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Chronic social defeat stress induces context avoidance in mice

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ABSTRACT

The scrutiny of social anxiety symptoms is necessary to overcome the obstacles encountered when managing this condition. Thus, it is important to study a new behavioral repertoire elicited by chronic social defeat stress (SDS), a social stressor, applied in a place conditioned fear protocol. In this study, we sought to develop a protocol that induced context avoidance after chronic SDS. Accordingly, the mice were re-exposed to an interaction apparatus (IA) to which they had been previously habituated. Between habituation and re-exposure phases, the mice were subjected to aggressive (stressed) or non-aggressive (control) encounters performed either in the IA (Exp. 1) or in a different apparatus (named social interaction box [SIB] – Exp. 2). To behaviorally assess this protocol, the mice were recorded based on spatiotemporal measures (frequency of entry and time spent in the home cage, tunnel, and surface [where the encounter took place] of the IA) and complementary measures (e.g., frequency of stretch-attend posture [SAP], rearing, and grooming). The results showed that defeated animals spent less time in the surface of the IA during the re-exposure phase as compared to the control group (Exp.1) or to conspecifics defeated elsewhere (Exp.2). In addition, these animals also presented increased number of risk assessment behavior (higher frequency of SAP) during the re-exposure phase, in comparison with its control group and mice defeated in the SIB. This shows an unprecedented place-dependent SDS-induced context avoidance. Taken together, the present study offers a relatively simple protocol capable of eliciting context avoidance induced by chronic SDS. It accounts for a novel behavioral repertoire that might be further explored within the scope of social affective disorders.

1. Introduction

Conditioned fear models are highlighted as an interesting approach to studying the neurobiology of affective disorders and fear memory in human and non-human individuals (Gilmartin et al., 2014). Studies have used multiple artificial stimuli (e.g., electric shock, restraint stress) to condition rodents to avoid situations (Bali and Jaggi, 2015; Baran, 2009; Barbayannis, 2017; Zu, 2019). Although useful, such approaches are limited in their ethological relevance, highlighting the need to investigate naturally occurring stressors capable of inducing conditioned avoidance.

In this regard, social defeat stress (SDS) represents a powerful naturalistic paradigm. Some studies used the social defeat paradigm to induce defensive reactions in conditioned hamsters (Potegal, 1993). Others sought to demonstrate that foot shock paired with social pursuit induced social fear conditioning in male mice (Toth et al., 2012). There is also data showing that defeated male Syrian hamsters become submissive and no longer defend their home territory (Huhman, 2003). However, to date, the literature has lacked a model of place-conditioned avoidance induced by chronic social defeat stress (SDS). In this context, SDS stands out as an ethological stimulus that models various behaviors in rodents. For instance, SDS stimulus seems to be consistent with increasing submissive behavior, decreasing aggressive and sexual behaviors, or altering anxiety behaviors in the open field or the elevated plus maze (Costa, 2016; Kudriavtseva, 1996; Kudryavtseva et al., 1991; Rodgers and Cole, 1993; Sapolsky, 2011; Van de Poll, 1982; Yoshimura and Kimura, 1991).

Moreover, previous studies have shown that male rodents that exhibit aggressive dominant behavior induce an increase in submissive responses in defeated conspecifics. The intruders change their aggressive impetus to active (e.g., walking backward) or passive (e.g., freezing) defense behaviors and show decreased mobility as well as weight loss

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(Albonetti and Farabollini, 1994). In this way, the social defeat paradigm, such as the conflict between conspecifics, establishes a dominant–subordinate relationship (de Boer, 2018; Toth and Neumann, 2013). Moreover, chronic exposure to various types of stress has been suggested to influence the onset of anxiety and fear-related behaviors. For example, rodents subjected to chronic stress have potentiated defensive reactions when exposed to aversive stimuli (Franklin, 2017; Lukas, 2011; Lumley, 2000; Razzoli, 2011; Venzala, 2012). Together, these findings support SDS as an ethologically grounded tool for probing how chronic social stress shapes defensive and affective responses, paving the way for models that integrate context-dependent learning.

Altogether, these observations justify the use of SDS episodes to induce avoidance behavior in a social context. However, despite extensive use of SDS, the literature still lacks models addressing place-conditioned avoidance specifically induced by chronic social defeat. This is a critical gap, as recent work has shown that chronic SDS (CSDS) produces broad neurobehavioral outcomes, including sex-specific effects linked to the estrous cycle (van Doeselaar et al., 2021), memory impairments (Jianhua et al., 2017), and site- and cell type–specific changes in CRF receptor signaling (Guo et al., 2020 Apr 20). Furthermore, CSDS appears to encompass a conditioned fear component directed toward social cues (Lee et al., 2024), with resilience playing a central role in the regulation of social avoidance behaviors (Ayash et al., 2020). These findings underscore the importance of exploring whether the context in which defeat occurs can itself acquire associative value, thereby broadening the behavioral repertoire relevant to stress-induced affective dysfunction.

Building on this rationale, our laboratory previously reported that defeated mice exposed to the interaction apparatus (IA) used in the rat exposure test (RET) did not change their spatiotemporal behaviors after a single episode of aggressive conflict with a conspecific (Crestani et al., 2018). On the other hand, there was a significant increase in grooming (self-cleaning behavior) in the home chamber (protected area), presented by the socially defeated group, suggesting associative learning that the home chamber was a safe place to express this behavior. It is important to note, however, that rodent grooming is not merely a self-cleaning activity; rather, it also reflects emotional and motivational states, being sensitive to stress and arousal levels and often interpreted as a displacement or coping behavior under challenging conditions (Estanislau C et al., 2019; Kalueff, 2016; Song et al., 2016). In the same study, the authors suggested alternatives to make the social defeat protocol more robust, such as increasing the number of aggressive encounters or adding environmental cues, to facilitate contextual learning (Crestani et al., 2018). In this context, the present study attempted to extend our understanding of the repertoire of behavior elicited after contextual fear learning induced by SDS. We hypothesized that a protocol based on social defeat, with a greater number of confrontations in addition to sensory interactions and environmental cues, would be capable of generating associative learning in defeated mice exposed to the IA. Accordingly, we aimed to propose a protocol that induced context aversion.

2. Material and methods

2.1. Subjects

One hundred and forty male Swiss mice (Univ. Estadual Paulista UNESP, SP, Brazil) weighing 25–35 g at testing were used in this study. Ninety-eight mice were assigned as experimental subjects or intruders, and they were housed in groups of 10 per cage (size: 41 ×34 x 16 cm) and maintained under a 12-h light cycle (lights on at 7:00 a.m.) in a temperature-controlled environment (23 ± 2 ºC). Twelve conspecifics (25–35 g) were used to induce a social encounter without aggression and they were housed with the intruders. Ten mice (25–35 g) were used to train the aggressors to attack an intruder before tests commenced. Finally, twenty aggressor mice (47–73 g) were housed alone in polypropylene cages (size: 24 ×37 x 24 cm) and maintained under a similar light cycle and temperature-controlled environment. Food and water were freely available except during the brief test periods. All mice, except the aggressors, were naïve at the beginning of the experiments and they were used once. All efforts were made to minimize animal suffering; thus, we permanently assessed their apparent health such as fur integrity, bruises, bleeding, and weight loss. The Ethical Committee for Use of Animals of the School of Pharmaceutical Science/UNESP approved housing conditions and experimental procedures (protocol CEUA/FCF/Car/UNESP 41/2016), which complies with Brazilian and international (ARRIVE) guidelines for animal use and welfare.

2.2. Interaction apparatus (IA)

The interaction apparatus (IA) (Fig. 1 A) was adapted from the conspecific interaction test described by Crestani et al. (2018), which in turn was behaviorally (Yang, 2004) and pharmacologically (Campos, 2013) validated as the rat exposure test (RET). Testing procedures were conducted in a 23 cm × 24 cm × 21 cm clear polycarbonate cage (i.e., the exposure chamber) covered with a black polycarbonate lid. A wire mesh screen divided the exposure chamber into two equal-sized compartments (the surface A and B, each measuring 11.5 cm×24 cm x 21 cm). The home cage was a (7 cm × 7 cm × 12 cm) box made of black Plexiglas on three sides and clear Plexiglas on the fourth side to allow videotaping. The home cage was connected to the exposure cage by a clear Plexiglas tube tunnel (4.4 cm in diameter, 13 cm in length, and raised 1.5 cm above the floor of the two chambers).

In our protocol all compartments could or not, depending on our convenience, be separated by an external guillotine door operated by the experimenter. The surface areas were reduced by half (i.e. 11.5 cm×24 cm x 21 cm) compared to that used in the protocol validated by Yang (2004), aiming at increasing the probability of social interaction.

2.3. Social interaction box (SIB)

In the second experiment, to assess whether social-induced avoidance is dependent on the context where the defeat occurred, mice were socially defeated in a different apparatus from that where the re-exposure took place. The stress was applied in the light compartment of the light/dark box, widely used as an animal model of anxiety (Crawley and Goodwin, 1980). It consists of a Plexiglas box (46 cm×28.5 cm×26 cm) with two compartments separated by a small door. While the first compartment is made of black walls (one-third of the box) the other is made of white walls (two-thirds of the box). For our purposes, we blocked the passage door and used only the white compartment (30.5 cm×28.5 cm×26 cm) of the light/dark box. Hereinafter, this compartment is referred to as social interaction box (SIB), a new environment to which the social defeat stress was applied (Fig. 1B). During the two episodes of sensory exposure that flanked the social defeat stress (SDS), we inserted a wire mesh to avoid physical contact between the intruder and the resident aggressor or non-aggressor (for more details, please see Section 2.4 below).

2.4. Protocol

Experimental sessions were conducted during the light phase under an 80 W lamp, providing 15 lux at floor level. Before each session, animals were placed in an adjacent room for 20 min habituation to the experimental conditions. Mice were first habituated to the interaction apparatus (IA). They were then exposed to repeated social interactions, either with an aggressive or a non-aggressive conspecific, occurring either in the same apparatus (Experiment 1) or in a distinct environment (Experiment 2). Finally, all animals were re-exposed to the IA to assess context-dependent avoidance behaviors.

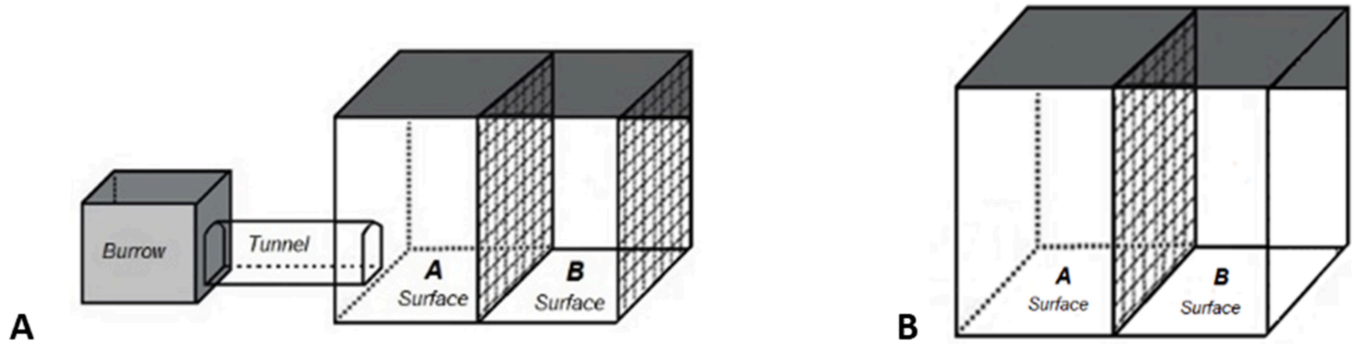

Fig. 1. (A) Interaction apparatus (IA) derived from that validated to the Rat Exposure Test. (B) Social interaction box (SIB) obtained by isolating the light compartment of the Light/Dark box to create a novel environment for Experiment 2.

2.4.1. *Experiment 1: Behavioral assessment of mice subjected to non-aggressive (control situation) or aggressive (social defeat) interactions and re-exposed to the interaction apparatus (IA)*

This experiment was designed to determine whether repeated episodes of social defeat within the same apparatus could induce context-dependent avoidance behaviors in mice.

Habituation phase (days 1–3): mice were confined in the IA home cage for 30 s via a guillotine door to minimize defensive responses after handling, then allowed to freely explore the IA for 10 min. This procedure was repeated for three consecutive days (24 h apart). On day 3, behavior was recorded (Hab) for comparison with post-interaction measures. Throughout habituation, wood shavings from each subject's home cage were placed in the IA as contextual cues.

Interaction phase (days 4–7): before each trial, intruders were confined in the home cage for 30 s, then allowed to explore the IA until reaching Surface A, at which point the door was closed to initiate the interaction. Each session comprised one 3-min encounter with either an aggressive or a non-aggressive conspecific in Surface A, flanked by two 5-min sensory (non-physical) interactions with the conspecific placed in Surface B behind a wire mesh. Wood shavings from the conspecific's cage were also added. Four daily sessions (24 h apart) were performed, each with a different conspecific to avoid habituation.

Re-exposure phase (day 8): twenty-four hours after the last interaction, mice were re-exposed individually to the IA for 10 min, without conspecifics, to freely explore. Behavior was recorded (ReE). Wood shavings from the last conspecific remained in the IA, which was cleaned with 20 % alcohol and dried between sessions.

2.4.2. *Experiment 2. Behavioral assessment of mice subjected to non-aggressive (control) or aggressive (social defeat) interactions into the social interaction box (SIB) and exposed to the IA*

This experiment aimed to test whether social defeat-induced avoidance depends on the specific context in which the stress occurred, by assessing animals defeated in a different environment and re-exposed to the IA. It followed the same protocol as Experiment 1, except that the interaction phase (days 4–7) was conducted in the SIB (Fig. 1B) located in a different experimental room.

2.4.3. *Behaviors and general procedures*

A camera mounted frontally to the IA recorded all sessions on the 3rd (Hab) and 8th (ReE) days of experiment. Data were stored in an SD memory card to further analysis. Based on a pilot study, only the first 5 min of the experiment are necessary to reveal a consistent behavioral pattern in the IA test. Thus, the behaviors were analyzed through the software "X-plo-Rat 2005", developed by the laboratory of Dr. Silvio Morato of the University of Sao Paulo - USP of Ribeirão Preto-Brazil (the software can be freely downloaded in http://Scotty.FFCLRP.USP.br/X-plo-Rat.html) (Tejada et al., 2018). It was recorded the spatio-temporal measures, which comprises the frequency of entry and time spent in seconds in the different compartments (home cage, tunnel, and surface A). It was also recorded the following complementary measures: the frequency of stretch-attend posture (SAP), and rearing, as well as, frequency and duration of grooming and time of defensive occultation. The SAP is described as the risk assessment behavior, in which the animal projects its body forward using the front legs while the hind legs remain in the initial position. Rearing on the surface is defined as the behavior in which the animal rises on the hind legs, standing uprightly. Grooming is a self-cleaning behavior that generally follows a cephalocaudal projection (Edmunds, 1974), and finally, defensive occultation is a behavior in which the animal digs and/or pushes the wood shavings towards the aggressor's compartment.

2.5. *Statistical analysis*

Data were submitted to the Levene homogeneity test. In cases where data indicated significance, they were log or square root transformed. Afterward, when homogenous, results were analyzed through the analysis of variance (ANOVA) followed by the Duncan posthoc test. Data that did not present a normal distribution were submitted the non-parametric Kruskal-Wallis analysis followed, when necessary, by Dunn's posthoc test. Data were considered significant when $p < 0.05$. All statistical analyses are presented in the Tables.

3. Results

Table 1 links the results with their corresponding Figures and statistical analyses. In the upper part of each Figs. (2–3), it has been inserted a timeline of the procedures to make easier the comprehension.

3.1. *Experiment 1. Effects of social defeat stress (SDS) on the behavior of mice re-exposed to the context (IA)*

Fig. 2 (A-B) shows the spatio-temporal measures of mice, during habituation (Hab – third day) and re-exposure (ReE – 8th day) phases of the test, exposed in this meantime to four aggressive (defeated) or non-aggressive (control) social encounters. Importantly, in this Exp. all phases took place at the same apparatus, i.e. the interaction apparatus (IA). According to Fig. 2 (A-B), results showed that the defeated group presented less time in the surface compartment during ReE as compared to the Hab phase ($p < 0.05$). Furthermore, the defeated group also spent less time on the surface compared to the control group during the re-exposure phase ($p < 0.05$). Mirrored to that, defeated animals spent more time on the home chamber during re-exposure compared to both the Hab phase and to its control group ($p < 0.05$). The same analysis showed that during the re-exposure phase, animals increased the frequency of entries in the tunnel area of the IA as compared to the other compartments (Fig. 2B; $p < 0.05$).

Following the behavioral analysis of Exp.1, Fig. 2 (C-H) shows the

Table 1
Summary of the Experiments, assessed behaviors, data normalization, corresponding Figures and statistical analysis performed throughout the study.

Exp.	Behavior	Fig.	Data	Statistic	Table
1	Time spent	2	Square root	3-way ANOVA	3
	Frequency of entries		Log	3-way ANOVA	3
	Frequency of grooming		Square root	3-way ANOVA	3
	Time of grooming		Square root	3-way ANOVA	3
	Frequency of SAP		Raw	Kruskal-Wallis	-
	Time of defensive occultation		Raw	Kruskal-Wallis	-
	Frequency of rearing		Raw	2-way ANOVA	2
	Time of rearing		Raw	2-way ANOVA	2
2	Time spent	3	Raw	3-way ANOVA	3
	Frequency of entries		Raw	3-way ANOVA	3
	Frequency of grooming		Raw	3-way ANOVA	3
	Time of grooming		Raw	3-way ANOVA	3
	Frequency of SAP		Raw	2-way ANOVA	2
	Time of defensive occultation		Raw	2-way ANOVA	2
	Frequency of rearing		Raw	2-way ANOVA	2
	Time of rearing		Raw	2-way ANOVA	2

complementary measures presented by the control and defeated groups during Hab and ReE phases. Accordingly, this analysis accounts for the risk assessment, locomotor, and self-cleaning behaviors.

Fig. 2 shows the frequency (C) and time (D) of grooming performed by control and defeated animals in the Hab and ReE phases of the test. Results systematically reproduce the spatio-temporal analysis, i.e., while defeated animals decreased the frequency and time of grooming in the surface area as compared to its control group and Hab phase, they increase this behavior in the home chamber as compared to its control group and the Hab phase ($p < 0.05$).

In addition, Fig. 2 shows the frequency of SAP (E) and the time of defensive occultation (F) of defeated animals in the Hab and ReE phases of the test. This analysis revealed that defeated mice displayed increased frequency of SAP during the ReE as compared to the Hab phase [$H(3,48) = 47$; $p < 0.05$]. Fig. 2 (F) reveals that defeated animals displayed less defensive occultation during the ReE phase as compared to its control group and the Hab phase [$H(3,56) = 11.5$; $p < 0.05$].

Finally, the lower part of Fig. 2 (G-H) accounts for the frequency and time of rearing performed by defeated mice during the Hab and ReE phases. These data are consistent with a decrease of vertical exploration displayed by the defeated animals compared only to their control group ($p < 0.05$).

Tables 2 and 3 shows the analysis of variance performed in Exp. 1.

3.2. Experiment 2. Effects of social defeat stress (SDS) performed in a novel environment (SIB) on the behavior of mice re-exposed to the IA

Following the same rationale of a display pattern of Exp. 1, Fig. 3 (A-B) shows the spatio-temporal measures of defeated and non-defeated mice, exposed to the conspecific in the social interaction box (SIB), nevertheless, re-exposed to the IA (ReE phase), where they were previously habituated (Hab). Results shown in the upper histograms (A) revealed that these animals, regardless of their condition (i.e. defeated or non-defeated) and the phase of the test (i.e. ReE or Hab), spent more time in the surface area of the apparatus ($p < 0.05$). Moreover, Fig. 3B shows that the frequency of entries was increased in the tunnel area regardless of the condition and the phase of the test ($p < 0.05$).

Consistent with Exp.1, spatio-temporal and complementary measures of Exp.2 are displayed similarly and their statistical analysis can be found in Tables 2 and 3.

Following data analysis, Fig. 3 shows the frequency (C) and time (D) of grooming, during Hab and ReE phases, performed by control and defeated animals, which were submitted to the social interactions in the SIB. Contrasting to Exp. 1, complementary data from Exp. 2 did not point to any effect, either in the frequency or in the time of grooming behavior ($p > 0.05$) when animals were stressed elsewhere. The same happens to the SAP (E; $p > 0.05$) and defensive occultation (F; $p = 0.05$) behaviors.

Finally, in the lower panel, histograms represent the frequency (G) and the time (H) of rearing behavior during Hab and ReE phases, performed by control and defeated mice. Interestingly, animals, regardless of the stimulus they were subjected to, i.e., SDS or non-aggressive encounter, increased vertical exploration of the IA, during ReE, as compared to the Hab phase ($p < 0.05$).

4. Discussion

The main results showed that the present protocol of SDS induced context avoidance in mice re-exposed to an IA where they were previously stressed. Importantly, this effect was dependent on the location where the SDS took place, as mice subjected to SDS in another environment, that is, the social interaction box (SIB), did not alter their behavior in the IA during the re-exposure (ReE) phase. The complementary measures of risk assessment corroborated the spatiotemporal data, pointing to increased context-induced avoidance in defeated mice re-exposed to the same apparatus where the SDS took place. As expected, complementary data from animals defeated in the SIB and re-exposed to the IA corroborated the lack of effect during ReE. Altogether, these data seem to offer a new method for behaviorally assessing a type of context-induced avoidance.

The first experiment was designed to assess an effective protocol for inducing context avoidance in defeated mice. It showed that four episodes of SDS with environmental cues (i.e., wood shavings from the aggressor's cage) and sensory interactions induced context avoidance in the mice, as evaluated by both spatiotemporal and complementary measures. Accordingly, defeated mice spent less time on the surface area of the IA, where the SDS was previously carried out, and, by extension, more time in the home chamber, a protected compartment, during ReE, compared to habituation (Hab), indicating context avoidance acquisition. It is important to highlight that ReE *per se* does not affect mouse behavior. This was shown by the lack of difference in the behavior of both groups (control and defeated) habituated to the IA compared to control animals re-exposed to the apparatus after a non-aggressive interaction. In this context, it was necessary to re-expose specific defeated mice from the context previously paired with SDS to detect avoidance. This context-induced avoidance with signs of the aggressor was also examined by Berton (2006), who observed that animals exposed to aggressive interactions for 10 days presented robust avoidance responses compared to animals exposed to a non-animated object. The researchers proposed a protocol of 10 min of physical aggression and 24 h of sensory aggression per day for 10 days. Exposure only occurred four weeks after the first SDS episode. The present results and other studies (Golden, 2011; Macedo, 2018) corroborate that the demand for social interaction is impaired after social defeat. This behavioral profile displayed by mice was accompanied by a preference for the home cage compartment (increased time spent in the home cage), thereby showing a mirrored effect between the preference for protected areas and the avoidance of the surface where the SDS took place.

Regarding general activity, there seemed to be no locomotion

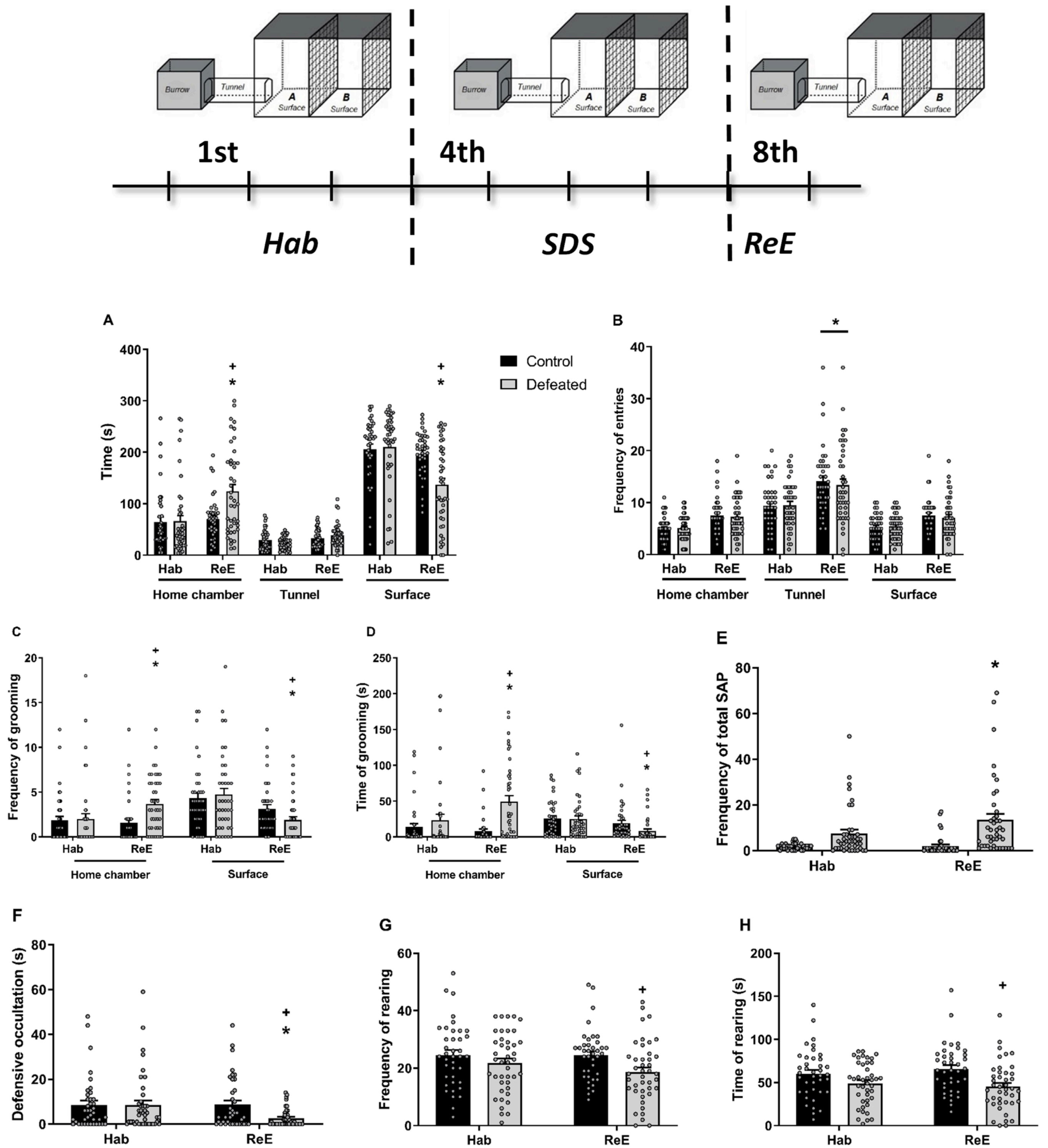

Fig. 2. The upper panel shows a timeline of Exp.1, indicating that habituation (days 1–3), social defeat or control interactions (days 4–7), and re-exposure (day 8) all occurred in the same interaction apparatus (IA). The histograms show the effects of the SDS or non-aggressive encounters in the (A-B) spatio-temporal, in the (C) frequency and (D) time of grooming, in the (E) frequency of SAP, in the (F) time of defensive occultation and in the (G) frequency and (H) time of rearing behaviors of mice re-exposed to the social interaction context. N = 39–42. $^{+}p < 0.05$ compared to the respective control group. $^{*}p < 0.05$ compared to the Hab phase.

impairment that could bias the interpretation of context-induced avoidance in Exp. 1. In this context, there was an increased number of entries to the tunnel compartment by both the control and defeated groups during the ReE phase. This result corroborates the argument promoted by other authors (Yang, 2004; Crestani et al., 2018) that the tunnel is a place of passage between the compartments (home chamber and surface) and therefore must present higher exploration. Complementary to that, vertical exploration, assessed by the time and frequency of rearing, decreased in the defeated group re-exposed to the IA as compared to the control group. Although we did not perform a correlation analysis, it could be argued that this decrease reflected the reduced time spent by the animals in this compartment, that is, the surface, as there were no differences in the absolute number of entries.

The complementary analysis corroborated the spatiotemporal data,

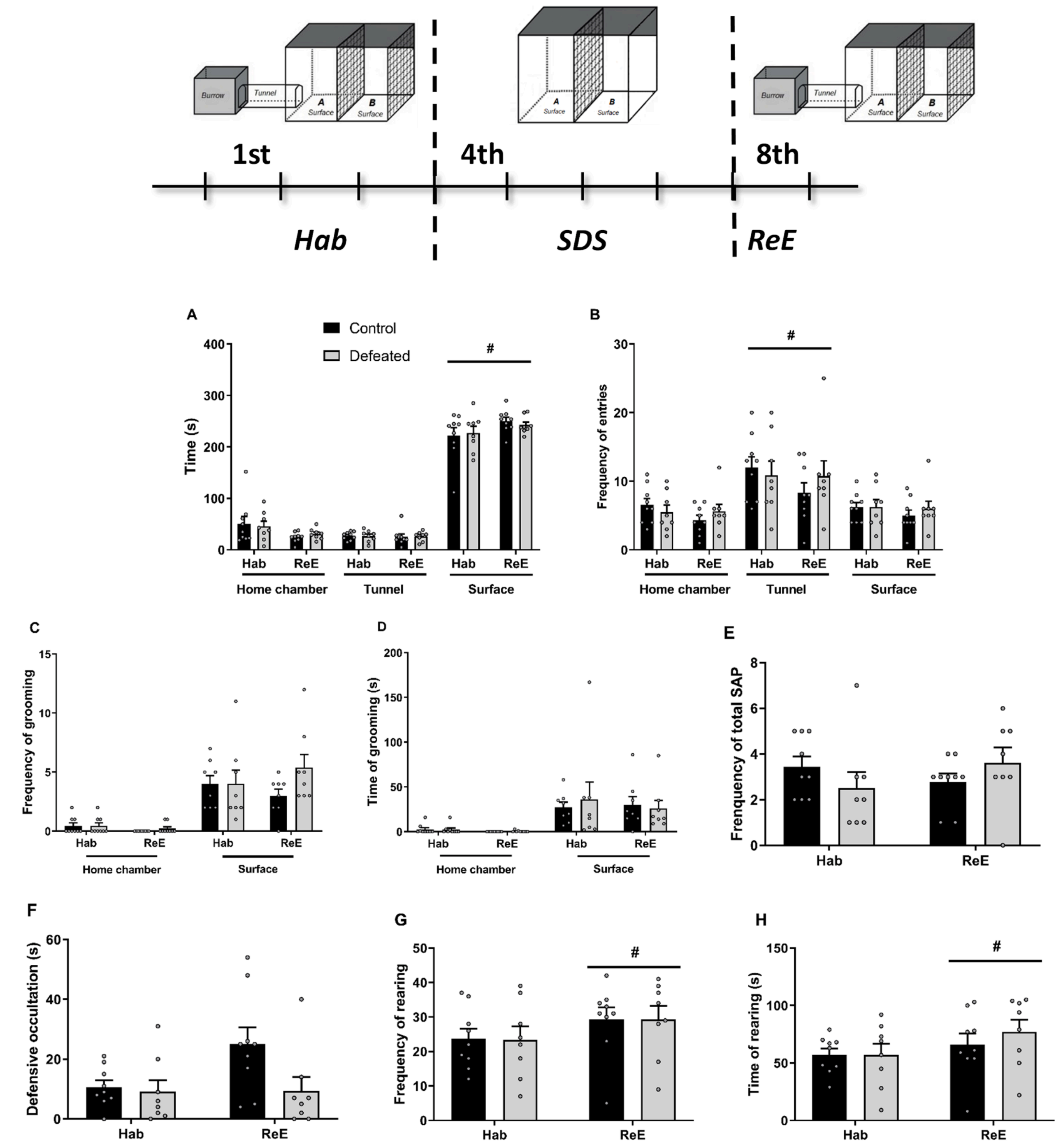

Fig. 3. The upper panel shows a timeline of Exp.2, indicating that habituation (days 1–3) and re-exposure (day 8) took place in the interaction apparatus (IA), whereas the social defeat or control interactions (days 4–7) were conducted in a distinct environment, the social interaction box (SIB). The histograms show the effects of the SDS or non-aggressive encounters in the (A-B) spatio-temporal behaviors, in the (C) frequency and (D) time of grooming, in the (E) frequency of SAP, in the (F) time of defensive occultation and in the (G) frequency and (H) time of rearing behaviors of mice submitted to the social interactions within the SIB and re-exposed to the IA. N = 8–9. $^{\#}p < 0.05$ compared to the other compartments.

as defeated animals increased the number of SAPs, a behavior related to risk assessment, during re-exposure, compared to the habituation phase. In this connection, SAP is largely accepted as a valid index of anxiety (Holly et al., 2016; Mascarenhas et al., 2013; Rodgers and Johnson, 1995) and its robust increase indicated a conflict between the avoidance and the approach to the surface area of the IA. Regarding defensive occultation, the defeated group in Exp. 1 decreased this behavior during ReE compared to both the Hab phase and its control group. Given that the time animals spent performing defensive occultation often *increased* (rather than *decreased*) in anxious animals exposed to the RET (Yang, 2004), one can speculate that this particular behavior may be related to prey–predator interactions, rather than to conspecific encounters. It did

Table 2
two-way ANOVA.

Exp.	behavior	factors	dfn, dfd	*F*	*p*
1	Frequency of rearing	Condition	1158	7.02	**0.008**
		Phase	1158	0.94	0.33
		Phase vs condition	1158	0.91	0.33
1	Time of rearing	Condition	1158	13.77	**0.0002**
		Phase	1158	0.08	0.77
		Phase vs condition	1158	1.15	0.28
2	Frequency of total SAP	Condition	1,30	4.00	0.05
		Phase	1,30	2.94	0.09
		Phase vs condition	1,30	2.7	0.10
2	Defensive occultation	Condition	1,30	0.007	0.92
		Phase	1,30	0.17	0.67
		Phase vs condition	1,30	2.69	0.11
2	Frequency of rearing	Condition	1,30	0.002	0.95
		Phase	1,30	2.60	0.11
		Phase vs condition	1,30	0.0008	0.97
2	Time of rearing	Condition	1,30	0.37	0.54
		Phase	1,30	2.60	0.11
		Phase vs condition	1,30	0.39	0.53

dfn: degrees of freedom numerator; dfd: degrees of freedom denominator

not seem to be evoked toward the place-induced avoidance in this protocol as it was for SAP or grooming behaviors. This may be because the present study approached contextual-induced avoidance, rather than the exposure of mice to a predator, a situation in which defensive occultation is likely to occur. With respect to displays of grooming, the results consistently showed that defeated animals increased this behavior in the home chamber during the ReE compared to the Hab and control groups. This was mirrored by the same group decreasing this behavior in the surface compartment during ReE compared to the Hab and control groups. The interpretation of grooming is complex. It seems to be modulated by stress and anxiety because it is an adaptive and stress-prepared behavior (Kalueff and Tuohimaa, 2005). In chronic protocols of aggressive interaction in rats and mice, the grooming response was reported to increase. In addition, Crestani et al. (2018) suggested that increased expression of grooming in a protected area is related to a reduction in excitement after an aggressive interaction, which could indicate an activity aimed at relieving anxiety after an aggressive confrontation (Crestani et al., 2018; Kalueff, 2016). In the context of our results, because defeated animals during the ReE phase showed robust context-induced avoidance, the increase in grooming performed in the home chamber (a protected area) seen in SDS-exposed mice seemed to be related to the expression of behavior aimed at relieving context-induced aversion. Taken together, the data from Exp. 1 consistently pointed to a protocol that induced avoidance to the context, whereby four episodes of SDS with an aggressor's cues and sensory interactions could represent a useful method for further investigating behaviors that might be implicated in social affective disorders and fear memory.

The second experiment aimed to evaluate the influence of the SDS location in context-induced avoidance. Accordingly, animals subjected to SDS in a new environment, that is, an SIB, did not alter their behavior when re-exposed to the IA to which they had previously been habituated. In this context, both the control and defeated groups, regardless of the test phase, explored the surface area of the IA more, corroborating the lack of context-avoidance when the SDS was applied elsewhere. Given that mice defeated in a different environment did not show measurable levels of avoidance, it might be argued that we effectively achieved a protocol that was robust to induce avoidance specifically dependent on the context (Exp.1). Moreover, corroborating the

Table 3
Three-way ANOVA.

Exp.	behavior	factors	dfn, dfd	*F*	*p*
1	Time spent	Condition	1158	1.57	0.21
		Phase	1158	12.27	**0.0005**
		Condition vs phase	1158	0.49	0.48
		Compartment	2316	231.71	**< 0.0001**
		Compartment vs condition	2316	6.06	**0.002**
		Compartment vs phase	2316	12.58	**< 0.0001**
		Compartment vs condition vs phase	2316	7.40	**< 0.0001**
1	Frequency of entries	Condition	1158	0.4	0.52
		Phase	1158	16.79	**< 0.0001**
		Condition vs phase	1158	0.89	0.34
		Compartment	2316	735.99	**< 0.0001**
		Compartment vs condition	2316	0.75	0.47
		Compartment vs phase	2316	4.18	**0.01**
		Compartment vs condition vs phase	2316	1.48	0.22
1	Frequency of grooming	Condition	1158	0.23	0.62
		Phase	1158	1.97	0.16
		Condition vs phase	1158	0.56	0.45
		Compartment	1158	15.16	**0.0001**
		Compartment vs condition	1158	6.45	**0.012**
		Compartment vs phase	1158	12.04	**0.0006**
		Compartment vs condition vs phase	1158	8.57	**0.003**
1	Time of grooming	Condition	1158	6.32	**0.012**
		Phase	1158	0.28	0.59
		Condition vs phase	1158	3.00	0.08
		Compartment	1158	1.30	0.25
		Compartment vs condition	1158	13.94	**0.0002**
		Compartment vs phase	1158	10.43	**0.001**
		Compartment vs condition vs phase	1158	8.85	**0.003**
2	Time spent	Condition	1,30	1.22	0.27
		Phase	1,30	−8.06	1.00
		Condition vs phase	1,30	3.60	0.06
		Compartment	2,60	4.84	**< 0.0001**
		Compartment vs condition	2,60	1.27	0.98
		Compartment vs phase	2,60	3.99	**0.02**
		Compartment vs condition vs phase	2,60	3.14	0.73
2	Frequency of entries	Condition	1,30	0.12	0.72
		Phase	1,30	1.03	0.31
		Condition vs phase	1,30	0.89	0.35
		Compartment	2,60	97.49	**< 0.0001**
		Compartment vs condition	2,60	0.23	0.78
		Compartment vs phase	2,60	1.13	0.32
		Compartment vs condition vs phase	2,60	1.30	0.27
2	Frequency of grooming	Condition	1,30	2.25	0.14
		Phase	1,30	0.06	0.80
		Condition vs phase	1,30	2.45	0.12
		Compartment	1,30	67.87	**< 0.0001**
		Compartment vs condition	1,30	1.11	0.29
		Compartment vs phase	1,30	0.26	0.60
		Compartment vs condition vs phase	1,30	1.52	0.22
2	Time of grooming	Condition	1,30	0.06	0.79
		Phase	1,30	0.39	0.53
		Condition vs phase	1,30	0.15	0.69

(continued on next page)

Table 3 (*continued*)

Exp.	behavior	factors	dfn, dfd	F	p
		Compartment	1,30	23.98	**< 0.0001**
		Compartment vs condition	1,30	0.04	0.83
		Compartment vs phase	1,30	0.04	0.83
		Compartment vs condition vs phase	1,30	0.18	0.67

dfn: degrees of freedom numerator; dfd: degrees of freedom denominator

interpretation of Exp. 1, the tunnel area was a passage compartment, and control and defeated mice revealed increased tunnel entries in both the Hab and ReE phases of the test.

The complementary analysis of risk assessment corroborated the lack of effect observed in spatiotemporal behaviors; that is, mice defeated in the SIB did not change their risk assessment during ReE in the IA compared to the control group or the Hab phase. Supporting the lack of effect, we highlight the increase in the frequency and time of grooming on the surface of both the control and defeated groups, suggesting that these animals did not show any level of avoidance that might lead them to perform grooming in a protected environment to attenuate it, as seen in Exp. 1. In this context, the grooming behavior displayed by the mice in Exp. 2 could be placed in a different dimension from the interpretation in Exp. 1, as it might indicate various other behaviors, such as those related to coitus, self-cleaning, and so on. In other words, because the second protocol did not induce context avoidance, as measured by spatiotemporal data, it is highly unlikely that these animals would display grooming as aversion-relieving behavior. Consistently, SAP and defensive occultation behaviors did not change, regardless of the group of animals or test phase, adding more evidence to support the lack of context avoidance in mice defeated elsewhere. SAP behavior is particularly important in the expression of anxiety, as it robustly indicates risk assessment and is closely related to spatiotemporal analysis (Rodgers and Johnson, 1995; Sorregotti, 2013; Sorregotti, 2018).

The last complementary measure analyzed in the second experiment was rearing behavior, which showed that both groups of mice increased their vertical exploration during the ReE compared to the Hab phase. This corroborated the lack of locomotor impairment observed in the spatiotemporal analysis, in which the mice showed no difference in entrance frequency to the compartments compared to the Hab and ReE phases. Moreover, it strengthened the correlation discussed in Exp. 1, in which animals that spent less time on the surface might also display less rearing behavior. In the context of Exp. 2, given that the mice spent more time on the surface area of the IA, one might expect an increase in their vertical exploration. Overall, Exp. 2 suggested that our protocol induced avoidance according to the context paired with the SDS and that the stress protocol itself was not sufficient to induce any effect, as assessed in the present study.

Recent CSDS work indicates that social avoidance can emerge through Pavlovian fear to social cues, such as the aggressor phenotype, with stimulus specificity and extinction (Lee et al., 2024). In contrast, our study highlights context as the central driver of associative learning: by pairing social defeat with a specific environment and re-exposing mice to the same apparatus, we reveal CSDS–induced context avoidance, expressed through reduced exploration of the defeat-paired surface, increased use of the protected compartment, and distinctive defensive behaviors. This place dependence distinguishes contextual memory of defeat from cue-directed social fear and fills a gap left by cue-focused CSDS models, offering a simple, ethologically grounded protocol to probe context-dependent learning. Importantly, we did not phenotype animals as stress-susceptible or resilient. Instead, we analyzed the group as a whole, a choice that better reflects the natural variability of human populations, where some individuals adapt successfully to stress while others do not. This approach strengthens the face and construct validity of our model and provides behavioral parameters that can be further explored in the context of social anxiety and related affective disorders.

5. Conclusion

The present results point to an unprecedented protocol that is effective in eliciting a social context-induced avoidance which might be implicated in social affective disorders.

CRediT authorship contribution statement

Diego Cardozo Mascarenhas: Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Data curation, Conceptualization. **Jeimmy Marion Penagos Gil:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nathalia Santos-Costa:** Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ricardo Luiz Nunes-de-Souza:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare no conflicts of interest related to the research or the manuscript.

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Data availability

Data will be made available on request.

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