Title: BEHAVIORAL COPING STRATEGIES PREDICT TUMOR DEVELOPMENT AND BEHAVIORAL IMPAIRMENT AFTER CHRONIC SOCIAL STRESS IN MICE

Authors' information:

Olatz Goñi-Balentziaga[a] . **E-mail address:** olatz.goni@ehu.eus

Larraitz Garmendia[a] . **E-mail address:** l.garmendia@ehu.eus

Ainitze Labaka[a] . **E-mail address:** ainitze.labaca@ehu.eus

Andrea Lebeña[a] . . **E-mail address:** andrea.lebena@ehu.eus

Garikoitz Beitia[a] . **E-mail address:** garikoitz.beitia@ehu.eus

Eneritz Gómez-Lázaro[a] . **E-mail address:** eneritz.gomez@ehu.eus

*Oscar Vegas[a], **E-mail address:** o.vegas@ehu.eus. **Phone number:** +34 943 015 733. **Fax number:** +34 943 311 055. **Postal address:** *Department of Basic Psychological Processes and their Development, University of The Basque Country, 70 Tolosa Av., 20018 San Sebastian, Spain.*

[a]*Department of Basic Psychological Processes and their Development, University of The Basque Country. 70 Tolosa Av., 20018 San Sebastian, Spain.*

*Corresponding Author

Behavioral coping strategies predict tumor development and behavioral impairment after chronic social stress in mice

The aims of this study were to identify behavioral strategies to cope with social defeat, evaluate their impact on tumor development and analyze the contributions of both to changes in physiology and behavior produced by chronic defeat stress. For this purpose, OF1 mice were inoculated with B16F10 melanoma cells and subjected to 18 days of repeated defeat stress in the presence of a resident selected for consistent levels of aggression. Combined cluster and discriminant analyses of behavior that manifested during the first social interaction identified three types of behavioral profiles: active/aggressive (AA), passive/reactive (PR) and an intermediate active/non-aggressive (ANA) profile. Animals that showed a PR coping strategy developed more pulmonary metastases at the end of the social stress period than animals in other groups. The ANA but not AA group also showed higher tumor metastases than non-stressed subjects. In addition, the ANA group differed from the other groups because it displayed the highest corticosterone levels after the first interaction. Chronic stress reduced sucrose consumption, which indicates anhedonia, in all the stressed groups. However, the PR subjects exhibited a longer immobility time and swam for less time than other subjects in the forced swim test (FST), and they travelled a shorter distance in the open field test (OFT). In this test, the ANA group also travelled smaller distances than the non-stressed group, but the difference was more moderate. In contrast, tumor development but not stress increased behaviors associated with anxiety in the OFT (e.g., time in the center) in all tumor-bearing subjects. In summary, although the effects of social stress and tumor development on behavior were rather moderate, the results indicate the importance of behavioral coping strategies in modulating the effects of chronic stress on health.

Keywords: chronic social stress, coping strategies, depressive like-behavior, tumor development, repeated defeat.

1. Introduction

The physiological and behavioral responses to acute stress can be adaptive, but exposure to chronic stress, particularly chronic psychosocial stress, can have negative consequences and increase susceptibility to chronic diseases, including depression (Hollis, Isgor, & Kabbaj, 2013) and cancer (Sommershof, Scheuermann, Koerner, & Groettrup, 2017). One of the most common chronic stressors in humans and other social animals is stress emerging from social interactions (Boersma et al., 2017; Brown, 2002; Kessler, 1997). Thus, losses of social rank, status and/or control are examples of chronic stressors that are increasingly recognized as risk factors for depression (Gotlib & Hammen, 2008). Likewise, a large body of evidence shows that chronic psychosocial stress affects the development of cancer and increases the mortality rate associated with various types of cancer (Chida, Hamer, Wardle, & Steptoe, 2008; Moreno-Smith, Lutgendorf, & Sood, 2010). In addition, the high prevalence of depressive disorder among patients with cancer (Lutgendorf & Andersen, 2015; Spiegel & Giese-Davis, 2003) suggests the existence of a relationship between both pathologies (Cardoso, Graca, Klut, Trancas, & Papoila, 2016; Satin, Linden, & Phillips, 2009) that is not exclusively explained by the psychosocial stress associated with this disease (Sotelo, Musselman, & Nemeroff, 2014).

Therefore, a link between stress, depression and cancer exists, but the possible physiological mechanism is not yet known. A relatively recently proposed mechanism is the inflammatory response produced by stress-induced neuroendocrine changes and the presence of the tumor itself (Antoni & Dhabhar, 2019; Dantzer, 2017; Santos & Pyter, 2018; Soung & Kim, 2015). In relation to stress, a number of factors modulate the physical and psychological responses to chronic psychosocial stressors (Wohleb et al., 2011). Apart from factors such as the duration, severity and controllability of the stressor (Charmandari, Tsigos, & Chrousos, 2005; Segerstrom & Miller, 2004), the strategies that an individual uses to cope with stress also have significant effects on the characteristic activation profile of the hypothalamic-pituitary-adrenal

(HPA)/sympathomedullary (SAM) axis (Azpiroz, De Miguel, Fano, & Vegas, 2008; De Miguel et al., 2011) and consequently on the immune balance (Antoni & Dhabhar, 2019).

Animal models using the resident-intruder paradigm, applied in different ways, have been very valuable in studying individual differences in patterns of behavioral and physiological responses to chronic social stress (Wood & Bhatnagar, 2015). Many of the studies using these models focus on the consequences of chronic defeat stress and sort subjects into resilient and susceptible groups based on their social behavior in the social avoidance test (Golden, Covington, Berton, Russo, & Russo, 2011; Krishnan et al., 2007) or into active and passive groups according to the different behavioral profiles manifested by subjects after chronic defeat stress (Gómez-Lázaro et al., 2011; Hammels et al., 2015; Peréz-Tejada et al., 2016). These studies have provided important data about behavioral and physiological changes associated with susceptible and/or passive subjects, which could be relevant to human pathology.

However, individual differences in the repercussions of chronic social stress are potentially linked to differences in the behavioral strategies and/or personalities that characterize each individual (Berton, Hahn, & Thase, 2012; Wood & Bhatnagar, 2015). Coping styles, which describe how an individual faces stressors in their environment, may allow identification of individuals with a higher susceptibility to future psychosocial stressors. Thus, resilience/susceptibility may reflect individual differences in behavioral coping strategies (Dantzer, Cohen, Russo, & Dinan, 2018; Russo, Murrough, Han, Charney, & Nestler, 2012); some individuals will be able to cope with chronic stress, while others may experience a pathology upon chronic stress exposure. Koolhaas et al. (1999) described two coping strategies: active/proactive and passive/reactive, with high and low levels of aggressive or offensive behavior, respectively (Koolhaas et al., 1999; Koolhaas, de Boer, Coppens, & Buwalda, 2010). However, social interaction and defeat encompass a large number of behaviors, such as the approach to a threatening situation, the reaction to an aggressive encounter, escape behavior and returning to a secure environment, and immobility (De Miguel et al., 2011; Wood & Bhatnagar,

2015), whose manifestations are part of the coping style. These coping strategies have not only been shown to be relevant factors associated with the vulnerability to depression (Berton et al., 2012; Buwalda et al., 2005) but are also associated with tumor development, as suggested by previous studies by our group (Cacho, Garmendia, Vegas, & Azpíroz, 2008; Vegas, Fano, Brain, Alonso, & Azpiroz, 2006) and other authors (Armaiz-Pena, Cole, Lutgendorf, & Sood, 2013; Feller, Khammissa, Ballyram, Chandran, & Lemmer, 2019; Moreno-Smith et al., 2010).

Based on this evidence, the objective of the present study was to identify coping strategies based on the behaviors initially manifested in interactions with a resident opponent and analyze their involvement in tumor development. The study also aimed to analyze the contributions of these strategies and tumor development to changes in the physiology and behaviors of animals exposed to chronic social stress. Therefore, the animals were subjected to repeated social defeat stress using the previously reported sensory contact social stress model that maintains a situation of psychosocial stress while minimizing physical harm (Kudryavtseva et al., 1991), with some modifications (Vegas, Beitia, Sánchez-Martin, Arregi, & Azpiroz, 2004). Body weight and corticosterone level were measured throughout the stress period. Behavioral changes were evaluated using the sucrose preference test (SPT), forced swim test (FST), open field test (OFT) and a social interaction test at the end of the stress period. After the behavioral tests, the animals were sacrificed, and the spleen and lung were harvested to determine spleen weight and lung tumor metastasis. We postulated that subjects subjected to chronic social stress who adopted a passive strategy would manifest greater tumor development and anxious/depressive behaviors. This information might enable the early administration of interventions to susceptible individuals to prevent or minimize the ultimate consequences of stress.

2. Methods

2.1. Subjects and husbandry

Six-week-old OF1 outbred male mice (Charles River, Oncins, France) were individually housed in transparent plastic cages measuring 24.5 × 24.5 × 15 cm. Food and water were available ad

libitum. The holding room was maintained at a constant temperature of 20°C with a reverse 12-h light/dark cycle (white lights on from 19:00 to 07:00 h) to enable the testing of these nocturnal animals during their active phase (1 h after the beginning of the dark cycle). All experimental procedures were conducted under dim red lighting in a room adjacent to the holding facility. All procedures involving mice were performed according to the European Directive (2010/63/EU) on the protection of animals used for scientific purposes (September 22, 2010). Provincial Council of Gipuzkoa (PRO-AE-SS-062) and the Ethical Committee of the Basque Country University (CEBA) approved the procedures for Animal Welfare.

2.2. Experimental procedure

The experiment began after a 7-day adaptation period. A basal blood sample and body weight was obtained for all mice (n = 102), and after 4 days, animals were randomly allocated to two groups that were inoculated with B16F10 melanoma cells (n = 51) or not inoculated (n = 51). Each group was separated into two subgroups, resulting in four experimental groups: stressed-tumor (n = 37), stressed-non-tumor (n = 36), non-stressed-tumor (n = 14) and non-stressed-non-tumor (n = 15). The stress period lasted 18 days (see below), and non-stressed mice were housed individually during this period. On the day the stress period ended, behavioral analyses were performed on consecutive days: the social approach test on day 18, the SPT on day 19, the OFT on day 20, and the FST on day 21. Immediately after completing these tests, the animals were sacrificed, and the lung and spleen were removed. Blood samples and body weights were also obtained on days 1, 9 and 21 (Fig. 1).

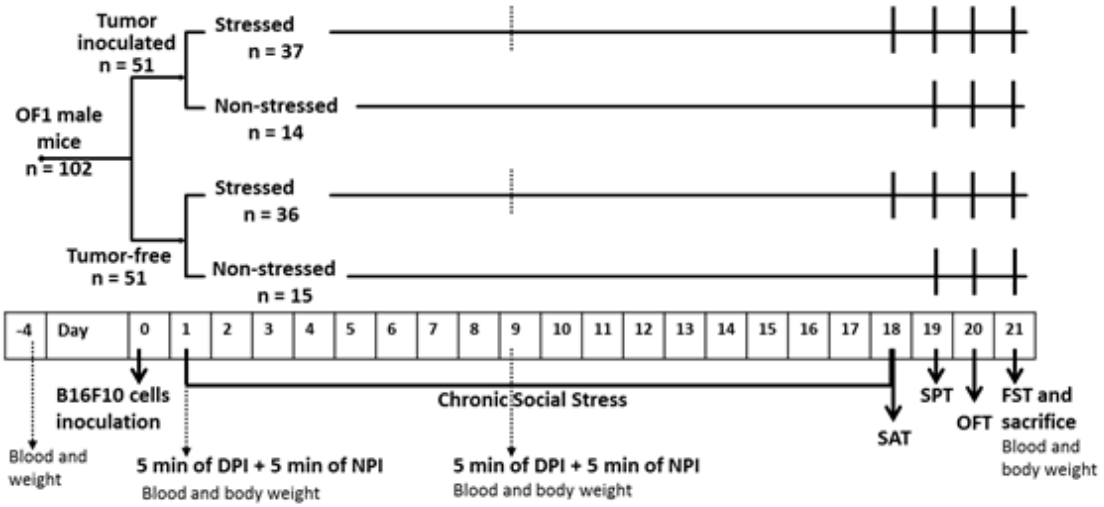

Figure 1. Experimental procedure. Notes: DPI = direct physical interaction; NPI = non-physical interaction; SAT = social approach test; SPT = sucrose preference test; OFT = open field test; FST = forced swim test.

2.3. Stress procedure

Animals in the stressed group (both inoculated and non-inoculated) were exposed to the sensory contact social stress model based on the resident-intruder paradigm (Kudryavtseva et al., 1991), with some modifications (Vegas et al., 2004). The experimental subject was allowed to interact with different, highly aggressive, resident mice that had been previously selected and trained and that socially defeated the intruder in a direct physical interaction (DPI) daily for 18 days. Additionally, on days 1 and 9, following DPI (5 min), the mice were subjected to another 5 min of non-physical interaction (NPI). In the NPI, the resident was covered with a wire mesh container, which prevented the resident from attacking the experimental subject and allowed the experimental animal to explore the environment while being protected from attacks. The direct and indirect interactions on days 1 and 9 were recorded to analyze the animals' behaviors. On subsequent days, the physical interactions were stopped after the first attack to avoid physical injuries. After the interactions, the intruders were separated from residents by perforated methacrylate barriers, which bisected the cage and allowed sensory (non-physical) contact outside the direct confrontation periods.

2.4. Experimental tumor induction

Tumors were induced by inoculating mice with B16F10 murine melanoma cells. These cells arrest in lung following intravenous injection, which makes them an ideal choice for studying lung-specific metastasis in mice (Brown, Welch, & Rannels, 2002). The B16F10 cells were maintained in vitro by subculturing the tumor cells at 37°C in a humidified atmosphere of 5% CO_2 in 75-cm^2 cell culture flasks (Corning Inc., Corning, NY, USA) with RPMI-1640 culture medium supplemented with HEPES and L-glutamine (Lonza, Basel, Switzerland) at a density of 10^5 cells/ml. Adherent B16F10 cells were detached by incubation with 0.02% EDTA for 5 min and subsequently washed in RPMI-1640 medium. Mice that had been preanesthetized via intraperitoneal injection of Nembutal (sodium pentobarbital; 60 mg/kg) were inoculated with 5×10^4 viable B16F10 cells in 0.1 ml of medium via the lateral tail vein using a 30.5-gauge needle, after the tail had been previously heated with a thermal pillow. All subjects received the complete 0.1-ml dose in one injection. To minimize the welfare impact on animals, a tumor cell line with slight in vitro development was selected for use in this experiment.

2.5. Behavioral assessment

2.5.1. *Analysis of the behavioral profile during social interactions*

The interactions conducted on days 1 and 9 were filmed with a video camera (Panasonic RX66, Osaka, Japan). Behavioral evaluations were performed using Observer XT 14 software (Noldus, ITC, Wageningen, The Netherlands), with a specific configuration based on the ethogram for the mouse developed by Brain, McAllister and Walmsley (1989) and modified by Vegas et al., 2006. This ethogram covers 51 behavioral elements grouped into 12 broad categories: *attack* (chasing, rushing towards or biting the opponent), *threat* (aggressive cleaning, vertical or lateral offense or hitting with tail), *non-social exploration* (exploration of the physical environment), *social investigation* (social exploration of the opponent by following or establishing physical contact, sniffing or cleaning), *exploration from a distance* (paying attention to the opponent from a distance), *digging* (moving the sawdust with front or back

187 legs), *body care* (self-cleaning), *avoidance* (remaining at a prudential distance from the
188 opponent), *flee* (running away when the opponent approaches), *defense/submission* (passive
189 avoidance of an attack by making signs of submission), *sexual behavior* and *immobility*
190 (remaining frozen). Furthermore, immobility and explorations during the NPI were also
191 included in the behavioral assessment.

192 *2.5.2. Social approach test (SAT)*

193 At the end of the stress period, the resident mouse was covered with a wire mesh container
194 to observe how the stressed animal behaved towards the aggressive mouse. Interactions were
195 recorded for 5 min, and the time the stressed animal spent in the area of the aggressive mouse
196 was evaluated using Observer XT 14.

197 *2.5.3. SPT*

198 All mice were offered a free choice between two bottles for 24 h; one bottle contained a
199 0.8% sucrose solution and the other bottle contained water. The position of the bottles was
200 counterbalanced to avoid possible effects of a side preference when drinking. The animals were
201 not deprived of food or water before the test. The consumption of the sucrose solution and water
202 was measured by weighing the bottles at the beginning and end of the test. The consumption of
203 sucrose was reported in relation to body weight. The sucrose preference was calculated as
204 percentage of sucrose consumption vs. sucrose plus water consumption.

205 *2.5.4. OFT*

206 Mice were placed in a black Plexiglas box (40 × 40 × 30 cm) and allowed to explore for 5
207 min. The test was performed 1 h after the SPT and was recorded for subsequent assessment. The
208 time spent in the center of the box, the average distance from the center, the distance covered
209 and the time spent immobile were analyzed using ANY-maze© 4.96 software (Stoelting
210 Europe, Dublin, Ireland). The apparatus was cleaned with a solution of 0.5% acetic acid
211 between tests in order to hide animal clues.

212 *2.5.5. FST*

213 Individual mice were placed in glass cylinders (height 18.5 cm and diameter 12.5 cm)
214 containing 13.5 cm of water at 25 ± 1°C for 5 min. The following behaviors were assessed:
215 immobility, swimming and climbing. The time spent engaged in each behavior was recorded
216 manually using Observer XT 14 by an experimenter blinded to the stress condition.

217 *2.6. Physiological assessments.*

218 *2.6.1. Determination of pulmonary metastatic foci*

219 After several days of incubation in Bouin's solution, the upper lobe of the left lung was
220 separated, and the number of metastatic foci was determined using an Olympus SZ30 Zoom
221 Stereo Microscope (Olympus, Tokyo, Japan).

222 *2.6.2. Blood collection and plasma isolation*

223 Blood was collected from the submandibular vein between 8:45 and 9:45 a.m. to measure
224 corticosterone levels. Blood samples were collected 4 days before the inoculation, 40–45 min
225 after the direct interactions (days 1 and 9) and 5–10 min after the FST (immediately before
226 sacrifice). The blood was stored in a heparinized container and then centrifuged at 1800 × g for
227 15 min at 4°C. The resulting plasma was collected and stored at −70°C until further analysis.

228 *2.6.3. Determination of plasma corticosterone concentrations*

229 The plasma corticosterone concentrations (ng/ml) were determined using a commercially
230 available enzyme immunoassay kit (Assay Designs, Ann Arbor, MI, USA) and a Synergy HT
231 microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). The sensitivity of the assay
232 was 5pg/ml, and the intra-assay and inter-assay coefficients of variation were 7% and 8%,
233 respectively.

234 *2.6.4. Spleen and body weight*

235 Animals were weighed 4 days before the inoculation, 1 and 9 days after inoculation, and
236 before sacrifice. After sacrifice, the spleen was harvested and weighed.

2.7. Statistical analysis

All statistical analyses were performed with the SPSS 24.0 for Windows software package (SPSS Inc., Chicago, IL, USA), and the level of significance was set to $p < 0.05$. The behavioral and physiological variables were analyzed using 1-way or 2-way ANOVA. The corticosterone levels and the time-dependent behavioral changes were analyzed using 1-way or 2-way ANOVA for repeated measures. When appropriate, specific comparisons were analyzed with a post hoc Tukey test. Cohen's *d* test for the effect size was performed to estimate the strength of the effects between two groups ("*d*" values > 0.8 are considered large effects, values between 0.5 and 0.8 are considered moderate effects, values < 0.5 are considered small effects). A partial eta-square (η^2) test for the effect size was used for analyses with more than two groups and interactions (η^2 = 0.01: small; η^2 = 0.09: moderate; η^2 = 0.25: large). A Chi-square test was performed to test for a difference in the distribution of coping strategies between tumor and tumor-free groups. The relation between corticosterone level and tumor development was analyzed using bivariate Spearman correlation.

3. Results

3.1. Strategies for coping with social stress

A cluster analysis using the mean percentage of time allocated to each assessed behavioral element during the first interaction was performed on all stressed subjects in terms of the behavioral characteristics they showed in the social stress situation, resulting in three clusters: active/aggressive (AA, n = 32), active/non-aggressive (ANA, n = 25) and passive/reactive (PR, n = 14) (Fig. 2). A multivariate discriminant analysis was performed to investigate the integrity of the groups derived from the cluster analysis and to determine which behavioral variables most efficiently discriminated the clusters. The discriminant model applied here accounted for 94.4% of groups obtained from the cluster analysis, thus confirming the statistical validity of these groups and their behavioral descriptions. Immobility in the DPI was the variable that best discriminated the three clusters, followed by flee, non-social exploration, defense/submission

and threat in the DPI and immobility in the NPI. No differences were observed in the distribution of coping styles as a function of tumor presence ($X^2(2) = 5.513$, $p = 0.064$).

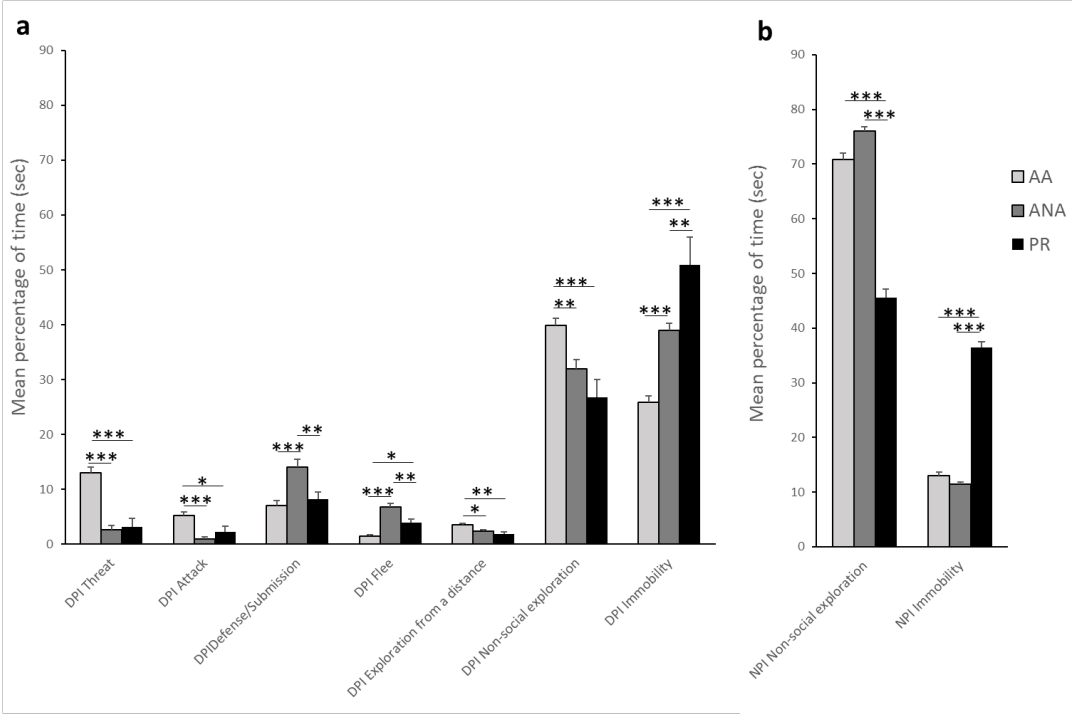

Figure 2. The mean percentage of time (mean ± standard error of the mean (SEM)) dedicated to each of the behaviors evaluated during (a) the DPI and (b) the NPI on day 1 analyzed in terms of group membership: AA (n = 32), ANA (n = 25) and PR (n = 14). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

3.2. Time-dependent behavioral changes

One-way ANOVA with repeated measures for each strategy showed significant time-dependent differences in behavior (Table 1). Animals employing all coping strategies modified their behaviors during interactions on day 9, and a reduction in non-social exploration (AA: $F(1,30) = 36.519$, $p < 0.001$, $\eta^2 = 0.549$; ANA: $F(1,24) = 13.285$, $p = 0.001$, $\eta^2 = 0.356$; y PR: $F(1,13) = 6.704$; $p = 0.022$, $\eta^2 = 0.340$) and an increase in immobility (AA: $F(1,30) = 163.141$, $p < 0.001$, $\eta^2 = 0.845$; ANA: $F(1,24) = 118.256$, $p < 0.001$, $\eta^2 = 0.831$; y PR: $F(1,13) = 17.801$, $p = 0.001$, $\eta^2 = 0.578$) were observed for all groups in the DPI. Furthermore, in the DPI, AA mice showed a reduction in their characteristic behaviors of threat ($F(1,30) = 133.210$, $p < 0.001$, $\eta^2 = 0.816$) and attack ($F(1,30) = 57.514$, $p < 0.001$, $\eta^2 = 0.657$), increasing the active behaviors of flee ($F(1,30) = 39.891$, $p < 0.001$, $\eta^2 = 0.571$); meanwhile, the ANA group exhibited reduced

threat ($F(1,24) = 11.139$, $p = 0.003$, $\eta^2 = 0.317$), attack ($F(1,24) = 8.046$, $p = 0.009$, $\eta^2 = 0.251$) and defense/submission behaviors ($F(1,24) = 6.066$, $p = 0.021$, $\eta^2 = 0.202$). In the NPI, the ANA mice but not AA or PR group mice showed a remarkable increase in immobility ($F(1,24) = 16.784$, $p < 0.001$, $\eta^2 = 0.412$) and a reduction in non-social exploration ($F(1,24) = 27.894$, $p < 0.001$; $\eta^2 = 0.538$) (Table 1).

Analysis of differences in behaviors between groups employing different strategies on day 9 only revealed differences in the behaviors of non-social exploration in the DPI ($F(1,67) = 3.291$, $p = 0.043$) and NPI ($F(1,67) = 5.745$, $p = 0.005$), and immobility in the DPI ($F(1,67) = 4.962$, $p = 0.010$) and NPI ($F(1,67) = 6.671$, $p = 0.002$) between the AA and PR groups (DPI non-social exploration: $p = 0.039$, Cohen's $d = 0.89$; NPI non-social exploration: $p = 0.007$, Cohen's $d = 0.97$; DPI immobility: $p = 0.008$; Cohen's $d = 0.93$; NPI immobility: $p = 0.003$; Cohen's $d = 1.04$). Additionally, the ANA group was not different from the PR group (Fig. 3).

291 **Table 1.** The mean percentage of time dedicated to each of the behaviors on day 1 and day 9 for each cluster.

	Coping strategy	Day 1 interaction *M*	*SD*	Day 9 interaction *M*	*SD*	*F*	η^2
DPI Threat	*AA*	12.97	±1.1	0.05	±0.0	133.210***	0.816
	ANA	2.66	±0.8	0.00	±0.0	11.139**	0.317
	PR	3.21	±1.5	0.24	±0.2	3.915	
DPI Attack	*AA*	5.19	±0.7	0.02	±0.0	57.514***	0.657
	ANA	1.01	±0.4	0.00	±0.0	8.046**	0.251
	PR	2.2	±1.1	0.01	±0.0	4.325	
DPI Defense / Submission	*AA*	7.11	±0.8	9.20	±0.9	2.361	
	ANA	14.07	±1.4	9.63	±1.0	6.066*	0.202
	PR	8.2	±1.4	10.37	±2.0	0.901	
DPI Flee	*AA*	1.48	±0.3	6.89	±0.8	39.891***	0.571
	ANA	6.75	±0.7	5.41	±0.5	2.634	
	PR	3.98	±0.7	4.75	±0.8	0.853	
DPI Exploration from a distance	*AA*	3.55	±0.3	1.49	±0.2	25.734***	0.462
	ANA	2.37	±0.3	1.43	±0.2	7.651	
	PR	1.88	±0.4	0.73	±0.2	12.129**	0.483
DPI Non-social exploration	*AA*	39.94	±1.2	26.60	±1.9	36.519***	0.549
	ANA	31.96	±1.7	22.35	±2.4	13.285**	0.356
	PR	26.82	±3.3	17.86	±2.4	6.704*	0.340
DPI Immobility	*AA*	25.93	±1.1	54.62	±2.1	163.141***	0.845
	ANA	38.92	±1.4	59.77	±2.0	118.256***	0.831
	PR	50.91	±5.0	65.77	±3.2	17.801**	0.578
NPI Non-social exploration	*AA*	70.9	±1.8	67.14	±2.9	1.205	
	ANA	75.99	±1.5	56.63	±3.3	27.894***	0.538
	PR	45.64	±2.7	50.08	±5.0	0.999	
NPI Immobility	*AA*	13.05	±1.3	15.71	±2.2	1.906	
	ANA	11.49	±1.0	25.53	±3.3	16.784***	0.412
	PR	36.49	±2.7	32.68	±5.2	0.503	

The data are presented as the means ± SEM. *$*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.*

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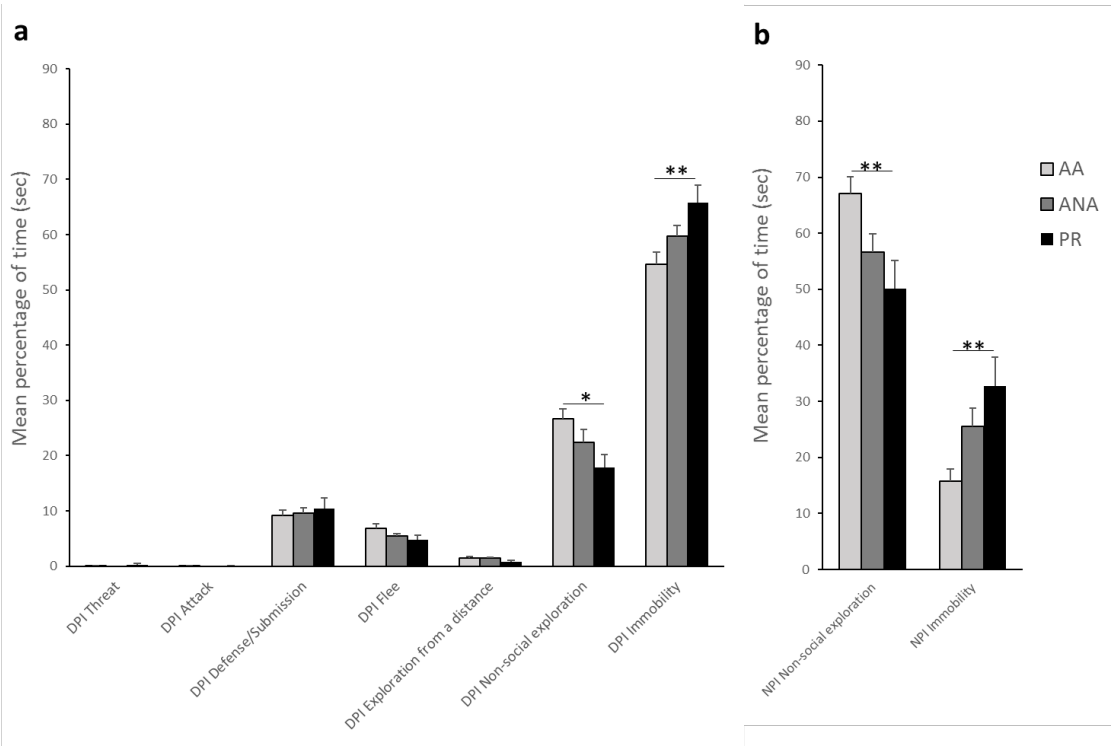

Figure 3. The mean percentage of time (mean ± SEM) dedicated to each of the behaviors evaluated during (a) the DPI and (b) the NPI on day 9 analyzed in terms of group membership. AA (n = 32), ANA (n = 25) and PR (n = 14). *$p < 0.05$ and **$p < 0.01$.

3.3. Effect of tumor development on time-dependent changes in behavior

Two-way ANOVA (tumor and time) with repeated measures showed significant differences for the time × tumor interaction in three behaviors: *attack* ($F(1,71) = 11.666$, $p = 0.001$, $\eta^2 = 0.143$) and *flee* ($F(1,71) = 4.376$, $p = 0.040$, $\eta^2 = 0.058$) in the DPI and *non-social exploration* ($F(1,68) = 6.500$, $p = 0.013$, $\eta^2 = 0.087$) in the NPI (Table 2).

Table 2. The mean percentage of time dedicated to DI attack, DPI flee and NPI non-social exploration behaviors during day 1 and day 9 interactions for tumor and tumor-free groups.

	Tumor / Tumor-free	Day 1 interaction		Day 9 interaction	
		M	***SD***	***M***	***SD***
DPI Attack	*Tumor*	1.76	±0,4	0.00	±0.0
	Tumor-free	4.43	±0,7	0.02	±0.0
DPI Flee	*Tumor*	4.67	±0,6	5.39	±0.6
	Tumor-free	3.1	±0,6	6.49	±0.7
NPI Non-social exploration	*Tumor*	69.48	±2.4	56.14	±2.8
	Tumor-free	65.65	±2.5	63.88	±3.2

The data are presented as the means ± SEM.

3.4. Effects of the chronic social defeat (CSD) and stress coping strategies (SCS) on pulmonary metastasis of B16F10 melanoma cells

Analysis of variance showed that chronically defeated mice had more tumor foci than non-stressed mice ($F(1,49) = 4.178$, $p = 0.046$, Cohen's $d = 0.71$). Furthermore, differences were observed between groups stratified according to stress coping strategies ($F(3,45) = 7.021$, $p = 0.001$). Post hoc analysis revealed that PR mice had more tumor foci than non-stressed ($p < 0.001$, Cohen's $d = 2.54$), AA ($p = 0.008$, Cohen's $d = 1.46$) and ANA ($p = 0.035$, Cohen's $d = 1.64$) subjects. In addition, ANA mice presented more tumor foci than non-stressed animals ($p = 0.010$, Cohen's $d = 1.51$); meanwhile, AA mice did not show differences compared with non-stressed group mice ($p = 0.100$) (Fig. 4).

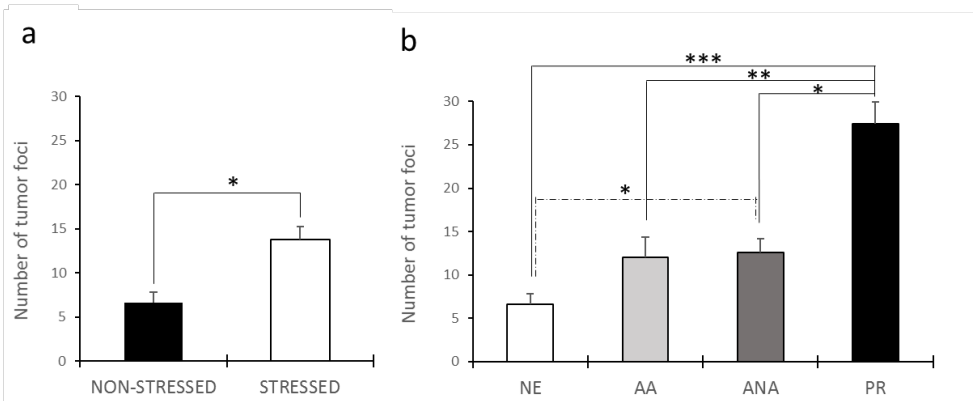

Figure 4. Number of tumor foci observed in (a) stressed (n = 37) and non-stressed (n = 14) animals and in (b) the AA (n = 12), ANA (n = 17), PR (n = 6) and non-stressed (NE) (n = 14) groups. The data are presented as the means ± SEM. **p < 0.05, **p < 0.01, and ***p < 0.001.*

3.5. Effects of tumors, CSD, and SCS on the SPT

According to the ANOVA results, chronically stressed mice consumed less of the sucrose solution in relation to body weight than non-stressed mice ($F(1,97) = 13.723$, $p < 0.001$, Cohen's $d = 0.80$); meanwhile, no differences in water consumption were observed ($F(1,97) = 0.213$, $p = 0.646$) (Fig. 5). When sucrose preference was analyzed, non-stressed mice showed a preference of 67.32% compared with 48.52% for the stressed mice, but this difference did not reach the level of significance ($F(1,97) = 3.046$, $p = 0.084$). No differences were observed in sucrose consumption between groups stratified according to the presence of tumors ($F(1,97) =$

1.427, $p = 0.235$), tumor × stress interaction ($F(1,97) = 0.004$, $p = 0.952$) and SCS ($F(2,68) = 1.151$, $p = 0.323$).

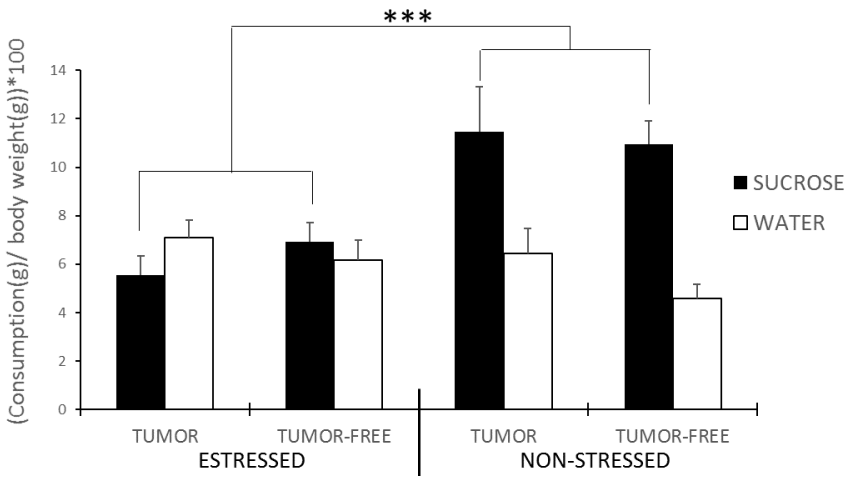

Figure 5. Sucrose and water consumption relative to body weight are presented for the stressed-tumor (n = 37), stressed-non-tumor (n = 36), non-stressed-tumor (n = 14) and non-stressed-non-tumor (n =15) groups. Numbers above the bars indicate the corresponding percent preference for the sucrose solution: $\left[\frac{\text{g of sucrose}}{\text{g of sucrose + g of water}} \text{x } 100\right]$. The data are presented as the means (±SEM). ***$p < 0.001$.

3.6. Effects of tumors, CSD and SCS on the FST

ANOVA showed no differences between the effects of stress and the tumor on the time spent immobile (*stress*: $F(1,98) = 0.236$, $p = 0.628$; *tumor*: $F(1,98) = 1.326$, $p = 0.252$; *tumor × stress interaction:* $F(1,98) = 1.576$, $p = 0.212$), swimming behavior (*stress*: $F(1,98) = 0.000$, $p = 0.989$; *tumor:* $F(1,98) = 0.413$, $p = 0.522$; *tumor × stress interaction:* $F(1,98) = 1.962$, $p = 0.164$), or climbing behavior (*stress*: $F(1,98) = 0.093$, $p = 0.762$; *tumor*: $F(1,98) = 3.602$, $p = 0.061$; *tumor × stress interaction*: $F(1,98) = 0.569$, $p = 0.453$). However, the ANOVA revealed differences in the time spent immobile in the FST in groups stratified according to SCS ($F(3,96) = 3.181$, $p = 0.027$). According to the post hoc analysis, PR mice spent more time immobile than AA ($p = 0.023$, Cohen's $d = 1.18$) and ANA mice ($p = 0.040$, Cohen's $d = 0.89$). On the other hand, the opposite results were obtained for swimming behavior ($F(3,96) = 5.536$, $p = 0.002$). In this case, PR mice spent less time swimming than AA ($p = 0.002$, Cohen's $d =$

1.41), ANA (p = 0.002, Cohen's d = 1.37) and non-stressed (p = 0.008, Cohen's d = 1.07) animals. No differences in climbing behavior were observed between SCS groups (F(3,96) = 0.676, p = 0.569) (Fig. 6).

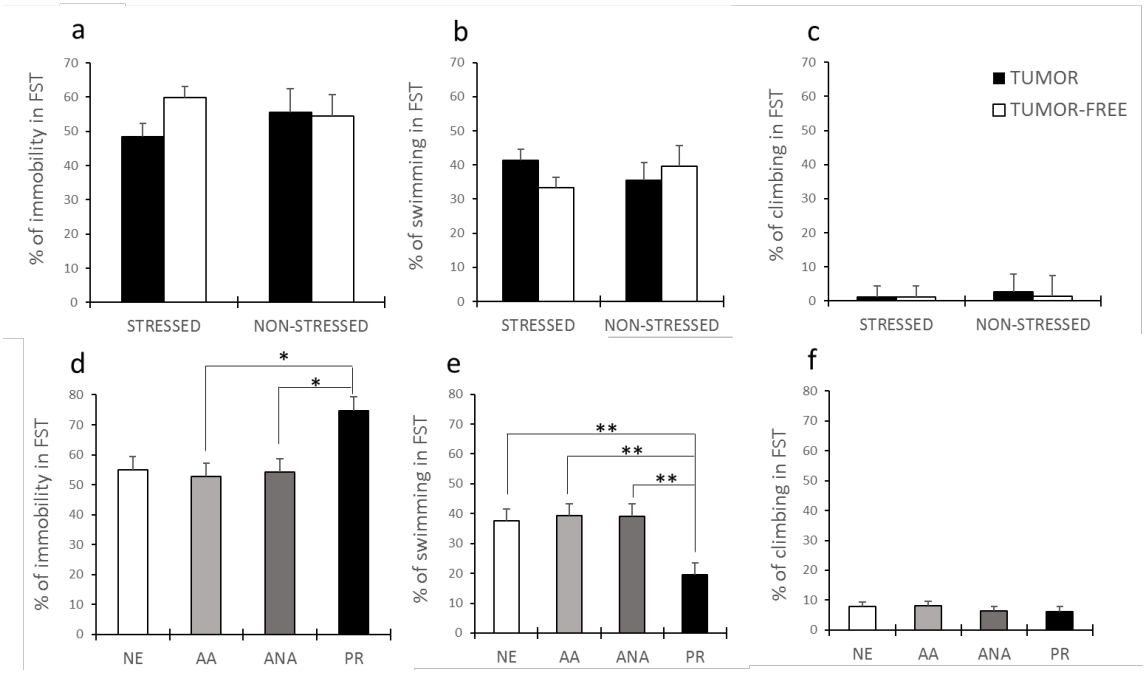

Figure 6. The percentage of (a) immobility, (b) swimming, and (c) climbing behaviors in the FST are presented for the stressed-tumor (n = 37), stressed-non-tumor (n = 36), non-stressed-tumor (n = 14) and non-stressed-non-tumor (n = 15) groups. The percentage of (d) immobility, (e) swimming, and (f) climbing behaviors in the FST are presented for the AA (n = 32), ANA (n = 25), PR (n = 14) and NE (n = 29) groups. The data are reported as the means ± SEM. *$p < 0.05$ and **$p < 0.01$.

3.7. Effects of tumors, CSD and SCS on the OFT

ANOVA showed no differences in immobility or distance travelled in groups stratified according to stress exposure (immobility: F(1,98) = 2.248, p = 0.137; distance travelled: F(1,98) = 3.385, p = 0.069), tumor presence (immobility: F(1,98) = 0.720, p = 0.398; distance travelled: F(1,98) = 0.064, p = 0.800) or stress × tumor interaction (immobility: F(1,98) = 0.098, p = 0.755; covered distance: F(1,98) = 0.032, p = 0.859). However, the tumor group spent less time in the center of the cage than the tumor-free mice (F(1,98) = 6.943, p = 0.010, Cohen's d = 0.46) and travelled a farther distance from the center (F(1,98) = 14.006, $p < 0.001$, Cohen's d = 0.96). No differences were observed in the time spent in the center and in the average distance from the center between the stressed and non-stressed groups (time in the center: F(1,98) =

0.356, $p = 0.552$; distance from the center: $F(1,98) = 2.505$, $p = 0.117$), in the stress × tumor interaction (time in the center: $F(1,98) = 1.589$, $p = 0.211$; distance from the center: $F(1,98) = 2.177$, $p = 0.143$) or in the groups stratified according to SCS (time in the center: $F(2,68) = 0.403$, $p = 0.670$; distance from the center: $F(2,68) = 2.930$, $p = 0.060$). The ANOVA revealed differences in the time spent immobile ($F(3,96) = 4.260$, $p = 0.007$) and the distance travelled ($F(3,96) = 3.527$, $p = 0.018$) between groups stratified according to SCS. According to the post hoc analysis, PR mice spent more time immobile than the non-stressed ($p = 0.018$, Cohen's $d = 0.94$) and AA group ($p = 0.015$, Cohen's $d = 1.02$) mice, and they travelled less distance than the non-stressed ($p = 0.015$, Cohen's $d = 1.00$) and AA group ($p = 0.046$, Cohen's $d = 0.89$) mice (Fig. 7).

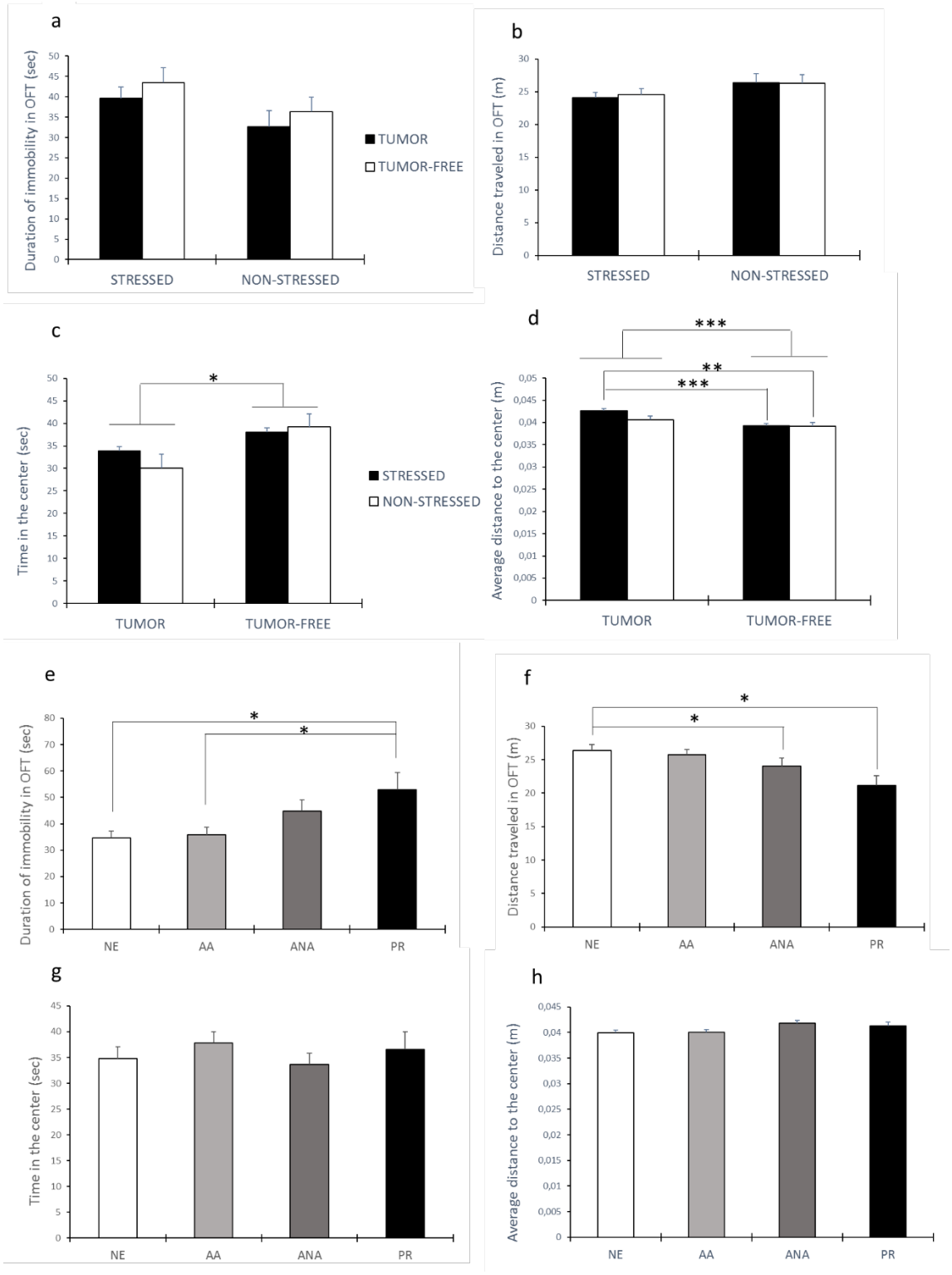

Figure 7. (a) The duration of immobility, (b) distance travelled, (c) time spent in the center and (d) average distance to the center in the OFT are presented for the stressed-tumor (n = 37), stressed-non-tumor (n = 36), non-stressed-tumor (n = 14) and non-stressed-non-tumor (n = 15) groups. (e) The duration of immobility, (f) distance travelled, (g) time spent in the center and (h) average distance to the center in the OFT are presented for the AA (n = 32), ANA (n = 25), PR (n = 14) and NE (n = 29) groups. The data are presented as the means ± SEM. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

3.8. Effects of tumors, CSD and SCS on animals' body weights

Two-way ANOVA (stress and time) with repeated measures showed significant differences in the weight gained during the experiment for the time factor ($F(1,85) = 420.702$, $p < 0.001$, $\eta^2 = 0.832$) and time × stress interaction ($F(1,85) = 22.231$, $p < 0.001$, $\eta^2 = 0.207$); stressed mice exhibited a lower body weight at the end of the experiment ($F(1,85) = 7.035$, $p = 0.010$, Cohen's $d = 0.55$). The groups stratified according to tumor presence did not show differences in body weight gained ($F(1,85) = 3.700$, $p = 0.058$) or in the final body weight ($F(1,85) = 0.402$, $p = 0.528$) (Fig. 8). The interaction between stress and tumor was not significant for the weight gained ($F(1,83) = 1.088$, $p = 0.300$) and the final body weight ($F(1,83) = 2.326$, $p = 0.131$). Furthermore, differences in the weight gained ($F(2,56) = 1.424$; $p = 0.249$) and final body weight ($F(1,56) = 0.608$, $p = 0.548$) were not observed in mice stratified according to coping strategies.

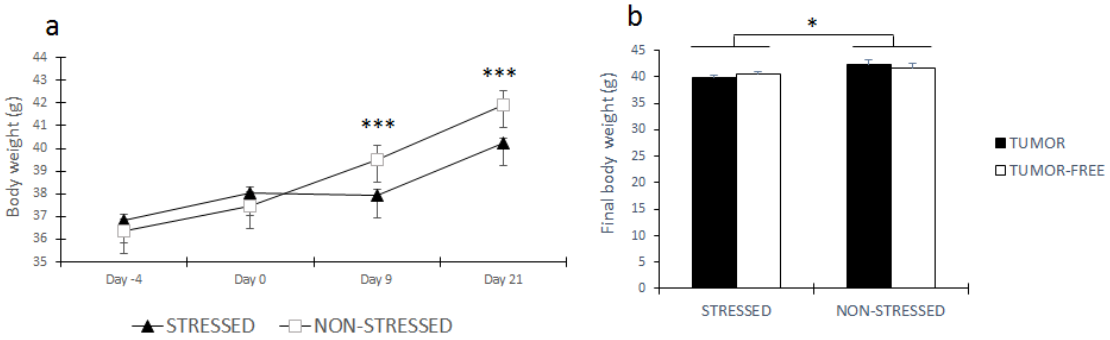

Figure 8. (a) Changes in the animals' body weights are presented for the stressed (n = 61) and non-stressed (n = 26) groups. (b) The percentage of the weight gained at the end of the experiment is presented for the stressed-tumor (n = 37), stressed-non-tumor (n = 24), non-stressed-tumor (n = 14) and non-stressed-non-tumor (n = 12) groups. The data are presented as the means ± SEM. *$p < 0.05$ and ***$p < 0.001$.

3.9. Effects of tumors, CSD and SCS on corticosterone levels

Two-way ANOVA (stress and time) with repeated measures showed significant differences in corticosterone levels for the time factor ($F(1,97) = 6.373$, $p = 0.013$, $\eta^2 = 0.062$) and for the time × stress interaction ($F(1,97) = 34.908$, $p < 0.001$, $\eta^2 = 0.265$) 40 min after the first

interaction, and 40 min after the interaction on day 9 (time: $F(1,95) = 6.606$, $p = 0.016$, $\eta^2 = 0.060$; time x stress interaction: $F(1,95) = 30.959$, $p < 0.001$, $\eta^2 = 0.246$). Furthermore, stressed mice had higher corticosterone levels after the FST ($F(1,96) = 11.933$, $p = 0.001$, Cohen's $d = 0.77$). No differences were observed in any corticosterone measurement between the tumor groups (after the interaction on day 1: $F(1,98) = 1.226$, $p = 0.271$; after the interaction on day 9: $F(1,96) = 3.637$, $p = 0.060$; after the FST: $F(1,96) = 1.325$, $p = 0.253$). Moreover, there was no correlation between the number of tumor foci and corticosterone level after the interactions or the FST (after the interaction on day 1: $r_s(51) = 0.269$; $p = 0.057$; after the interaction on day 9: $r_s(49) = 0.262$; $p = 0.069$; after the FST: $r_s(49) = 0.039$; $p = 0.778$). Differences were observed between groups stratified according to SCS after the first interaction ($F(1,68) = 5.716$, $p = 0.005$); the ANA group showed higher corticosterone levels than the AA group ($p = 0.005$, Cohen's $d = 0.82$) (Fig. 9).

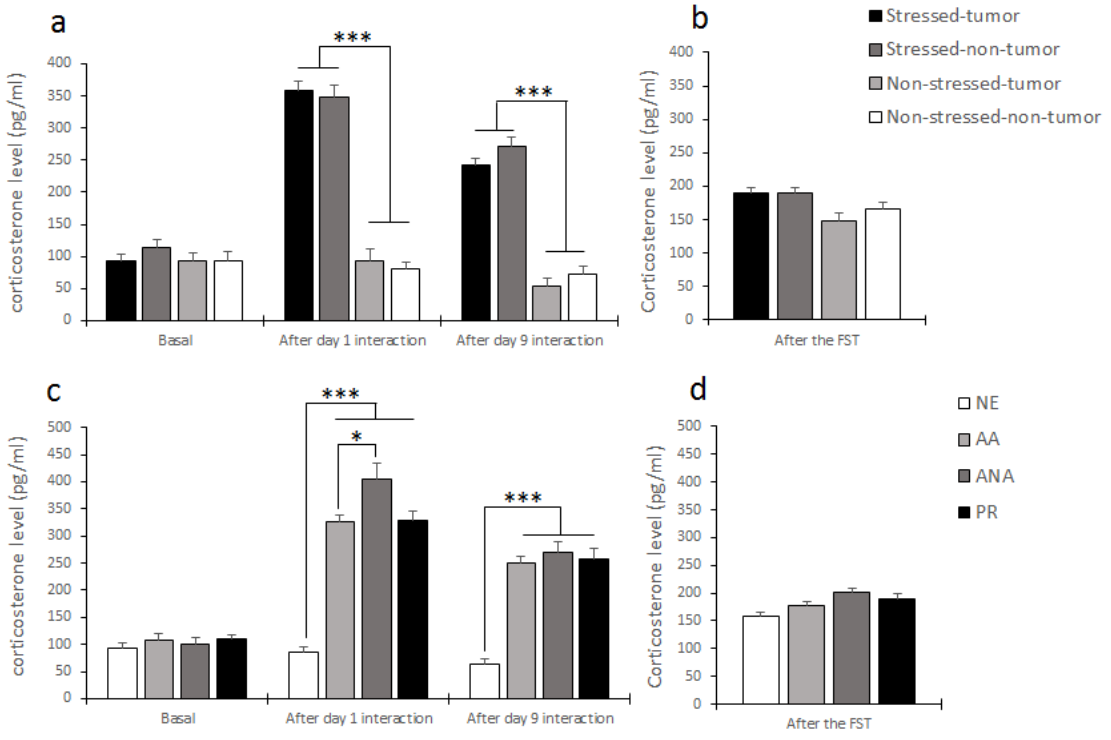

Figure 9. (a) Plasma corticosterone levels (ng/ml) measured on day -4 and after the day 1 and day 9 interactions are presented for the stressed-tumor (n = 37), stressed-non-tumor (n = 36), non-stressed-tumor (n = 14) and non-stressed-non-tumor (n = 15) groups. (b) Plasma corticosterone levels (ng/ml) measured after the FST are presented for the stressed-tumor (n = 37), stressed-non-tumor (n = 36), non-stressed-tumor (n = 14) and non-stressed-non-tumor (n = 15) groups. (c) Plasma corticosterone levels (ng/ml) measured on day -4 and after the day 1 and day 9 interactions are presented for the AA (n = 32), ANA (n = 25), PR (n = 14) and NE (n = 29) groups. (d) Plasma corticosterone levels (ng/ml) measured after the FST are presented for the AA (n = 32), ANA (n = 25), PR (n = 14) and NE (n = 29) groups. The data are reported as the means ± SEM. *$p < 0.05$ and ***$p < 0.001$.

3.10. Effects of tumors, CSD and SCS on spleen weight

According to ANOVA, the weight of the spleen from stressed mice was increased compared with non-stressed subjects ($F(1,98) = 47.480$, $p < 0.001$, Cohen's $d = 1.43$). No differences were observed between tumor groups ($F(1,98) = 0.048$, $p = 0.828$, Cohen's d), stress × tumor interaction ($F(1,98) = 0.215$, $p = 0.644$) or groups with different coping strategies ($F(2,68) = 0.571$, $p = 0.568$), but the weight of the spleen was greater in all coping strategy groups than in non-stressed mice ($F(3,96) = 15.697$, $p < 0.001$): AA ($p < 0.001$, Cohen's $d = 1.52$), ANA ($p < 0.001$, Cohen's $d = 1.30$) and PR ($p < 0.001$, Cohen's $d = 1.33$) (Fig. 10).

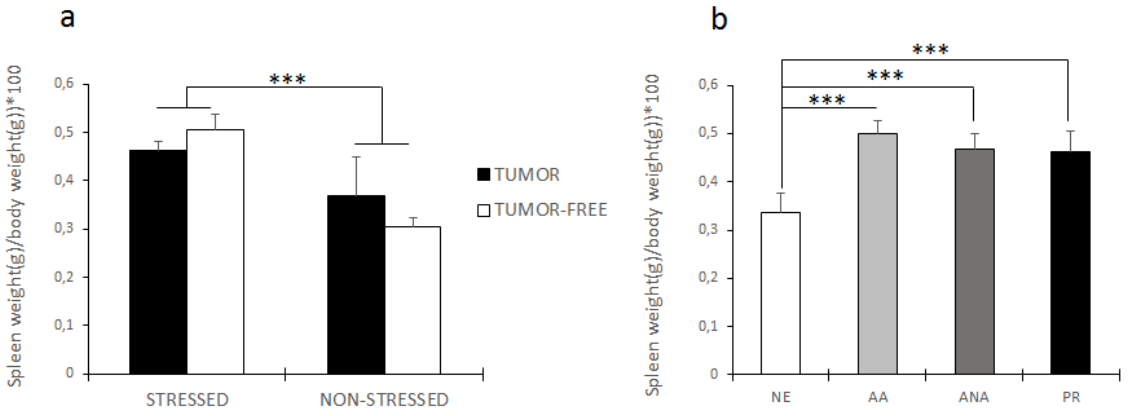

Figure 10. (a) Spleen weights (g) are presented for mice in the stressed-tumor (n = 37), stressed-non-tumor (n = 36), non-stressed-tumor (n = 14) and non-stressed-non-tumor (n = 15) groups. (b) Spleen weights (g) are presented for the AA (n = 32), ANA (n = 25), PR (n = 14) and NE (n = 29) mice. The data are presented as the means ± SEM. ****p < 0.001.*

3.11. Effects of tumors and SCS on the SAT

ANOVA conducted only in stressed subjects showed that tumor-bearing mice spent less time near the aggressive mouse in the SAT ($F(1,70) = 8.477$, $p = 0.005$, Cohen's $d = 0.76$). No differences were observed between groups stratified according to SCS ($F(1,69) = 1.165$, $p = 0.318$) (Fig. 11).

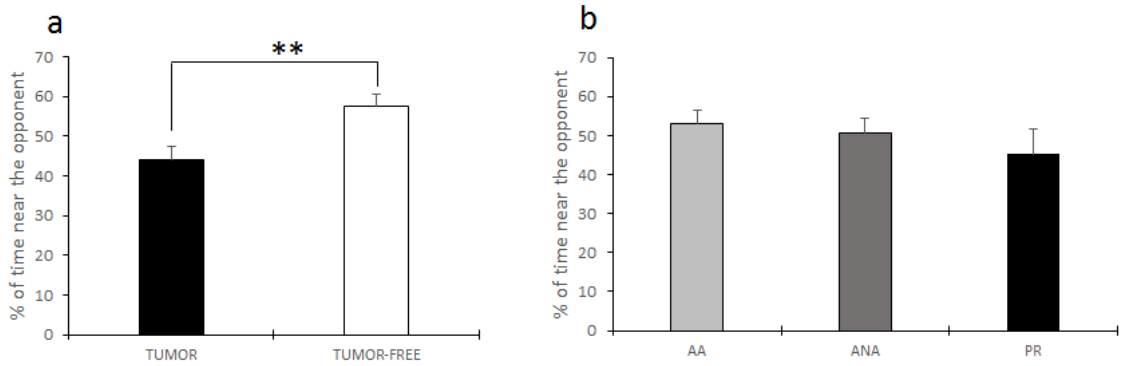

Figure 11. The percentage of time spent near the opponent is presented for (a) the tumor-stressed (n = 37) and non-tumor-stressed (n = 36) groups and (b) for the AA (n = 32), ANA (n = 25) and PR (n = 14) groups. The data are presented as the mean ± SEM. $**p < 0.01$.

4. Discussion

To the best of our knowledge, the results of the present study are the first to show the effect of chronic social defeat stress on tumor development, the impact of SCS on tumor development and the effect of the interaction of both factors on behavioral and physiological variables, particularly the response of the HPA axis, body weight and spleen weight.

In this study, subjects were sorted into three clusters based on the behaviors manifested in the first social confrontation, revealing the existence of three different subject groups with characteristic behavioral profiles. One group of subjects, which we call the active/aggressive (AA) group, presented a clear proactive strategy behavioral pattern; in the direct interaction, this group spent the most time engaged in attack and threat behaviors. According to many authors, individual differences in the aggressiveness trait reflect general coping styles in other situations, with open aggression representing a component of a larger set of behavioral characteristics that constitute a proactive coping strategy (de Boer, Buwalda, & Koolhaas, 2017; Koolhaas, De Boer, Buwalda, & Van Reenen, 2007; Koolhaas et al., 1999). Thus, a greater investment in non-social exploration behavior and less time spent immobile were also characteristics of this cluster. In addition to failing to display attack and threat behaviors, another group presented low non-social exploration behavior and spent more time immobile than the other groups. This group exhibited a reactive strategy and thus was called the passive/reactive (PR) group. Finally,

the cluster analysis defined a third group of subjects, which we designated the active/non-aggressive (ANA) group. The ANA group presented minimal attack and threat behaviors, maintained a higher activity level than the PR group and tended to engage defense/submission and flight behaviors to a greater extent than the other groups. Notably, according to various authors, a proactive strategy may not be equally clearly expressed in all challenging situations (de Boer et al., 2017). Therefore, according to the hypothesis proposed by Homberg (2012), it is possible that the impossibility of being dominant in our model, did not allow ANA group to manifest their actual coping strategy. In this regard, the inclusion of the indirect interaction in the cluster analysis confirmed the existence of this third group, because when animals were allowed to move freely without the risk of being assaulted, ANA subjects explored the environment and remained immobile for short periods, similar to AA mice. Therefore, the ANA group corresponded to subjects in an intermediate group with characteristics of both the AA and the PR groups.

Exposure to repeated defeat stress increased the pulmonary metastasis of B16F10 melanoma cells, confirming findings from previous studies showing that psychosocial stress is a powerful modulator of cancer progression in different tumor models (Armaiz-Pena et al., 2013; Feller et al., 2019; Moreno-Smith et al., 2010; Payne, 2014). Other authors have reported a relationship between social defeat stress and the progression and metastasis of Lewis lung carcinoma (LLC) when animals were exposed to 10 days of repeated defeat stress prior to cancer cell inoculation (Wu et al., 2015). Furthermore, in the present study, the PR animals exhibited more extensive tumor development than subjects in the AA and ANA groups. Notably, the strategies were established before tumor development occurred, excluding the possibility that the tumor itself induced more passive behaviors. Sajti et al. (2004) also observed a greater number of large metastatic foci of subcutaneous tumors (MADS 106) in passive rats in an open field situation. On the other hand, AA subjects did not develop metastatic tumors, consistent with the results reported by Amkraut and Solomon (1972), where animals responding with spontaneous fighting developed smaller virus-induced sarcoma lesions than animals that

467 did not fight. Similar data were previously obtained in our laboratory when acute stress was
468 applied several days after inoculation of B16F10 tumor cells (Vegas et al., 2006). Animals
469 employing attack behaviors and presenting high levels of environmental exploration in
470 situations of social conflict exhibited lower levels of tumor development. These data suggest
471 that a proactive strategy, accompanied by offensive responses and elevated exploratory activity,
472 protects against the effects of chronic stress on tumor development. In agreement with this
473 notion, the ANA subjects, who did not manifest offensive behaviors but displayed exploratory
474 activity, showed moderate tumor development. These data suggest that a generally active coping
475 strategy, but not the specific aggressive behavior displayed, play a key role in protecting mice
476 from tumor development.

477 Social defeat stress increased activation of the HPA axis throughout the entire stress period,
478 although the increase in corticosterone levels was less after 9 days of stress, confirming the
479 results published by other authors using other social defeat models (Blanchard, Sakai, McEwen,
480 Weiss, & Blanchard, 1993; Macedo et al., 2018). This change in corticosterone levels suggests
481 adaptation of the HPA axis to repeated stress, but the results reported by other authors (Norman
482 et al., 2015) and our group do not support this hypothesis. Specifically, data obtained in our
483 laboratory using a repeated defeat model similar to the model used in this study revealed equally
484 high levels even after 21 days of stress (Gómez-Lázaro et al., 2011; Pérez-Tejada et al., 2013).
485 The higher corticosterone levels in the stressed subjects at the end of social stress in the present
486 study might indicate an alteration in the HPA axis as a result of repeated exposure to defeat
487 stress, although reestablishment (Macedo et al., 2018) or a decrease (Gómez-Lázaro et al., 2011)
488 in basal levels in the animal after several days of exposure to any manipulation cannot be
489 excluded.

490 On the other hand, the highest corticosterone levels were observed in the ANA group after
491 the first direct interaction. Notably, these subjects did not present fight behaviors, such as attack
492 and threat, that could moderate the response of the HPA axis, as has been observed in other

493 studies (Walker, Masters, Dielenberg, & Day, 2009). In subjects with a proactive predisposition
494 who do not have the opportunity to manifest such behaviors, defeat is likely more stressful,
495 resulting in a greater reactivity to stress. This hypothesis might explain why these subjects had
496 higher corticosterone levels than AA and PR mice after the first stressful interaction. Thus, the
497 findings would support the hypothesis that coping styles influence the outcome of experienced
498 stress due to social subordination (Boersma et al., 2017). Although corticosterone levels were
499 approximately equal in all stressed groups after nine days of stress, we cannot exclude that a
500 long-term effect on other components of the HPA axis and/or on other physiological variables
501 related to the function of the HPA axis (brain-derived neurotrophic factor, immune system, etc.)
502 are responsible for the behavioral changes observed upon exposure to prolonged stress. Thus, in
503 the ANA group, immobility in the day 9 interaction, which was not affected by tumor
504 development and was the behavior that best discriminated the different groups, was similar to
505 that in the PR group and was distinct from that in the AA group. This finding might indicate a
506 change or transition in the ANA coping strategy towards a more reactive strategy. Moreover,
507 Paul et al. (2011) reported that repeated social defeat stress results in a change from proactive
508 coping behaviors to reactive coping behaviors. Our results also showed changes in behavior
509 after repeated exposure to defeat stress in all groups (a decrease in non-social exploration and an
510 increase in immobility), which reduced the individual differences observed in the first
511 confrontation. However, we should consider that tumor development, although minimal, might
512 also contribute to changes in some behaviors.

513 The weight of the spleen, an indirect measure of immune activation, was increased in all
514 the stressed subjects, regardless of the presence of the tumor and the type of coping strategy
515 employed. This result does not imply that the immune state (activation) was similar in all
516 stressed subjects because the type of parameters and the changes produced in these measures
517 (proliferative capacity of T and B cells, production of cytokines, etc.) might differ according to
518 the manifested strategies (Gómez-Lázaro et al., 2011; Pérez-Tejada et al., 2013). In addition, an

519 effect of the presence of the tumor on any of these parameters cannot be excluded (Lebeña et
520 al., 2014).

521 Based on new evidence, the glucocorticoids and catecholamines released during the stress
522 response play important roles in many of the stages required for cancer metastasis by altering
523 immune activity (Armaiz-Pena et al., 2013; Dhabhar, 2014, 2018; Feller et al., 2019). However,
524 the results from this study showed no relation between corticosterone level and tumor
525 development, with similar corticosterone levels observed between in tumor-bearing and non-
526 tumor-bearing subjects in both the stressed group and the control group. Furthermore, subjects
527 with greater tumor development at the end of the chronic stress period (subjects initially
528 employing a PR strategy) did not present different corticosterone levels than subjects in other
529 behavioral groups. Nevertheless, we cannot exclude the possible effects of the HPA axis,
530 catecholamines, or other mechanisms or pathways on modulating the relationship between
531 stress, behavioral strategies, and tumor development (Azpiroz et al., 2008; Wu et al., 2015).

532 When the behavioral consequences of both stress and tumor development were analyzed,
533 the data appeared to indicate that the effects on the variables analyzed in this study generally
534 differed according to each of the two factors. Thus, chronic stress but not the presence of a
535 tumor reduced the consumption of sucrose, which indicates anhedonia. All stressed mice,
536 regardless of strategy, showed a reduction in consumption of the sucrose solution (0.8%)
537 measured over 24 h but maintained the same water consumption. Although some authors have
538 reported a reduction in the sucrose preference, others have found no effect (see Hammels et al.,
539 2015). These discrepancies may be due to differences in the protocol used and the duration of
540 exposure to the stressor, because a reduced preference was not observed when the defeat stress
541 was limited to five days (Croft, Brooks, Cole, & Little, 2005) or one day (Razzoli, Carboni,
542 Andreoli, Ballottari, & Arban, 2011). In the present study, the reduction in the duration of daily
543 physical interactions to avoid injuries might have affected the results obtained, and thus, we
544 were unable to observe differences between coping strategies. On the other hand, although other

545 authors have reported a reduction in sucrose consumption caused by tumor development (Pyter,
546 Pineros, Galang, McClintock, & Prendergast, 2009), we did not observe such a reduction, likely
547 due to the moderate tumor development observed in the experimental subjects.

548 Regarding social behavior, tumor presence was associated with less time spent in the
549 opponent's area. This behavior, which has been interpreted by other authors as a social
550 inhibition associated with a susceptibility to depression (Dadomo et al., 2011; Lagace et al.,
551 2010; Venzala, García-García, Elizalde, Delagrange, & Tordera, 2012), suggests the presence of
552 depressive-type symptomology in subjects with tumors. Given that the social behavior test was
553 conducted only in stressed mice, we could not establish a possible stress effect on depressive-
554 like behavior.

555 On the other hand, the body weight of all subjects increased over 21 days, although this
556 increase was greater in non-stressed subjects. In contrast to our expectations, the presence of a
557 tumor did not reduce weight gain; the weight of tumor-bearing subjects was similar to that of
558 non-tumor-bearing subjects at the end of the experiment. Notwithstanding, other authors have
559 also not observed differences in this parameter as a function of tumor development (Nashed,
560 Seidlitz, Frey, & Singh, 2015; Pyter et al., 2009, 2017).

561 The FST is commonly used to determine depressive behavior in mice and rats, which is
562 defined by an increase in immobility or a decrease in latency to immobility in this test. This
563 behavior appears to be influenced by the species and the procedure used in the test. Thus, an
564 increase in immobility has been reported in rats (Becker et al., 2008; Hayashida, Oka, Mera, &
565 Tsuji, 2010; Rygula et al., 2005) but not in mice (Kinsey, Bailey, Sheridan, Padgett, & Avitsur,
566 2007; Krishnan et al., 2007), after acute or chronic social defeat stress, although a reduction in
567 immobility parameters was observed when the behavior was recorded in mice in a second
568 swimming session, similar to observations in rats (Gómez-Lázaro et al., 2011, 2012; Tang, Yu,
569 Chen, Gao, & Xiao, 2018). In the present study, the behavior manifested in the FST was not
570 altered by stress, but an effect was observed as a function of coping strategy. The PR group

showed more immobility and spent less time swimming than the AA and ANA groups, and they were the only group that differed from the non-stressed group in swimming. These data suggest that chronic stress exerted greater effects on behavior in the FST in subjects initially employing a PR strategy. The presence of a tumor did not alter the behaviors of mice in the FST, in contrast with the findings reported by Nashed et al. (2015) and Norden et al. (2015). This discrepancy may be attributed both to differences in the experimental subjects (females) and in the experimental tumor model (mammary tumors) or, as mentioned above, the moderate tumor development observed in our study.

In the OFT, which is used both to determine levels of anxiety and to measure motor activity in rodents (Crawley, 1985), tumor development and not stress is the most important influence, but only as a function of the parameter analyzed. Specifically, tumor-bearing animals spent the least amount of time in the center and remained closest to the periphery, behaviors that suggest anxiety in this test. The presence of a tumor did not reduce the distance travelled, i.e., the motor activity was not altered, which is consistent with findings reported by other authors in other tumor models (Norden et al., 2015; Pyter et al., 2017). This result is surprising because fatigue is one of the symptoms observed in patients with cancer. However, according to Norden et al. (2015), fatigue would not be a consequence of general malaise but rather a lack of motivation and would be associated with an increase in immobility in the FST and a reduction in sucrose consumption, which was not altered by the tumor in the present study. In contrast with the presence of a tumor, chronic defeat stress did not alter parameters related to anxiety, consistent with findings reported by other authors in rats (Liu et al., 2017) and in contrast with other studies (Kinsey et al., 2007; Patki, Solanki, Atrooz, Allam, & Salim, 2013) conducted with mice and rats. Differences in the method used, the period of defeat stress, and the species used might be responsible for the contradictory results. However, although stress did not appear to exert a statistically significant effect on distance travelled, when considering the different groups as a function of coping strategy, the PR and ANA groups travelled smaller distances than non-stressed subjects, again showing that these animals were more affected than AA mice.

In summary, exposure to social stress results in behavioral manifestations that allow subjects to be grouped into three different profile or behavioral strategy categories: AA, PR and the third intermediate group ANA, which is also characterized by a greater initial response of the HPA axis. Moreover, the effects of chronic exposure to social stress appeared to be more negative when subjects initially adopted a passive strategy (PR) because these subjects presented greater tumor development and exhibited the greatest changes in behavior at the end of the stress period. Regarding the ANA subjects, the results suggest that an unconformity between the coping style and the demands of their surroundings results in negative health consequences because these animals also presented greater tumor development and lower locomotor activity in the OFT than the non-stressed subjects. Despite the observed differences in tumor development as well as in behavior based on coping strategies, our results failed to show a clear interaction between tumor presence and stress, possibly because of the moderate tumor development and/or the variability in the stress effects due to the different behavioral coping strategies.

5. Conclusion

This study contributes to identification of detailed behavioral profiles that allow us to predict different levels of vulnerability to chronic stress and might help researchers develop personalized intervention strategies that reduce the negative effects of social stress on health. However, more research is needed in this area to determine and measure physiological mediators indicative of this vulnerability.

Declaration of Conflicting Interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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