).
88. Friard, O. & Gamba, M. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* **7**, 1325–1330 (2016).
89. Friard, O. DORIS: Detection of Objects and tracking Research Interactive Software (2019).
90. Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**, 676–682 (2012).
91. Goldowitz, D. Allen Reference Atlas. A Digital Color Brain Atlas of the C57BL/6J Male Mouse. *Genes, Brain Behav.* **9**, 128–128 (2010).
92. Franklin, K. B. & Paxinos, G. *The Mouse Brain in Stereotaxic Coordinates* (2007), third edit edn.
93. Cullinan, W. E., Herman, J. P., Battaglia, D. F., Akil, H. & Watson, S. J. Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience* **64**, 477–505 (1995).
94. Hahn, J. D. & Swanson, L. W. Connections of the juxtaventromedial region of the lateral hypothalamic area in the male rat. *Front. Syst. Neurosci.* **9**, 66 (2015).
95. Hashikawa, K., Hashikawa, Y., Lischinsky, J. & Lin, D. The neural mechanisms of sexually dimorphic aggressive behaviors. *Trends Genet.* **34**, 755–776 (2018).
96. Polston, E., Gu, G. & Simerly, R. Neurons in the principal nucleus of the bed nuclei of the stria terminalis provide a sexually dimorphic gabaergic input to the anteroventral periventricular nucleus of the hypothalamus. *Neuroscience* **123**, 793–803 (2004).
97. Tovote, P. *et al.* Midbrain circuits for defensive behaviour. *Nature* **534**, 206–212 (2016).
98. Cumming, G. *Understanding The New Statistics* (New York, 2013), 1 edn.
99. Cumming, G. Cohen's d needs to be readily interpretable: Comment on Shieh. *Behav. Res. Methods* **45**, 968–971 (2013).
100. Scott, D. W. On Optimal and Data-Based Histograms. *Biometrika* **66**, 605–610 (1979).

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Author contributions statement

S.C.M. and A.P.A. conceived the project. A.P.A. conducted the experiments and collected the data (with additional input from S.C.M.). M.V.C.B., S.C.M. and A.P.A. did the statistical analyses. S.C.M. and A.P.A. wrote the manuscript (with editorial input from M.V.C.B.). All authors reviewed and approved the final manuscript.

Additional information

Competing Interests: The authors declare no competing interests.

Figures and Tables

Figure 1. Behavioural analysis of intruder animals submitted to the resident-intruder paradigm. A) Behavioural pattern of intruder animals submitted to one exposure of the resident-intruder paradigm or three exposures, during the first and third days. B) Time spent by intruder mice on the border and centre of the resident's home cage during paradigm sessions. C) Total distance covered and distance covered in the centre and the border by the intruders during the paradigm sessions. The data is represented as a combination of the kernel density estimate and the boxplot. The kernel is based on the Gaussian function. The scale factor to use when computing the kernel bandwidth was determined by the Scott method¹⁰⁰. The actual kernel size was determined by multiplying the scale factor by the standard deviation of the data within each bin. In the end, we chose to preserve an equal width between each violin. The white circle in the centre of the boxplots represents the median, the black rectangle involves values within the first and third quartiles and whiskers go up to 1.5 times the interquartile range below or above the first and third quartiles, respectively.

Figure 2. Time plot showing the behaviour of the same intruder mouse throughout its first exposure to the resident-intruder paradigm (A) and its last exposure (B).

Figure 3. Photomicrographs of transverse Fos-stained sections at the level of the VMH (A-C), MeAp (D-F), and PMD (G-I) from control animals (A, D, G), intruder animals submitted to one exposure to resident-intruder paradigm (B, E, H) and animals submitted to three exposures (C, F, I).

Figure 4. Photomicrographs of transverse Fos-stained sections at the level of the VMH (A-B) and PMD (C-D) from animals submitted to social stress (A, C) and restraint stress (B, D).

Figure 5. Pattern of neural activation in control animals, animals submitted 1 time to the resident-intruder paradigm or submitted 3 times. A) Septum; B) Medial amygdala; C) Paraventricular nucleus; D) Sexually dimorphic circuit of the hypothalamus; E) Lateral hypothalamic area; F) Dorsomedial hypothalamic nucleus and dorsal preammillary nucleus; G) Rostral portion of the periaqueductal grey matter and H) caudal portion. The data is represented as a combination of the kernel density estimate and the boxplot. The kernel is based on the Gaussian function. The scale factor to use when computing the kernel bandwidth was determined by the Scott method¹⁰⁰. The actual kernel size was determined by multiplying the scale factor by the standard deviation of the data within each bin. In the end, we chose to preserve an equal width between each violin. The white circle in the centre of the boxplots represents the median, the black rectangle involves values within the first and third quartiles and whiskers go up to 1.5 times the interquartile range below or above the first and third quartiles, respectively.

Figure 6. Pattern of neural activation in animals submitted to social or restraint stress. A) Septum; B) Medial amygdala; C) Paraventricular nucleus; D) Sexually dimorphic circuit of the hypothalamus; E) Lateral hypothalamic area; F) Dorsomedial hypothalamic nucleus and dorsal preammillary nucleus; G) Rostral portion of the periaqueductal grey matter and H) caudal portion. The data is represented as a combination of the kernel density estimate and the boxplot. The kernel is based on the Gaussian function. The scale factor to use when computing the kernel bandwidth was determined by the Scott method¹⁰⁰. The actual kernel size was determined by multiplying the scale factor by the standard deviation of the data within each bin. In the end, we chose to preserve an equal width between each violin. The white circle in the centre of the boxplots represents the median, the black rectangle involves values within the first and third quartiles and whiskers go up to 1.5 times the interquartile range below or above the first and third quartiles, respectively.

Figures

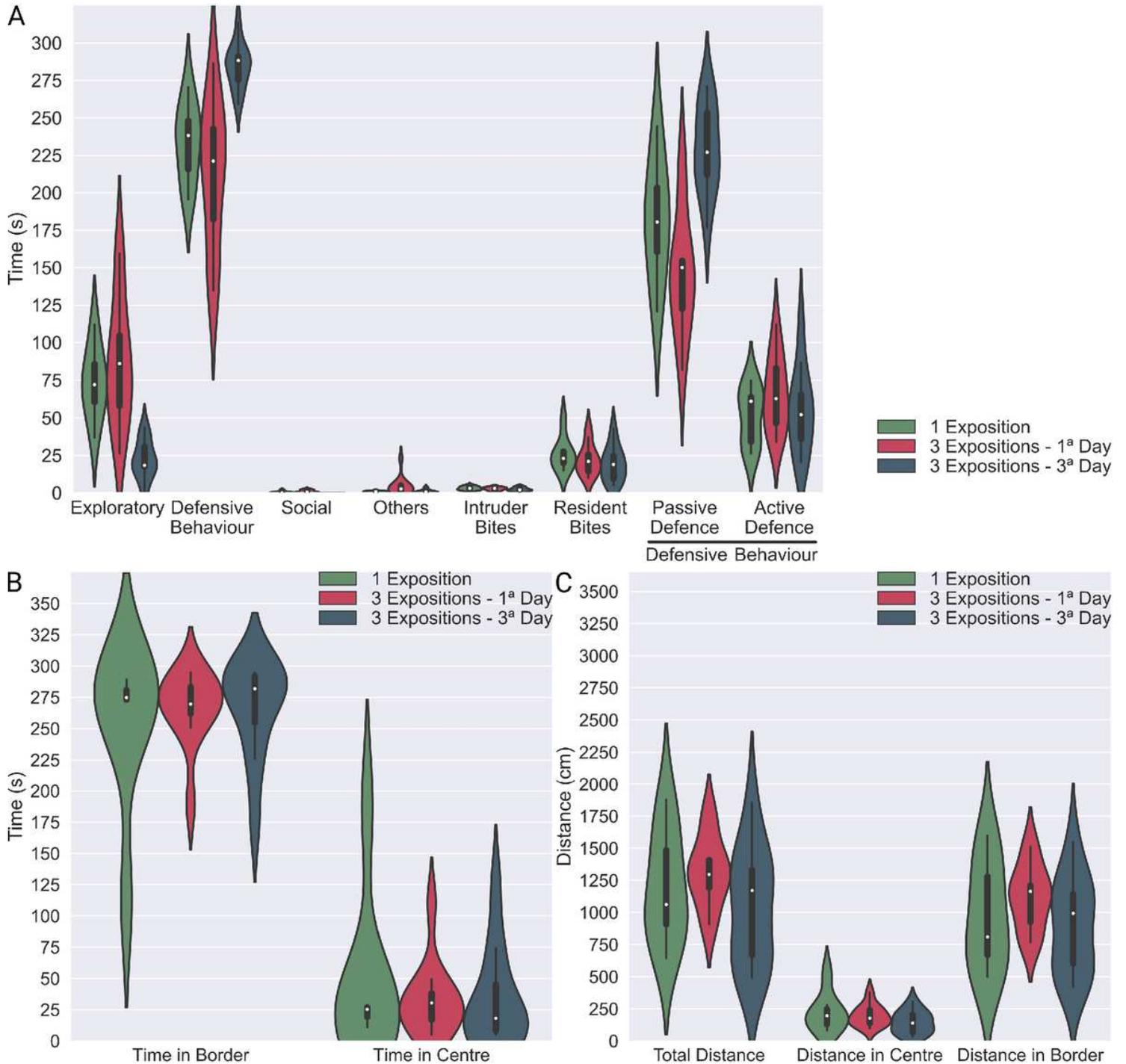


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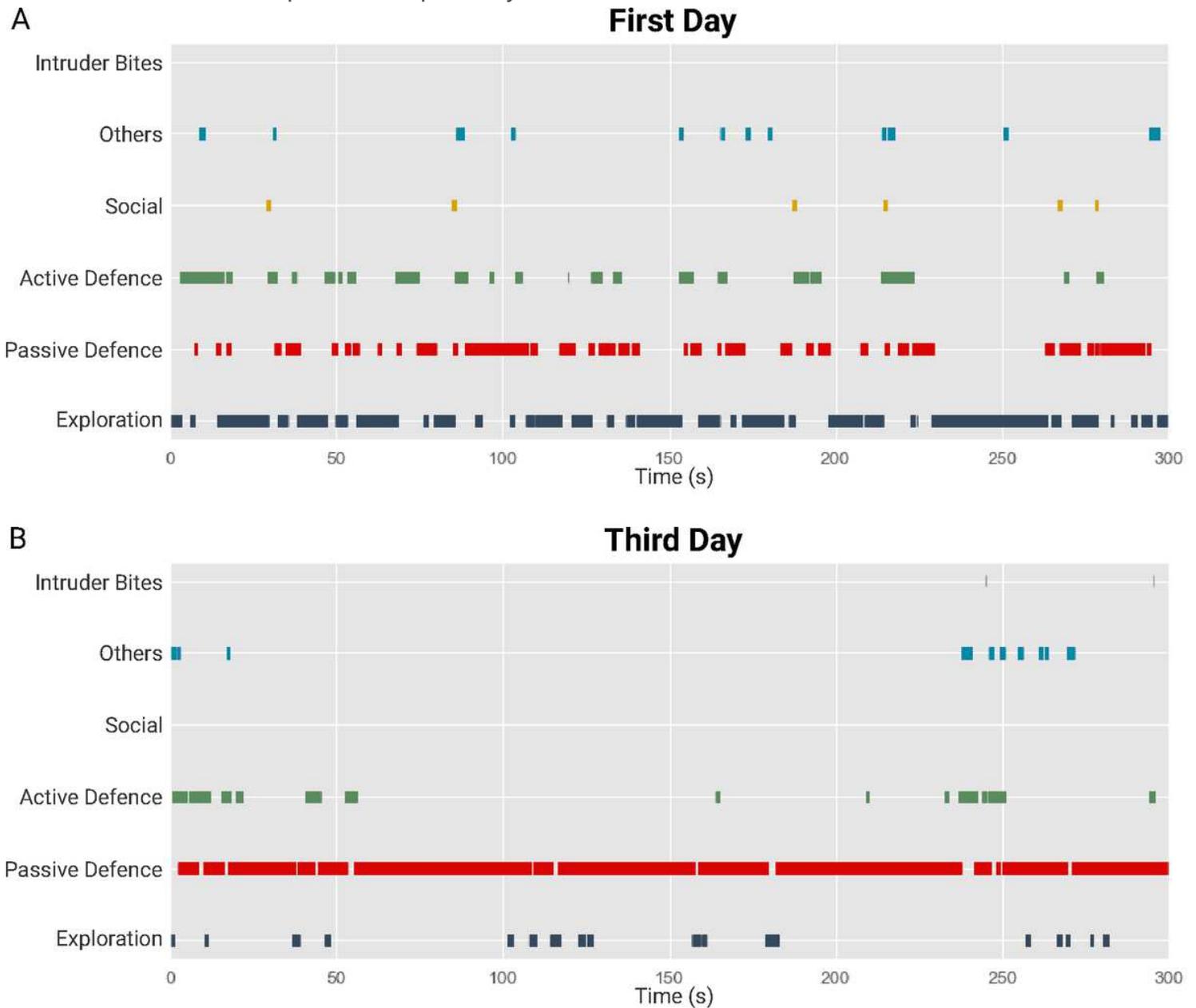


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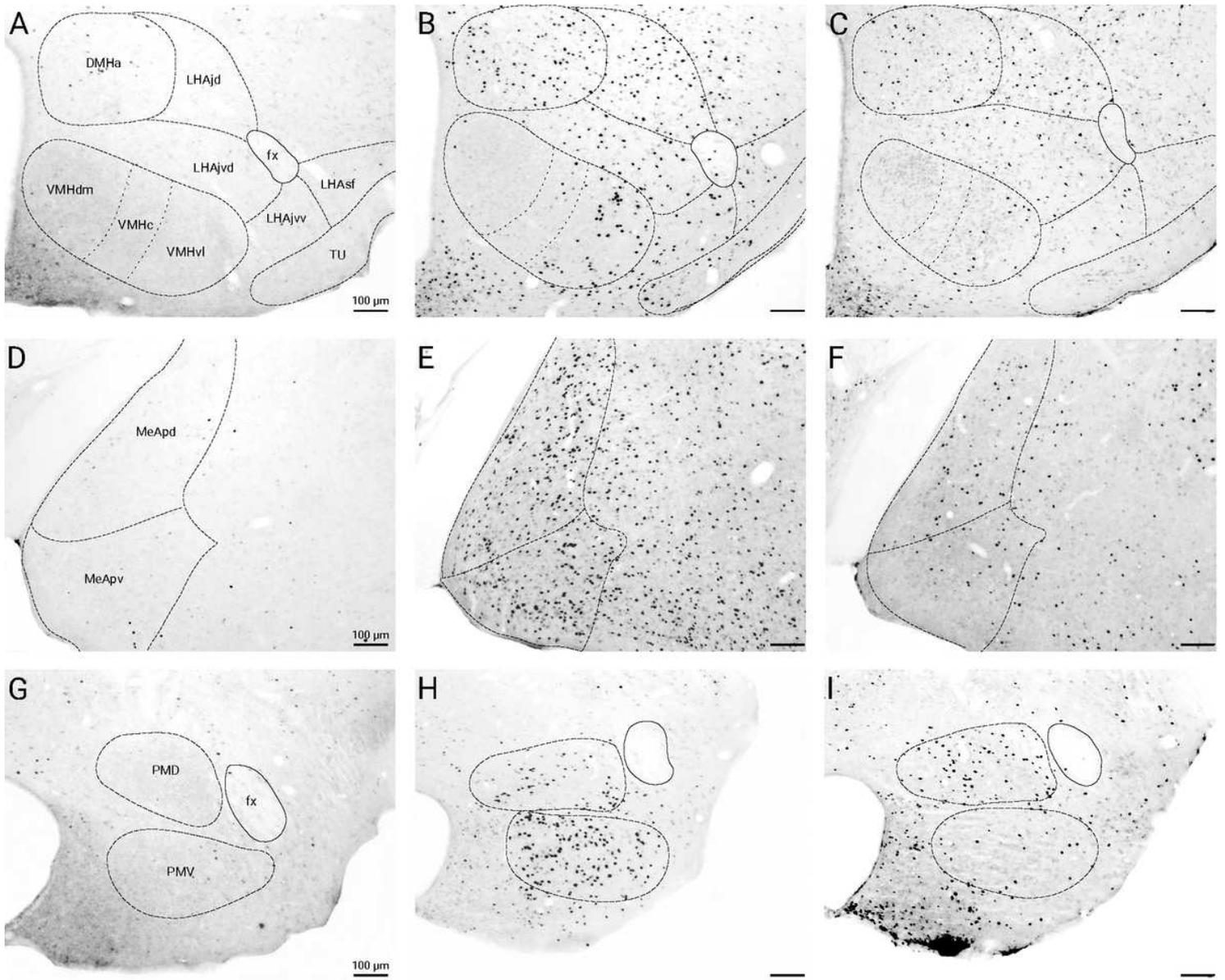


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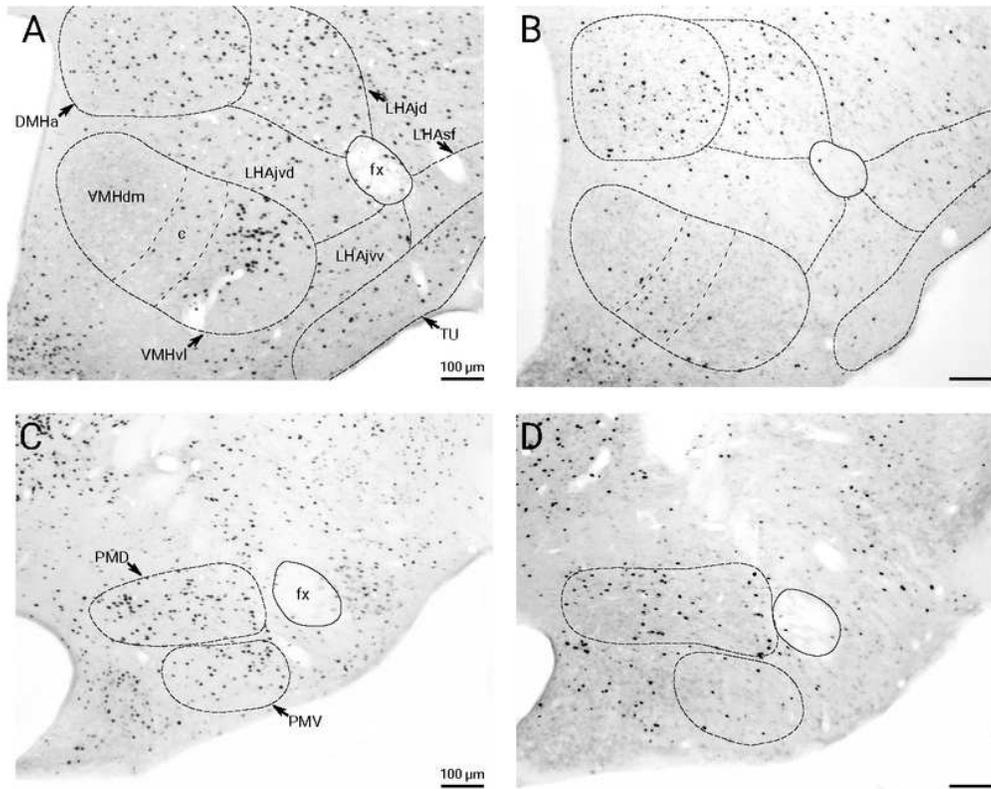


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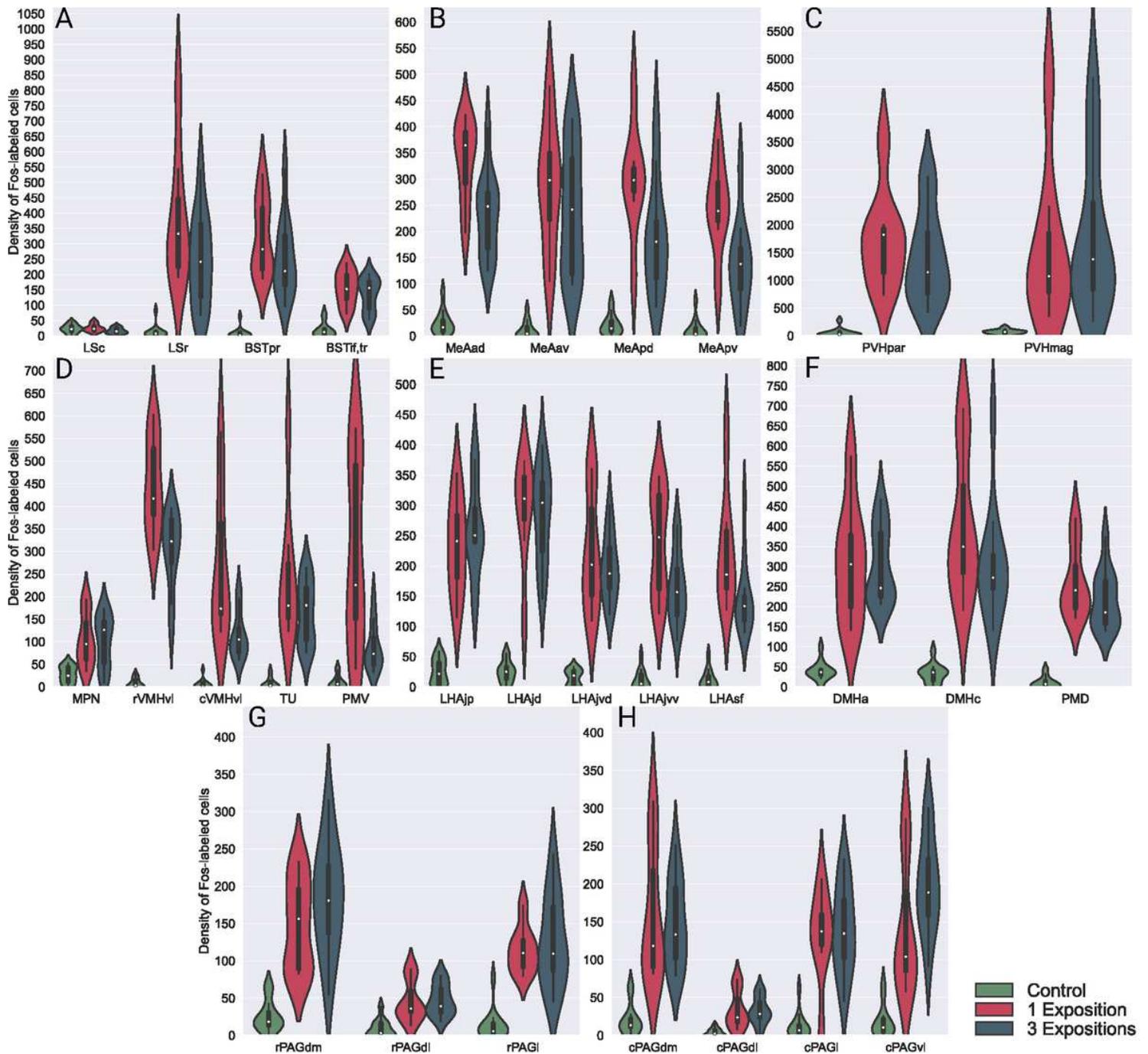


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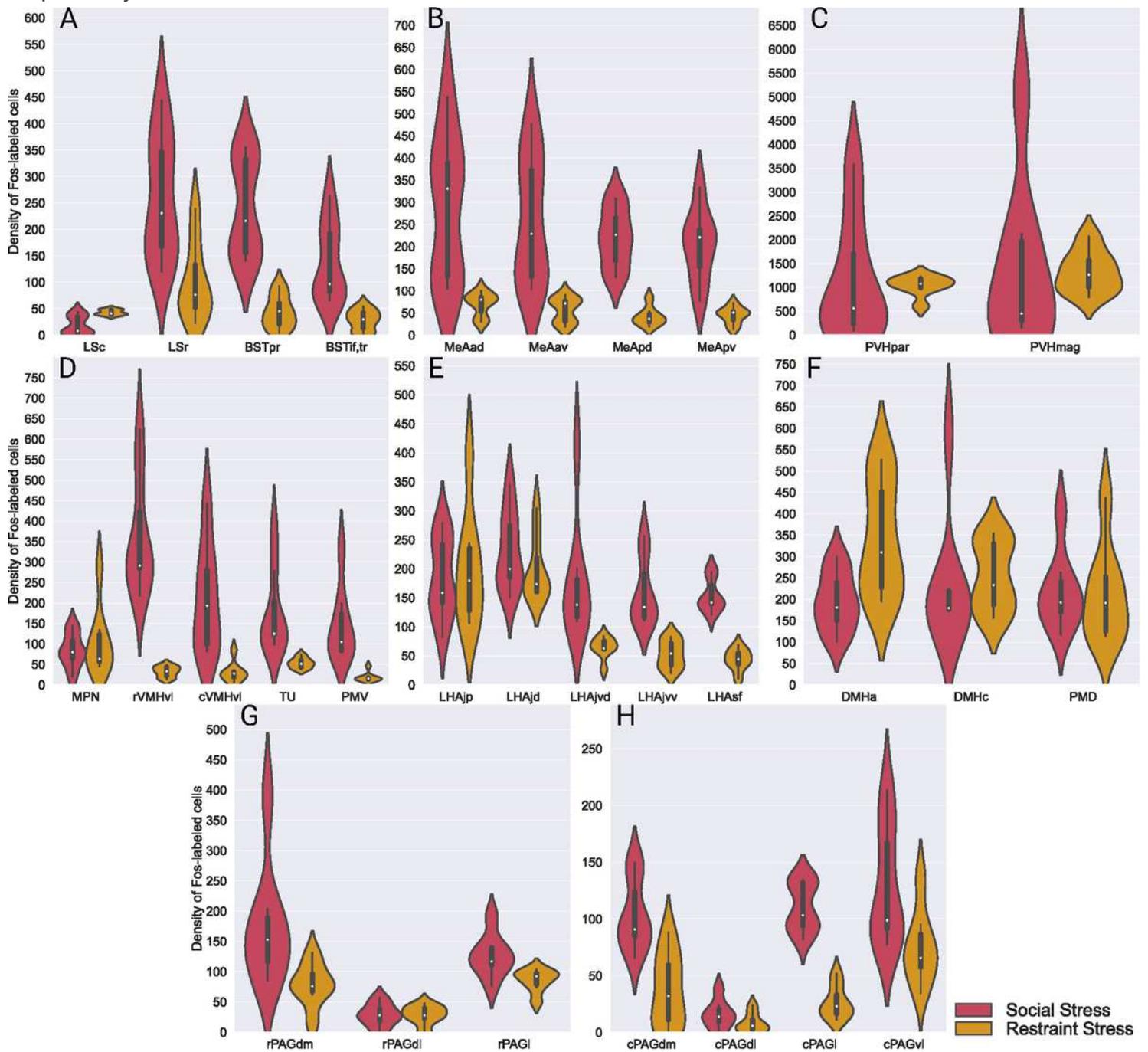


Figure 6

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