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Effects on Behavior by Different Exposure Durations of Predator Scent Stress

Efectos Conductuales por Exposición a Diferentes Duraciones de Estrés por Olor de Depredador

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Abstract

Stress is conceptualized as a systemic response triggered by a stimulus potentially harmful to an organism. Instead of an adaptive outcome, life-threatening experiences may contribute to the development of anxiety disorders and depression. Predator scent stress (PSS) is one of the most utilized rodent models of stress-induced psychopathology, in which rodents are exposed to a volatile predator cue that signifies imminent danger. It is unclear if the duration of a life-threatening experience could have differential consequences on the expression of anxiety-like and depression-like behaviors. For this reason, the goal of this present study was to evaluate the effect of different exposure durations (3 min., 10 min., or 20 min.) to the scent of bobcat urine. Wistar rats housed under 12/12 dark cycle in standard laboratory conditions were exposed to the PSS model and 24 hrs. after the stressor, behavioral consequences were evaluated in the open field test, saccharin preference test, and forced swim test. The results obtained show that a 10-minute exposure is sufficient to induce an anxiety-like and a depression-like behavioral profile. We conclude that the time exposure could be a major variable to obtain clear and trustable results and to avoid overexposure to stressor.

Keywords: Stress, Predators Scent, Behavior, Depression, Anxiety, Rats

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Resumen

El estrés es una respuesta sistémica desencadenada por un estímulo potencialmente peligroso para el organismo. Esta respuesta permite al organismo adaptarse a la condición estresante, sin embargo, experiencias que amenazan a la vida pueden incrementar el riesgo de desarrollar trastornos de ansiedad y depresión. La exposición al olor de depredador (EOD) es el modelo animal de patología inducida por estrés más utilizado. Consta de la exposición a una pista olfativa que significa peligro inminente. Aún no está claro si la duración a una experiencia que amenaza la vida puede generar diferencias en la expresión conductas tipo-ansiedad o tipo-depresión. Por esta razón, el objetivo de este estudio fue evaluar el efecto de diferentes duraciones de exposición (3 min., 10 min. o 20 min.) al aroma de lince. Se utilizaron ratas hembra de la cepa Wistar en un ciclo luz oscuridad 12/12 en condiciones estándar de laboratorio, los sujetos fueron evaluados en la prueba de campo abierto, preferencia de sacarina y nado forzado 24 hrs. después de terminado el estresor. Los resultados indican que la exposición a 10 min. es suficiente para inducir el perfil conductual tipo-depresión y tipo-ansiedad. Concluimos que el tiempo de exposición puede ser una variable de mayor importancia para obtener resultados confiables y prevenir exposiciones innecesarias al estrés.

Palabras Clave: Estrés, Olor de Depredador, Conducta, Depresión, Ansiedad, Ratas

A traumatic event (TE) is defined by the DSM-5 as an exposure to threatened death, serious injury or sexual violence and can have detrimental consequences in mental and physical health (American Psychiatric Association, 2013). In an epidemiology study carried out in 24 countries demonstrates that TEs are rather common worldwide, given that 70% of respondents were exposed at least one TE during their lives (Benjet et al., 2016). Traumatic experiences are linked to the development of anxiety disorders (McMillan & Asmundson, 2016; Price & Van Stolk-Cooke, 2015) and depression symptoms, such as anhedonia (Byllesby et al., 2017; Fani et al., 2019; Wang, Xu, & Lu, 2019) and helplessness (Hammack, Matthew, & Lezak, 2012). The former is lived as an inability to experience pleasure and disinterest for normally rewarding experiences, while the later reflects the inability or unwillingness to cope with intense stress.

Predator Scent Stress (PSS) is one of the most utilized rodent models of stress-induced psychopathology, which consists of a life-threatening experience similar to human traumatic experiences (Cohen, Kozlovsky, Alona, Matar, & Joseph, 2012; Leong & Packard, 2014; Manjoch et al., 2016; Roltsch et al., 2014; Török, Sipos, Pivac, & Zelena, 2019; Zohar, Matar, Ifergane, Kaplan, & Cohen, 2008). As a general description, rodents are exposed to a volatile cue in an inescapable enclosure, such as feline urine, used cat litter, or trimethylthiazoline (chemical compound obtained from fox feces). From an evolutionary standpoint, prey animals have developed fast-acting mechanisms that recognize and respond accordingly to the presence of a predator (Dielenberg & McGregor, 2001). The instinctive (i.e. not dependent on experience) detection and response to volatile predator cues in rodents is mediated by specific neurons in the olfactory cortex that route information to the amygdalo-piriform transition area, which in turn activates neurons of the paraventricular hypothalamus nucleus that initiate the hormonal response to stressors (Kondoh et al., 2016). Furthermore, other brain structures related to stress response show to be involved in response to PSS; like amygdala (Butler et al., 2011), bed nucleus of the stria-terminalis (Xu et al., 2012), hippocampus (Cohen, Kozlovsky, Matar, Zohar, & Kaplan, 2011), and prefrontal cortex (Smith, Davis, Gehlert, & Nomikos, 2006).

PSS in laboratory rodents induces a robust and long-lasting expression (i.e. several days) of defensive behaviors and circulating corticosterone (Cohen et al., 2012; Dias Soares et al., 2003; Fenchel et al., 2015; Mayer, Matar, Kaplan, Zohar, & Cohen, 2014; Siviy, Steets, & DeBrouse, 2010; Staples & McGregor, 2006; Whitaker & Gilpin, 2015). Defensive beha-

viors are a set of responses to threatening stimuli that have evolved as a means to deal with potential harm (Blanchard & Blanchard, 2008) and an enduring expression of these behaviors in the absence of the original trigger is an indicator of anxiety (Lutz, Marsicano, Maldonado, & Hillard, 2015). Examples of defensive behavior include freezing (Hubbard et al., 2004), heightened startle (Rajbhandari, Baldo, & Bakshi, 2015) and avoidance of open places (Whitaker & Gilpin, 2015; Wu et al., 2019). Additionally, PSS model has been reported to induce anhedonia by lowering the preference to a sweet liquid (Neumann et al., 2011). However, the consequences on PSS on forced swim test, an animal model of learned helplessness (Yankelevitch-Yahav, Franko, Huly, & Doron, 2015) and consequences of life-treating stressors (Török et al., 2019) remains poorly explored.

It is well known that the magnitude of an organism's behavioral response to an acute stressor can be reduced with longer exposures (i.e. habituation) (Benini, Oliveira, Gomes-de-Souza, & Crestani, 2019; Ruehle, Rey, Remmers, & Lutz, 2012). The process of habituation keeps in check the impact of stressors by downregulating the behavioral and physiological facets of stress response and an impairment of habituation has been linked to development of depression and the severity post-traumatic stress disorder (Herman, 2013; Kim et al., 2019). However, behavioral habituation to a life-threatening experience has not been explored thoroughly and entails an important avenue to understanding the development of stress-induced psychopathology. To address this problem, independent groups of rats were exposed to different durations of bobcat (Lynx rufus) urine inside an inescapable enclosure and anxiety-like and depression-like behavioral consequences were assessed.

Method

Subjects

Animal procedures were approved by the ethical research committee of the Faculty of Psychology, UNAM (FPSI/422/CEIP/449/2018). Female Wistar rats with 3 months of age were used (N=26) and were housed in communal cages (5 subjects per cage) in standard laboratory conditions with a light-dark cycle of 12/12 hours, lights on at 8:00 a.m. Sample size was chosen based on literature in the field. Access to food and water was *ad libitum* for the duration of the experiment. Each subject was randomly assigned to an experimental group: control (n=6), 3 minutes (n=6), 10 (n=6), or 20 minutes (n=6). Two subjects from the control group were removed from the experiment due to technical problems.

Materials

The stressor used for this study was cat litter soaked with lynx urine (90 ml) and contained in a small plastic container with wholes (Bobcat Urine, PredatroPee®, Hermon, ME 04401 USA). An acrylic exposure chamber of 50x50x40 cm. with two compartments (25x50x40 cm.) was used. The wall dividing the two compartments was opaque, so it did not allow visual contact between the two subjects. An acrylic lid was placed on top of the exposure chamber to prevent the dispersion of the volatile cue. An acrylic cylinder (height of 40cm. with a diameter of 40 cm.) was used for forced swim test. The acrylic arena for open field test was 100x100x50 cm. and was divided into 16 quadrants of 4x4 cm. The four central quadrants were denominated as the central area and the rest as the peripheral area.

General procedure

All manipulations were done in the light phase between 12:00 p.m. and 14:00 p.m., unless otherwise stated. All subjects were handled 5 days prior to experimental procedures and were weighted every day for the entire duration of the experiment (including the 5 days of handling). One day later (24 hrs.) to the last day of handling, rats were subjected to the PSS protocol. The control group was not introduced to the exposure chamber and instead remained in their home cages. Behavioral assessment commenced

24 hours after PSS and test sessions were distributed in 8 days. The order of sequence for open field test (OFT) and saccharin preference test (SPT) randomized among all subjects, while forced swim test (FST) was fixed to the last position for all subjects to avoid carry-over effects due the stressful implications of the assay. Henceforth, subjects could be assigned to two possible sequences: OFT-SPT-FST or SPT-OFT-FST.

PSS protocol

The exposure chamber was located in a separate room to where behavior was later assessed. The apparatus was sanitized before each exposure to avoid any contaminating odor cues. The small plastic container with the bobcat urine was randomly placed in one of the four corners in the exposure chamber for each animal. Each animal was placed in the center of the exposure chamber immediately after opening the lid of the small plastic container and were left there for 5, 10 or 20 minutes.

Open field test

Subjects were placed at the center of the open field arena for five minutes and behavior was recorded using an overhead camera. A cross into a quadrant was defined as corporal entry of more than 50%. Videos were scored offline by trained observers. The anxiety-like behaviors scored included: duration of permanence (seconds) in the central area, total number of crosses, and the duration of immobility and grooming (Kalueff et al., 2015; Prut & Belzung, 2003).

Forced swim test

This behavioral assessment is sensitive to depression-like behavior and has been shown to be responsive to treatment with anti-depressive drugs. The first day of habituation, subjects were placed in the water filled cylinder for 15 minutes. On the second day, subjects were placed in the water filled cylinder for 5 minutes and the session was recorded. Water temperature was maintained in a range of 23 - 26 C°. Videos of the second session were scored offline by trained observers. Immobility was defined as the minimal movements require to stay afloat with the head above water (Yankelevitch-Yahav et al., 2015).

Saccharin preference test

This behavioral assessment is sensitive to anhedonia induced by stress. Subjects were housed individually for the duration of the test (5 days). The first day at 18:00 hours, rats were permitted access to a single bottle of water (250 ml). Twenty-four hours later (18:00, day 2), basal water consumption was measured, and the bottle was replaced with another containing saccharin diluted in water (250 ml). Twenty-four hours later (18:00, day 3) basal saccharin consumption was measured. Also, at 19:00 hours animals were allowed access to two bottles containing water (250 ml. each) for an hour and at 20:00 consumption was measured to determine any preference (left or right bottle). For the next twenty-four hours, subjects were deprived of water and at 20:00 hours of the fourth day two bottles were placed (one with water and another with saccharin diluted in water). On the fifth day at 20:00, water and saccharin consumption were measured.

Statistical analysis.

SigmaPlot© for Windows Version 11 was used to elaborate all graphs and perform the statistical analysis. Results from OFT were analyzed with an one-way ANOVA; results of liquid consumption from SPT were analyzed with a two-way ANOVA for Factor Group (CON, 3MIN, 10MIN, & 20MIN) and Factor Liquid (water & saccharin); results of immobility duration presented in time bins were analyzed with a two-way ANOVA for repeated measures for Factor Group and Time bin (1,2,3,4, & 5). The post-hoc Tukey test was used to evaluate interactions in all analyses stated above.

Results

Open field test

The variance analysis of duration in the central area reported an effect by Group $F(3,20) = 7.107$, $p < 0.01$. Subjects with a PSS exposure of 10-min. and 20-min. spent significantly less time in the central area and this measurement did not differ between these two experimental groups (see Figure 1A). The 3 min. group did not differ from the control group. Furthermore, no effect on locomotion (as number of crosses) was observed among all groups (Figure 1B.). The decrease in the time spent in the central area and the lack of effect in locomotion observed in the 10-min. and 20-min. indicates an avoidance of a high-risk area that is characteristic of an anxiety-like phenotype previously described by other authors (Prut & Belzung, 2003). Furthermore, the impact of 10 and 20 mins. is statistically the same, hence it appears that just 10 minutes of exposure is needed to induce anxiety. Immobility $F(3,20) = 1.836$, $p > 0.05$ and grooming $F(3,20) = 2.057$, $p > 0.05$ behavior duration were not affected by the stressor (see Figures 1C and 1D, respectively).

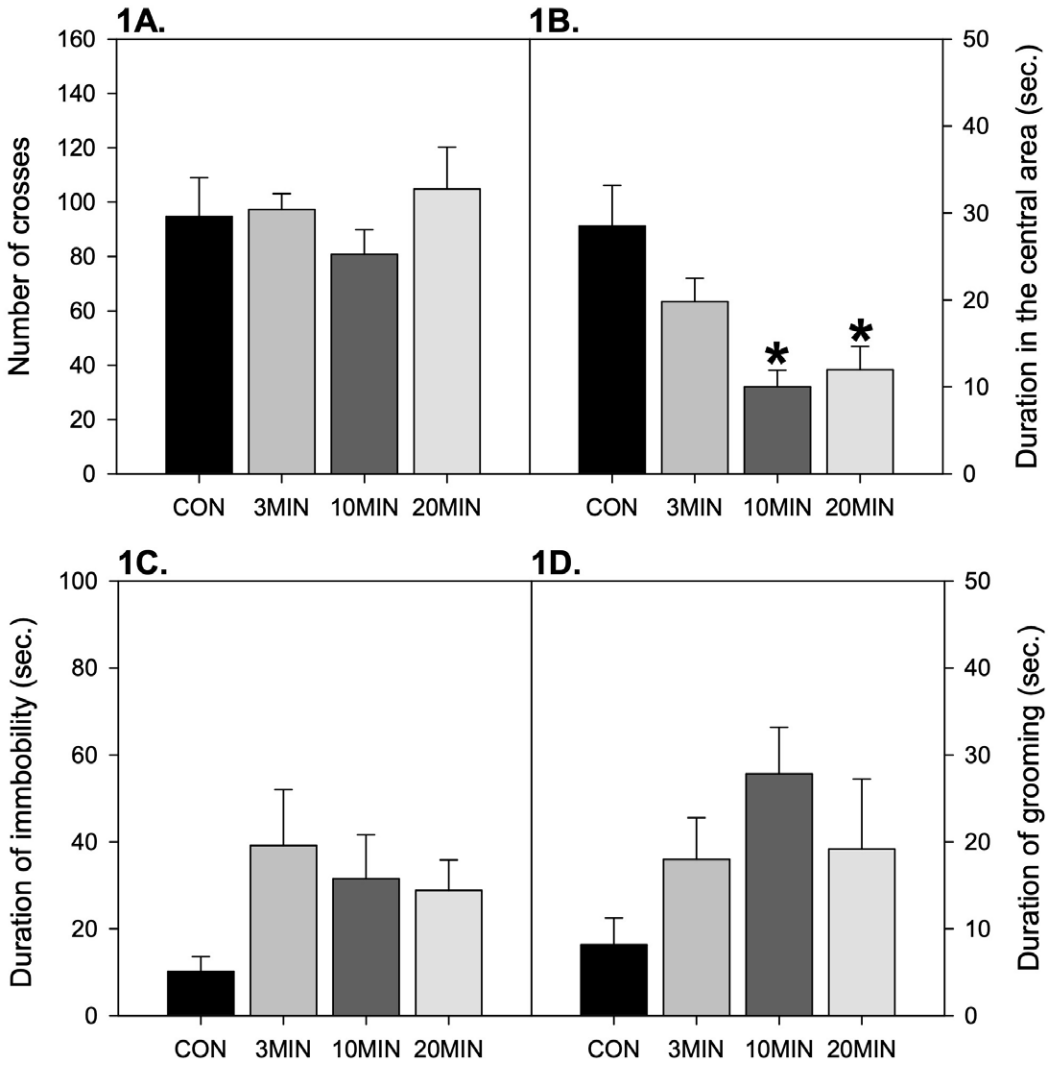

Figure 1. Open Field Test

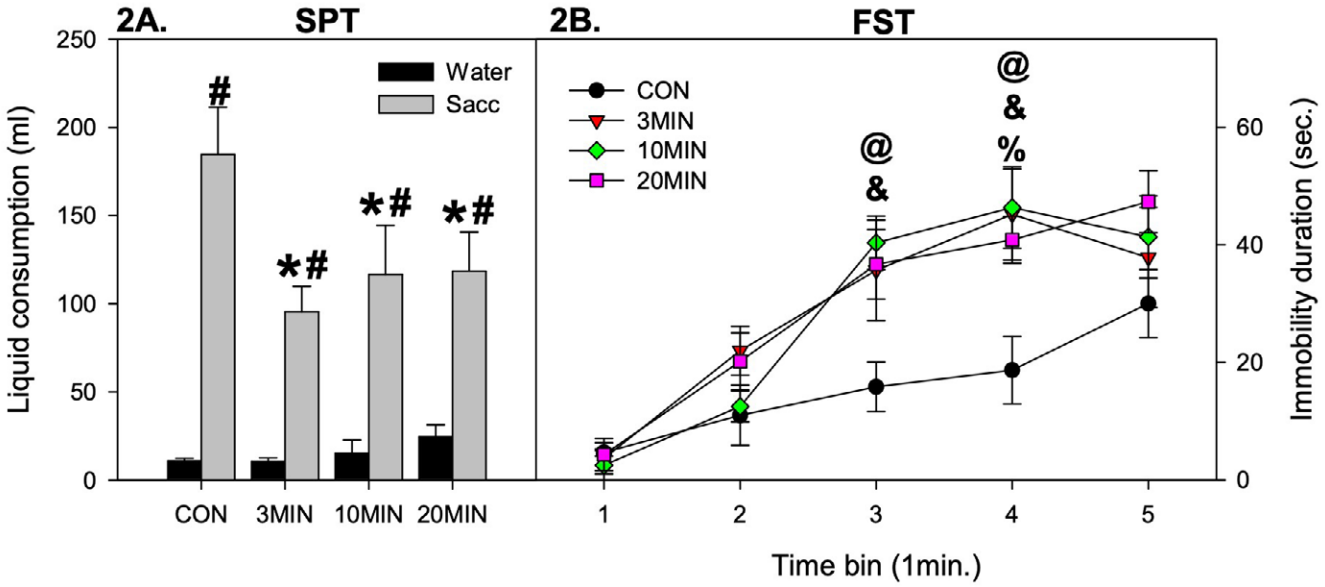

Figure 2. SPT and FST results. Effects of PSS on anhedonia and helplessness as depression-like behaviors. (2A) presents data of liquid consumption (saccharin and water) from the SPT. (2B) presents data of immobility duration throughout the duration of the test session of FST. (n= 6 per group; ANOVA two ways 2A and ANOVA two ways repeated measures 2B; Tukey method; *p<0.05, vs. CON; #p<0.05, vs. water consumption within group; %p<0.05, 3MIN vs. CON; & p<0.05, 10MIN vs. CON; @p<0.05, 20MIN vs. CON).

Saccharin Preference Test

Saccharin preference was present in all groups, given that all groups consumed statistically more saccharin than water $F(1,40) = 89.664$, $p < 0.001$. The statistical analysis of fluid consumption reported an interaction Group X Liquid $F(3,40)= 2.885$, $p > 0.05$ and the post-hoc analysis revealed that all three groups exposed to PSS had a lower saccharin consumption against the control group ($p < 0.05$). Water consumption did not differ between all groups, therefore PSS exposure affected specifically saccharin consumption independent of exposure duration (see Figure 2A).

Forced swim test

As in SPT, PSS exposure also seems to affect another depression-like marker regardless of duration. There was an effect by factor Group $F(4,80)= 3.363$, $p > 0.05$ and an interaction between factors $F(4,80)= 1.938$, $p > 0.05$, where groups 3-min., 10-min., and 20-min. show a greater duration of immobility in time bin 4 against the control group ($p < 0.05$). In time bin 3, only the groups 10-min. and 20-min. differed from the control group ($p < 0.05$). On the other hand, immobility increases as a factor of time $F(4,80)= 45.916$, $p < 0.001$, which is indicative of an accumulation of fatigue. The control group shows a clear gradual increase, where measurements from the last time bin is statistically higher to the measurements from bin 1 and bin 2 ($p < 0.05$). Considering that the onset of increased immobility was sooner in the stressed groups than the control group, we suggest that these results indicate a depression-like phenotype consistent with the results of SFT.

Discussion and Conclusion

PSS is a frequently used animal model that has several attractive characteristics for biomedical research (Cohen et al., 2012; Daskalakis, Yehuda, & Diamond, 2013). However, it is unknown if the temporal aspect of PSS could have distinct consequences in anxiety-like and depression-like behavior. Previous studies have reported that PSS of 10-min. is capable of inducing anxiety-like behaviors (e.g. avoidance) (Cohen et al., 2012; Hubbard et al., 2004; Mackenzie,

Nalivaiko, Beig, Day, & Walker, 2010; Manjoch et al., 2016)the diagnosis of PTSD is made only if an individual exhibits a certain number of symptoms from each of three quite well defined symptom clusters over a certain period of time. Animal behavioral studies, however, have generally tended to overlook this aspect and have commonly regarded the entire group of animals subjected to certain study conditions as homogeneous. Thus, in an attempt to develop animal models of long-term chronic behavioral responses to stress (i.e. PTSD, but it unclear if lower and higher durations have different effects.

In this current study we report that the exposure duration is a relevant dimension of PSS. Generally. the subjects exposed to 3 minutes did not show an anxiety-like profile, while exposure durations of 10 and 20 minutes induced undistinguishable anxiety-like and depression-like profiles. Henceforth, the possibility that longer durations of exposure could habituate the behavioral response was discarded. Considering that there was no difference between the 10- and 20-min. groups on time spent in the central area, we suggest a possible ceiling-effect induced by the stressor, i.e. a higher duration of PSS beyond 10 minutes does not have a further effect.

Consistent with the pattern observed in the OFT, the 10- and 20-min. groups induced greater duration of immobility in the FST before the 3-min. group that was distinguishable from the accumulation of fatigue. We suggest that a 10- and 20-minute exposures have a more robust effect on learned helplessness than a 3-min. exposure. The average consumption of saccharin was lower in all groups exposed to PSS regardless of the duration, which indicates that the reinforcing value of saccharin is the most vulnerable to the stressor. Taking together the results observed in the FST and SPT, it is suggested that only 10-min and 20-min exposures induce a consistent depression-like profile.

The magnitude and duration of defensive behaviors are determined by environmental risk evaluation (Lima & Dill, 1990). Consequently, prey animals display threat-sensitive responses to predator odors and the intensity of these responses correlates positively with the perceived level of risk based on the information contained in the chemical cues (Blanchard & Blanchard, 2008). PSS induces glucocorticoid release, which increase the metabolic rate that prepares animals for the fight-or-flight response (Lima & Dill, 1990; Torres-Carrillo et al., 2018). This response involves the hypothalamus-pituitary-adrenal axis and extra-hypothalamic areas, such as the amygdala, which disrupt the flow of information to the hippocampus and prefrontal cortex (Sanchez-Castillo et al., 2015).

Further experiments are needed to evaluate duration of the stressor on physiological markers. In another set of experiments from our laboratory, we measured the corticosterone levels of female Wistar rats 30 minutes after an exposure of PSS of 10 minutes and found an increase of circulating corticosterone (Torres-Carrillo et al., 2018). Rather counterintuitive, it is known that dampening the corticosterone release to stressors can have detrimental long-term effects on anxiety-like and depression-like behavior in rodents (Cohen et al., 2006; Sotnikov et al., 2014) and leads to the development of psychopathology in humans (Ayer et al., 2013; Melhem et al., 2017)it is not clear whether such dysregulation exists prior to or is a consequence of attempt. Studies also show an activation of inflammatory responses in suicidal behavior but often combine attempters with those with ideation. Methods The sample consisted of psychiatric inpatients, aged 15–30 years, admitted for suicide attempt (SA, n = 38. It has been demonstrated that corticosterone participates in negative feedback mechanism that terminate stress response and mitigate the behavioral consequences (Atsak et al., 2018; Di, Malcher-Lopes, Halmos, & Tasker, 2003). In a study in which rats were administered with Δ9-THC (an active component in marihuana) after an exposure to PSS, corticosterone release was damped, and subjects showed a long-lasting anxiety-like profile (Mayer et al., 2014). We hypothesize that exposure durations above 10 minutes exhaust corticosterone before the termination of the stressor and therefore the stress response endures to have a greater toll on the individual.

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