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Summary
The Behavioral Effects of Social Buffering in Rats

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Humans require social bonds to fulfill their higher-order needs as social beings. The most striking psychological effect of social bonds is the capability of decreasing acute psychological stress—a mental and/or emotional load or tension occurring during negative conditions (Holt-Lunstad, Smith & Layton, 2010; Kikusui, Winslow & Mori, 2006). This overall positive impact on mood is defined as *social buffering* (Cohen & Wills, 1985).

A particularly important (i.e. critical for survival) and well-studied effect of social buffering in animal models is strengthening the so-called *defensive behaviors* under life-threatening conditions or suppressing the opposite action, *risk-taking behavior*. This vital behavioral effect is likely related to the anxiolytic properties of social buffering. It is well-known that under stressful situations, social buffering directly modulates anxiety-related autonomic and physiological responses (Kiyokawa et al., 2004, 2007). On the other hand, the relationship between these autonomic features and the aforementioned complex behavioral effects of social buffering remains to be elucidated.

Psychological stress is divided into two as *chronic* and *acute*, depending on its occurrence frequency. These two types of stress can have different consequences on various behavioral paradigms. The differential behavioral and physiological effects of acute and chronic stress have been widely studied in animal models (Campos, Fogaca, Aguiar & Guimaraes, 2013; Donovan, Liu & Wang, 2018; Eşsizoğlu, Yıldırım, Mengi, Oral & Yurdakoş, 2009; Harris, 2015; Katz, Roth & Carroll, 1981; Lowy, Wittenberg & Yamamoto, 1995; Rai, Bhatia, Sen & Palit, 2003; Solomonow & Tasker, 2015; Suvrathan, Tomar & Chattarji, 2010; Takatsu & ark., 2013; Ueyama, Kawai, Nemoto, Sekimoto, Toné & Senba, 1997).

Given the variability in behavioral effects of different types of psychological stress, many preventive and therapeutic applications work under only a limited number of stressful situations. Social buffering is an exception, as it consistently decreases the negative emotional effects of many different forms of stress, whether acute or chronic. This prevalent positive psychological effect of social buffering is widely investigated in humans (Uchino, 2006) and other animals (Hennessy, Kaiser & Sachser, 2009; Hostinar, Sullivan & Gunnar, 2014; Hostinar & Gunnar, 2015; Kiyokawa & Hennessy, 2017) in natural settings as well as controlled laboratory environments.

This study investigates the behavioral effects of social buffering in rats by evaluating two critical phenomena for survival, namely risk-taking behavior and anxiety. Animals that have been provided with conditions for social support and control animals receiving no social support were compared for risk taking behavior in the Multivariate Concentric Square Field (MCSF; Augustsson & Meyerson, 2004) and anxiety-like behavior in the Elevated Plus Maze (EPM).

Methods

All experimental procedures were carried out following to the rules and regulations of the Boğaziçi University Institutional Ethics Committee for the Local Use of Animals in Experiments (BÜHADYEK).

Subjects

A total of 32 experimentally-naïve, adult, male Wistar rats (>3 months old; 300–380 g) were used. Animals were group-housed in standard rat cages on a 12:12 L/D cycle (Lights on: 8:00–20:00) at 21–22 ºC with ≈%55 humidity. Food and water were provided *ad libitum* throughout the experiment.

Experimental Groups

Animals from different home cages were assigned to experimental groups based on their starting weight prior to experiments. The experimental group received social buffering (center-starter pairs), whereas the controls (bridge-starters) did not. The animals were divided into

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subgroups based on their starting point in the MCSF (the center or the bridges), being alone or in pairs, and being exposed to acute stress or not (n = 4 per subgroup; Table 1).

Apparatus

Multivariate Concentric Square Field. The MCSF is a square field (100 x 100 x 40 cm) utilized to measure exploration, risk-taking, shelter-seeking and general mobility levels in rats (Meyerson et al., 2006). It consists of two equally illuminated (60 ± 10 lx) bridges, a hurdle, four dark boxes, slopes, corridors, an intensively illuminated confined space (500 ± 20 lx), and a neutral center compartment (Figure 1). Risk-taking behavior is assessed based on the time spent in the *risky zones*, such as the bridges. The time spent in the *safe zones,* like the dark boxes, defines shelter-seeking behavior, and the center compartment and corridors compose the *neutral zones*. In paired groups, the *physical interaction* denotes the total time when there is 1 cm or less between the two animals.

Mild Acute Stress Induction. A short-duration (30 min) immobilization stress was induced (Thai, Zhang & Howland, 2013) using a plexiglass cylinder (stress tube; diameter: 8cm; length: 20 cm). Paired-stress animals were placed into the stress tubes at the same time. Non-stress groups were transferred to a new cage and spent the same amount of time there.

Elevated Plus Maze. EPM is a widely used rodent anxiety paradigm (Aykaç et al., 2015). It consists of two open and two closed arms (arm length: 50 cm, arm width: 10 cm). Each animal is placed into the maze for 10 min from the center, and the time spent in open and closed arms is recorded. The time spent in closed arms (65 ± 5 lx), which are substantially darker than the open arms (165 ± 5 lx), defines anxiety-related behavior.

Procedure

Following acute stress induction for 30 min (non-stress groups spent the same amount of time in a standard cage), each rat was taken to its cage for 2 min and then placed into the MCSF for 20 min. Half of the rats started from the center compartment (n = 16), while the other half started from the bridges (n = 16). Rats were tested in the EPM 20 ± 5 min after the end of their MCSF session. Immobilization stress induction, the MSCF and EPM tests were carried out in different rooms.

Results

Mobility in the MCSF was rated by three blind observers with good interrater reliability (Cronbach's α = .87). The mean and standard deviation of the dependent variables used in statistical analyses are summarized in Table 2. One-way ANOVA results showed that there was no difference among single stress, paired stress, single non-stress, and paired non-stress groups, $F(3, 28) = .50$, $p < .68$. Thus, the immobilization stress did not influence general mobility scores.

No mobility difference was found after applying independent samples t test between the rats starting the MCSF from the center compartment (M = 48.87, SD = 30.47) and the bridges (M = 74.09, SD = 43.91), $t(30) = -1.92$, $p < .06$. In contrast, independent samples Mann-Whitney U test showed that the center-starters mostly spent their time in the safe (U = 59) and neutral zones (U = 38.5), while the bridge-starters mostly remained in risky zones (U = 248, $p < .01$; Figure 2). This difference did not change between paired non-stress and stress groups (for all U = 16, n = 4, $p < .05$). However, rats in the single-stress group spent similar time in different zones irrespective of their starting point (U = 14, n = 4, $p > .05$).

When physical interaction was compared for paired groups with independent samples t test, we found that the center-starters had significantly more interaction time than the bridge-starters, $t(16) = 3.29$, $p < .01$ (Figure 3). It should be noted that bridge-starters were placed into the maze from separate compartments (bridges), and accordingly, they could completely avoid being in the center compartment of the MCSF at the same time.

Utilizing this difference, we have then compared the anxiety levels of the groups in the EPM. We found that the time spent in open arms was inversely correlated to the time spent in risky zone in the MCSF according to the Pearson correlation (r = .365, $p < .04$). We found no anxiety difference between center-starter stress and non-stress groups. However, an independent samples Mann-Whitney U test showed that acute stress animals who started the MCSF from the bridges with limited physical interaction (M = 35.25, S = 29.05) showed significantly more anxiety-related behavior compared to the non-stress group (M = 431.75, S = 218.73), (U = 60, n = 16, $p < .02$; Figure 4). This difference, led by acute stress, was abolished for center-starters that had substantially more physical interaction (U = 20.5, n = 16, $p < .23$; independent samples Mann-Whitney U test), indicating the anxiolytic effect of social buffering.

Discussion

We showed that acute stress induction did not alter overall mobility levels of the animals, ensuring the validity of risk-taking behavior and anxiety measures. The MCSF is an animal model used to provide social buffering and measure risk-taking behavior. We found that the starting location in this model is a critical factor

for determining the time spent in different zones: animals starting from the bridges spent more time in risky areas; whereas the center-starters spent more time in safe and neutral areas. It should be noted that the paired bridge-starter rats were placed into the maze from different bridges and, thus, did not see each other at the first moment. This is likely the main reason for the limited physical interaction observed in this group.

Physical interaction is a common way of communication among rodents, but not the only one. It is well-known that rats can communicate via ultrasonic vocalization within a 30 cm distance (Smith, 1979). In our experiments, the distance between the starting spots on the two bridges was about 80 cm, ruling out this type of initial interaction. This led to a major difference between the center-starters and bridge-starters. The anxiogenic effect of acute stress was not observed in the paired center-starter rats, which had significantly more physical interaction than the isolated bridge-starter rats, indicating that short-term (20 min) social buffering can have a powerful anxiolytic effect.

Social environments of humans have a key role in the progress of depressive disorders (Hammen, Shih & Brennan, 2004; Sheeber, Hops & Davis, 2001). Social support provided by partners, children and people who have a close relationship with the support receiving individual is shown to be effective against clinical depression (Liu, Gou & Zuo, 2016). Pregnant women who are socially supported has a smoother labor with less frequency of postpartum depression (Collins, Dunkel-Schetter, Lobel & Scrimshaw, 1993). Under stress, social support may prevent development of various mental disorders and provide resistance to major depression (Dalgard, Bjork & Tambs, 1995). Social support was found to be negatively correlated with severity of the PTSD symptoms and positively correlated with negative social relations (Dirkzwager, Bramsen & van der Ploeg, 2003). These studies indicate that the acute anxiolytic effect observed in our study would even be greater if we assigned the paired rats from the same home cage.

Social buffering in humans is often studied and utilized as a preventive measure. Findings of this controlled animal study, on the contrary, indicate that the anxiolytic effects of social buffering can emerge much faster than observed in most human studies and thus, can be utilized for acute care. The next step would be to implement similar short-term social buffering conditions in clinical populations to investigate their fast-onset therapeutic effects.