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Title: SOCIAL INSTABILITY IN FEMALE RODENTS AS A MODEL OF STRESS RELATED DISORDERS: A SYSTEMATIC REVIEW

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SOCIAL INSTABILITY IN FEMALE RODENTS AS A MODEL OF STRESS RELATED DISORDERS: A SYSTEMATIC REVIEW

ABSTRACT (250 WORDS)

The risk of developing stress related disorders such as depression is two times higher in women than in men, and social stress is considered the principal etiology for this disorder. Social defeat animal model is the most common procedure to induce social stress in male rodents, but the stressful stimulus and the stress response can be different for each sex. In this regard, social defeat stress model does not fit the social nature of females, and the emerging evidence indicate that the social instability stress (SIS) model could be a suitable model to investigate this stress related disorder in females. This study aims to systematically review the effects of SIS on physiological and behavioral parameters involved in the pathophysiology of depression. A systematic review was undertaken following PRISMA method on PubMed, Medline and Web of Science. Sixteen studies met the inclusion criteria. The reported physiological measures comprised the hypothalamic-pituitary-adrenal axis activity, neurotrophic factors, immune and monoaminergic systems, vasopressin and oxytocin receptors, sex hormone levels and estrus cycle, while main behavioral measures involved sucrose preference test, forced swimming test, elevated plus maze, open field test and social interaction. Although several works found HPA axis hyperactivity and disrupted reward system, the methodological variability lead to different biological and behavioral results among studies.

MAX 5 KEY WORDS

Social instability stress; female; rodent; systematic review

INTRODUCTION

Depression is the leading cause of disability worldwide (World Health Organization, 2017). It presents high comorbidity with anxiety-related disorders (Kennedy, 2008; Kessler et al., 2005) and its prevalence is approximately two times higher in women than in men (Bekker and van Mens-Verhulst, 2007; Kessler, 2003). A consistent finding is that repeated social stress is the most common etiological factor in the precipitation of depression in humans (Kessler, 1997). Therefore, animal models involving chronic social stress have been widely used to study depressive-like disorder. In particular, social defeat is a commonly used model of social stress in

male rodents, and it is based on the resident-intruder paradigm, where subjects interact aggressively to establish dominance over the territory (Miczek, 1979). This model is considered ethologically appropriate since, according to the "social competition hypothesis", loss of rank and resources can lead to physiological and behavioral changes associated with depressive mood and anxiety (Price et al., 1994; Rohde, 2001; Sloman et al., 2003). Social defeat studies have, in fact, provided a better understanding of the underlying mechanisms of stress related disorders, as well as allowing for the testing of pharmacological treatments in males (Chaouloff, 2013; Keeney et al., 2006; Kudryavtseva et al., 1991; Levinstein and Samuels, 2014; Slattery and Cryan, 2017). However, three factors have been underestimated. First, women show a higher frequency of depression (Bekker and van Mens-Verhulst, 2007; Kessler, 2003). Second, there are evidence of animal and human studies of biological sex differences in the stress response and mechanisms involved in depression (Hughes, 2007; Labaka et al., 2018), such as the hypothalamic–pituitary–adrenal axis (HPA), serotonergic system, the inflammatory response and neurotrophic factors among others (Dalla et al., 2010; Hodes et al., 2017; Pitychoutis and Papadopoulou-Daifoti, 2010). Finally, adverse effects of various drugs are more common or severe in women than in men (Rogers and Ballantyne, 2008). Despite these evidences, females have been largely omitted as experimental subjects in neuroscience and a remarkable male bias characterizes many animal models of human diseases and traits (Beery and Zucker, 2011; Blanchard, 1995; Zucker and Beery, 2010).

One of the causes of this underrepresentation is the incorrect assumption that females are intrinsically more variables than males due solely to the reproductive cycle (Prendergast et al., 2014). In this regard, the majority of the works realized in females have used ovariectomized subjects, but the results obtained might be biased, given that the physiological and behavioral response to social stress differs in ovariectomized rodents (Al-Rahbi et al., 2013a; Al-Rahbi et al.,2013b). Applicable female studies are required in order to clarify this serious lack, since generalizations between sexes may not be valid. In this regard, some studies have applied the social defeat model in females, but what may be stressful for one sex is not necessarily stressful for the other, and this model appears to be less useful for evoking the stress-response in female rodents (Haller et al.; 1999; Palanza, 2001), who do not naturally exhibit territorial aggression unless they are defending their litters (Solomon, 2017). Although they also have a repertoire of moderate agonistic behaviors aimed at establishing a dominance hierarchy (e.g., chasing, pinning down, aggressively grooming and barbering) (Allen et al., 2010; Bartolomucci et al., 2005; Clipperton-Allen et al., 2011; Garner et al., 2004), laboratory female mice benefit from the "tend-and-befriend" strategy when coping with stress (Taylor et al., 2000; Vegas et al., 2012).

Moreover, females appear to be more resilient to the stress procedures usually applied in males (Kokras and Dalla, 2014; Palanza, 2001). Thus, taking into account the social nature of females, the disruption of the social network may be a more valid social stressor for this population. Haller et al. (1999) proposed for the first time the social instability stress (SIS) model in rats. This model consists of alternating isolation and crowding phases as well as membership rotation for crowding phases. SIS disrupts the social network stablished by females, and forces them to build a new hierarchical rank in each of the crowding phases. In this first work conducted by Haller et al. (1999), changes in the HPA axis (result that has also been confirmed in later studies) and depressive-like behavior were found (Haller et al., 1999; Herzog et al., 2009; Jarcho et al., 2016; Labaka et al., 2017; Schmidt et al., 2010). However, not all studies involving social instability have generated the same results, and the data appear to be inconclusive (Palanza and Parmigiani, 2017; Saavedra-Rodríguez and Feig, 2013). This is possibly due to the fact that the model is not yet sufficiently established and there are essential differences in the methodology that is employed, along with the measured variables.

This study aims to systematically review the current state of the findings obtained with the social instability stress model in female rodents when studying depressive and anxiety-like behavioral and physiological changes.

METHODS

An extensive systematic search of the published literature about the social instability model of stress in female mice or rats was conducted in PubMed, Medline and Web of Science databases, from inception up until December 19, 2017. We used the Preferred Reporting Items for Reviews and Meta-Analyses (PRISMA) flow sheet and checklist to ensure complete reporting of the evidence-based minimum reporting items (Liberati et al., 2009). Our search terms comprised the following key word combinations: social instability AND female. Articles selected for review met the following criteria: (1) used social instability as a model of stress; (2) included adult female rats or mice; and (3) published in English or Spanish. We excluded works that (1) studied other animals than mice or rats; (2) used a different animal model than SIS; (3) used ovariectomized subjects; and (4) focused on pharmacological interventions. Full-text not available and reviews were also excluded.

We identified 346 articles in our initial database search (Figure 1). All of these were imported into Mendeley Reference Manager to identify and remove duplicates (183 articles). There were 135 articles that were identified as clearly non-relevant, based on titles and abstracts revised, and were excluded because did not use social instability model (n = 18), were human

studies (n=39), included other animal models (n = 16), did not use female subjects (n = 34) or used pup subjects (n = 10), published in other language (n=2), were reviews (n=11), or full-text were not available (n=5). Then, 28 potentially relevant full-text articles were assessed for eligibility criteria, although 12 were excluded since they evaluated pharmacological interventions (n =2), used ovariectomized subjects (n = 6), did not use social instability model (n = 1), did not have control group (n = 1), was retracted (n = 1) or was a conference paper (n = 1). Finally, the authors reviewed each of the 16 full-text articles that met the study criteria. Data extracted from the eligible articles included author and year of publication, sample characteristics, social instability model information, and biological and behavioral findings (Table 1).

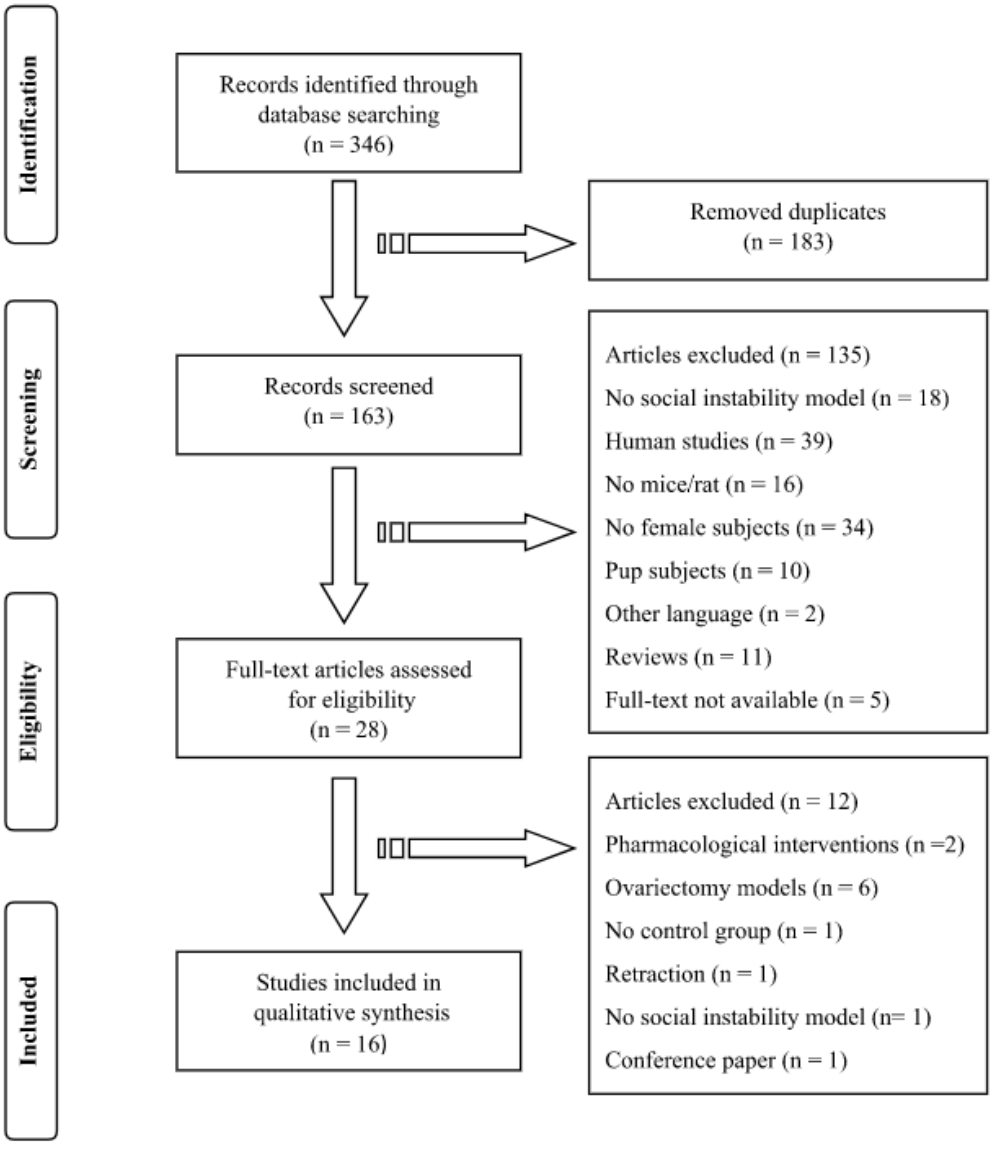

Figure 1. PRISMA flowchart of study selection.

RESULTS

Sixteen articles met the inclusion criteria (Table 1). These studies were published between 2004 and 2017. The sample sizes reported in the studies ranged from 22 to 93 subjects. The age of the rodents at the beginning of the stress period ranged from 27 to 84 days, although some studies did not specify the age of the subjects, and the stress periods varied from two to seven weeks.

Behavioral results

Most of studies screened different behavioral tests in order to analyze depressive-like behavior (Dadomo et al., 2017; Herzog et al., 2009; Labaka et al., 2017; Nowacka-Chmielewska et al., 2017c; Nowacka et al., 2014; Pittet et al., 2017), anxiety-like behavior (Baranyi et al., 2005; Dadomo et al., 2017; Jarcho et al., 2016; Nowacka-Chmielewska et al., 2017a; Nowacka-Chmielewska et al., 2017c; Saavedra-Rodríguez and Feig, 2013), sociability (Baranyi et al., 2005; Haller et al., 2004; McCormick et al.,2007; Pittet et al., 2017; Saavedra-Rodríguez and Feig, 2013), aggressiveness (Haller et al., 2004; Pittet et al., 2017) cognition and memory (McCormick et al.,2013; McCormick et al., 2010; Saavedra-Rodríguez and Feig, 2013).

The Sucrose Preference Test (SPT) and the Forced Swim Test (FST) are widely used models to analyze the depressive-like behavior, since they inform about two of the main depressive-like symptoms, anhedonia and locomotion activity, respectively. Dadomo et al. (2017) and Labaka et al. (2017) observed that stressed rodents showed a lower sucrose preference than controls, indicative of anhedonia. However, other studies did not find any difference between the two groups (Nowacka et al., 2014; Pittet et al., 2017) and Nowacka et al. (2017c) obtained the opposite results, observing that SIS rats showed a higher preference for sucrose. Regarding the FST, Labaka et al. (2017) found higher total duration and frequency of climbing, nevertheless, they did not found any difference in swimming and immobility behaviors. Similarly, Herzog et al. (2009) did not observe differences in floating time and latency between stressed and control rats.

The Elevated Plus-Maze (EPM), the Open Field Test (OFT) and the Social Interaction Test (SIT) are commonly used tools for the analysis of the anxiety-like behavior in rodents. Different results have been observed for its test. Nowacka et al. (2017b) found a decrease in the time spent in the open arms, in the entries to this open spaces and in the head dip frequency. Furthermore, Saavedra-Rodriguez and Feig (2013) showed that even 2 months after the SIS procedure was carried out, stressed mice spent less time in the open arms. However, Dadomo

et al. (2017) observed the opposite results: stressed mice spent more time in the open arms, less in the center of the EPM and showed a higher locomotor activity. Other studies did not find any difference in the time spent in the open arms (Baranyi et al., 2005; Jarcho et al., 2016). Regarding to the OFT, some authors found that stressed subjects showed a lower spontaneous locomotion, exploration, rearing and grooming (Nowacka-Chmielewska et al., 2017b; Nowacka-Chmielewska et al., 2017c) and spent less time in the center of the open field (Nowacka-Chmielewska et al., 2017c) and in the bright area (Dadomo et al., 2017). Similar to the EPM, Saavedra-Rodriguez and Feig (2013) showed a lower locomotor adaptation in the OFT two months after SIS exposure. Nevertheless, Jarcho et al., (2016), observed that stressed and non-stressed rodents spent the same amount of time in the center and periphery of the open field and Dadomo et al. (2017) did not find differences in the latency to enter the open field, time expended exploring the arena, time in the center area and locomotor activity. In the SIT, higher agonistic behavior and a lower social interaction was observed in stressed rats (Baranyi et al., 2005; Haller et al., 2004), and even 2 months later, stressed subjects spent less time interacting than the controls (Saavedra-Rodríguez and Feig, 2013). However, no differences were observed in the duration of resting, grooming and exploration (Baranyi et al., 2005) and no differences in the time spent proximal to the stimulus animal and in olfactory investigation of the stimulus during the approach test (Pittet et al., 2017). This study also assessed the social interaction and found that stressed subjects spend more time in social exploration (Pittet et al., 2017). Similarly, higher levels of social and non-social activity and lower levels of social inactivity were found in SIS mice soon after animals return from isolation (McCormick et al., 2007). The aggressiveness during the SIT was also analyzed and results differ among studies. Haller et al. (2004) observed higher duration of aggressive interactions (more bite attacks and dominant posture) in stressed rodents whereas lower levels of aggression were found in the study conducted by Pittet et al. (2017). In addition, Labaka et al. (2017) measured the whisking behavior, a type of hetero-barbering included in the repertory of social interaction behaviors that could indicate anxiety-like behavior. They observed that 83% of the stressed subjects showed shortened whiskers, whereas all the whiskers were intact in the control group.

Social Novelty Test is used to analyze the effects of stress in cognition and memory. Saavedra-Rodríguez and Feig (2013) observed that stressed mice performed worse in the Social Novelty Test than controls, since SIS mice spent less time with the unfamiliar mouse than with the already explored mouse. Similarly, adult rats which were subjected to stress procedure during adolescent spent less time investigating the novel object in the 1h inter-trial interval

comparing to controls, however, no differences were found when testing in adolescents (McCormick et al., 2010).

The fear conditioning is a useful model for investigating the neural circuitry underlying anxiety and contextual memory. McCormick et al. (2013) applied this procedure in adult rats after exposure to stressors in adolescence. Rats exposed to adolescent social instability stress froze more during the presentation of tone and in the inter-tone intervals during the cue extinction trials when it was carried out soon after the stress procedure. However, they did not differ on the memory for extinction of cue when tested two days later and neither other differences were found when the SIS was conducted in adulthood. In other words, stressed female rats show a reduction in memory only when tested soon after the stress exposures and there may be a higher sensitivity to this stress procedure in adolescence compared to in adulthood.

Biological results

We addressed the significant effects of social instability in nearly 45 biological parameters examined in each study (Table 1). Some of them focused on HPA axis variables (Baranyi et al., 2005; Dadomo et al., 2017; Haller et al., 2004; Herzog et al., 2009; Jarcho et al., 2016; Labaka et al., 2017; McCormick et al., 2007; Nowacka-Chmielewska et al., 2017b; Nowacka-Chmielewskaet al., 2017c; Nowacka et al., 2014; Nowacka et al., 2015), serotonin and dopamine related indicators (Labaka et al., 2017), vasopressin and oxytocin receptors (Nowacka-Chmielewska et al., 2017b), sex hormone levels and estrus cycle (Herzog et al., 2009; Labaka et al., 2017; Nowacka-Chmielewska et al., 2017b; Nowacka et al., 2015), and pro and anti-inflammatory cytokine levels (Labaka et al., 2017). Other authors studied growth and neurotrophic factors (Herzog et al., 2009; Nowacka-Chmielewska et al., 2017a; Nowacka-Chmielewska et al., 2017c; Nowacka et al., 2015; Nowacka et al., 2014). Body and organs weight (Baranyi et al., 2005; Dadomo et al., 2017; Herzog et al., 2009; Jarcho et al., 2016; McCormick et al., 2007; Nowacka-Chmielewska et al., 2017b; Nowacka-Chmielewska et al., 2017c; Nowacka et al., 2014), body temperature (Herzog et al., 2009), and food intake (Dadomo et al., 2017; Herzog et al., 2009) were also analyzed. Finally, one study measured proliferation related variables (McCormick et al., 2010). The variability of these results can be due to the great heterogeneity among the study samples and the model utilized.

Regarding HPA axis activity, most authors found higher corticosterone levels in stressed group after social instability period, both in plasma and in hair (Baranyi et al., 2005; Haller et al., 2004; Herzog et al., 2009; Jarcho et al., 2016; Labaka et al., 2017; Nowacka et al., 2015; Nowacka

et al., 2014), while other studies found similar corticosterone and adrenocorticotropin hormone (ACTH) levels in both groups (Dadomo et al., 2017; McCormick et al., 2007; Nowacka-Chmielewska et al., 2017b; Nowacka-Chmielewska et al., 2017c), and an increase in the plasma ACTH/corticosterone ratio in the stressed group (Nowacka-Chmielewska et al., 2017b). Regarding the corticosterone receptors, Labaka et al. (2017) found decreased hypothalamic glucocorticoid receptor (GR) expression in social instability stressed group, but no differences in hypothalamic mineralocorticoid receptor (MR) expression and in the ratio GR/MR. Only two researches focused on the study of corticotropin-releasing hormone (CRH) and its receptors (McCormick et al., 2007; Nowacka et al., 2017b). McCormick et al. (2007) found that, despite the fact that one hour of isolation decreased the CRH expression in parvocellular paraventricular nucleus (PVN) of the hypothalamus, after a social instability period of 15 days, there were no differences in PVN and central nucleus of amygdala (CeA) in females. With respect to CRH receptors, Nowacka et al. (2017b) revealed that stressed group showed lower CRH-R1 expression in the hippocampus and higher expression in the prefrontal cortex (PFC).

Among the studies found, only Labaka et al. (2017) studied variables related with the monoaminergic and immune system. Regarding to monoaminergic variables in hippocampus, stressed group had lower serotonin (5-HT), dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) levels, and higher 5-hydroxyindoleacetic acid (5-HIAA) levels. Stressed group also presented higher 5-HIAA/5-HT ratio and a higher ratio between 3-Methoxy-4-hydroxyphenylglycol (MHPG) and noradrenaline (NA), whereas there were no differences in levels of NA, MHPG, tryptophan (TRYP), kynurenine (KYN) and 3-hydroxy kynurenine (3-HK), neither in the DA/DOPAC ratio. On the other hand, the results showed stress related immune changes. Specifically, it was found that, despite there being no differences in the IL-1β, IL-6 and TNF-α mRNA levels, the stressed females presented lower expression of anti-inflammatory cytokine IL-10 than controls and higher pro-/anti-inflammatory ratios (IL-1β/IL-10, IL-6/IL-10 and TNF-α/IL10). They did not find stress-dependent differences in IDO expression, an enzyme that, activated by pro-inflammatory cytokines, metabolizes TRYP into KYN.

In relation to other neurotransmitters, the study carried out by Nowacka et al. (2017b) showed higher expression of pro-opiomelanocortin (POMC), arginine vasopressin receptor (AVPR1a), and oxytocin receptor (OXTR) in the amygdala following chronic social instability stress, while both in PFC and hypothalamus, POMC, AVPR1a, AVPR1b, and OXTR expression decreased.

Several studies have been conducted with regard to neurotrophic and growth factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and vascular

endothelial growth factor (VEGF). According to Herzog et al. (2009) and Nowacka et al. (2017a, 2015, 2014), in the hippocampus, olfactory bulbs, pituitary and plasma there were no differences in BDNF expression and protein levels. However, Nowacka et al. (2014) found that females rats that were submitted to chronic social instability stress presented lower BDNF mRNA in the amygdala. Focusing on LPS response in the hypothalamus, amygdala and pituitary gland, both BDNF and VEGF levels were higher in stressed group (Nowacka et al., 2015, 2014). In relation to VEGF expression and protein levels, studies have yielded contradictory results (Nowacka-Chmielewska et al., 2017c; Nowacka et al., 2015). The two studies found higher VEGF expression in the hippocampus, amygdala and hypothalamus and lower expression in the pituitary; however, while Nowacka et al. (2017c) found a decrease in serum protein concentration in the stressed subject group, these authors previously (2015) found no differences.

In relation to cell proliferation, the study carried out by McCormick et al. (2010) showed that social stressed group had reduced hippocampal cell proliferation compared to controls as indicated by BrdU immunoreactive cell counts, and did not differ in Ki67 immunoreactive cell counts.

Among the studies included in this review, four of them have measured sex hormones and estrous cycle. Thus, some studies showed that social instability stress produced changes in the regularity of estrous cycle in stressed group (Herzog et al., 2009; Labaka et al., 2017), however other authors found no differences (Nowacka-Chmielewska et al., 2017b). Nowacka et al. (2017b) and Labaka et al. (2017) found similar estradiol levels in both groups. These last authors also found no differences in hypothalamic and hippocampal estrogen receptor α and β expression (Labaka et al., 2017). Nowacka et al. (2017b) found no differences in plasma testosterone levels. Herzog et al. (2009) analyzed the plasma prolactin and luteinizing hormone (LH) levels, finding an increase in stressed group, however other authors did not encounter differences in prolactin levels (Nowacka et al., 2015).

In general, chronic social instability stress does not affect the body weight (Baranyi et al., 2005; Herzog et al., 2009; Jarcho et al., 2016; McCormick et al., 2007; Nowacka-Chmielewska et al., 2017b), however, one study found a decrease in stressed group (Dadomo et al., 2017). These authors also detected no differences in adrenal, spleen, thymus, ovaries, uterus, perigonadal, visceral and intra scapular adipose tissues weight (Dadomo et al., 2017), but other studies revealed an increase in adrenal and thymus glands weights (Herzog et al., 2009; Nowacka-Chmielewska et al., 2017c; Nowacka et al. 2014; Nowacka et al., 2015). Some works found that stressed female consumed significantly less food (Dadomo et al., 2017; Herzog et al.,

2009), whereas Labaka et al. (2017) did not observe differences in this variable. In relation to body temperature, Herzog et al. (2009) detected a decrease in stressed group compared to control and that the peak body temperature observed in the middle of the light phase disappeared in the stress group after 4 weeks of stress.

DISCUSION

We identified 16 works that employed social instability stress in female rodents, and even if the targeted measures and the methodology vary across the studies, the most consistent findings include SIS induced disruption of the reward system and HPA axis alteration. Reward system is considered the most applicable domain to compare stress related disorders in animal models, given that the brain circuits that control pleasure and reward are largely shared between humans and rodents (Berridge and Kringelbach, 2008). In this regard, anhedonia - the lack of interest in pleasurable or rewarding stimuli - is considered a core symptom of depression and is manifested by decreased sucrose consumption in rodents. Similarly, food intake and social interaction display high hedonic value for rodents (and humans) (Slattery and Cryan, 2017), and the five works included in this review that evaluated social interaction found a number of social defects, including social anxiety or social withdrawal, loss of preference for social novelty, maladaptive tolerance to intruders in lactating dams and less preference for novel objects (Baranyi et al., 2005; Haller et al., 2004; McCormick et al., 2007; McCormick et al., 2010; Pittet et al., 2017; Saavedra-Rodríguez and Feig, 2013).

In addition, the increased agonistic behaviors found in social interaction by Baranyi et al. (2005) and Haller et al (2014) may indicate altered social-network dynamic, along with the whisking behavior found by Labaka et al. (2017). This last behavior is a type of hetero-barbering in which the so-called barber holds a cage mate down and cuts its vibrissae with its incisors (Kalueff et al., 2006; Sarna, 2000), and may be specially interesting to asses in SIS applied females, since it has been linked to the establishment of social hierarchies (Kalueff et al., 2006). Those affiliative and aggressive behaviors could have been exacerbated during the stress response by endocrine interactions between the HPA axis and neuropeptides such as arginine vasopressin and oxytocin (Champagne, 2010; Nephew, 2012; Nephew and Bridges, 2008). Additionally, estrogen levels of amygdala and hippocampus and central estrogen receptors have been found to modulate social recognition memory and agonistic behaviors in females (Ervin et al., 2015; Sánchez-Andrade and Kendrick, 2011).

None of the two works that assessed immobility in the FST found stress-related differences. Although immobility in the FST is usually labeled as a despair symptom, some authors defend that it actually reflect the coping strategy and adaptation of the subject (De Kloet

and Molendijk, 2016). However, one study found an increased climbing in this test, as well as increased serotonergic and noradrenergic activity, indicative of a high arousal state (Labaka et al., 2017). In the same direction, most articles found anxiety-like behaviors in the EPM and OFT parameters in stressed mice. Interestingly, anxiety symptoms overlap with depressive symptoms more frequently in women than in men (Keers and Aitchison, 2010; Marcus et al., 2008). To note, when female rodents perform behavioral tests, researchers have noticed a number of depressive-like and anxiety-like behaviors different from those traditionally registered in males, such as rearing in the OFT (Jarcho et al., 2016), climbing in the FST (Labaka et al., 2017), and head shaking (Rahona et al., 2014). Detecting sex specific behavioral indicators can be crucial to adjust measurable indexes in both sexes (Kokras and Dalla, 2017).

Other robust finding among the articles included in this review, is the hyperactivity of the HPA axis, manifested by high levels of corticosterone. In line with these results, some authors found changes in GR and CRH-R1. Apart from mediating behavioral and cognitive changes, HPA axis activation can inhibit trophic factors such as BDNF and VEGF in the central nervous system, and those factors are implicated in the cascade of events triggered on stress-induced affective disorders (Hansson et al., 2003; Nowacka et al., 2014). However, it has to be taken into account that stress-induced changes in trophic factors can be sex-dependent. For instance, in BDNF knockout mice, locomotor activity was perturbed in males, while females showed reduced anxiety levels with an increase in depression-like behavior (Monteggia et al., 2007). BDNF can also be affected through the immune system activation in the stress response. In this regard, pro-inflammatory cytokines have been related with neuron damage while anti-inflammatory cytokines such as IL-10 show neuroprotective characteristics (Sharma et al., 2011; Zhou et al. 2009). The downregulation of IL-10 in the stress response seems to be especially characteristic in female subjects (Labaka et al., 2017; Voorhees et al., 2013), while the traditionally reported pro-inflammatory activation in males may not be so robust in females (Bekhbat and Neigh, 2016; Pyter et al., 2013). In this regard, the only work that measured IDO and cytokines expression and monoamine levels, found changes in monoamines, but not in IDO (Labaka et al., 2017). Further studies are required in order to clarify the interactions between HPA axis, endocrine and trophic factors, and monoamine and immune systems in the development of depressive-like behavior in females.

The major limitation of current evidence consists on the variability of the results seen across the studies. This may simply be the result of methodological differences; although the stress regimen of the majority of works include alternating phases of crowding and isolation, three of them did not include isolation phases (Dadomo et al., 2017; Pittet et al., 2017; Saavedra-Rodríguez and Feig, 2013). According to Herzog et al. (2009), the alternation of both phases

makes this model more robust, as isolation or crowding *per se* can be insufficient to induce a stress response in females (Benton and Brain, 1981; Brown and Grunberg, 1995). The works that included both phases also present differences in the duration of the phases and the isolation conditions, as well as in the cage-composition of the crowding phases. Furthermore, the duration of the stress vary from two weeks to seven weeks, and, some authors assessed long-term consequences several weeks after the stress procedure had ended (McCormick et al., 2013; McCormick et al., 2010; Saavedra-Rodríguez and Feig, 2013), whereas others measured the targeted variables immediately after. In addition to this, variations in response and magnitude may be due to the differences between rat and mice.

In conclusion, this systematic review provides an up-to-date summary of the existing literature on the biological and behavioral consequences of social instability stress model in female rodents. Though the nature of stress and adversity factors linked to mental illness is complex (Bagot et al. 2014; Howes and Murray 2014), the reviewed studies indicate that the most robust findings found with this model are the HPA axis hyperactivity and disrupted reward system. Given the growing body of preclinical evidence showing that the stress-related disorders' physiopathology presents sexual dimorphism, we suggest that further work in this area focus on monoaminergic, immunity, neurotrophic and behavioral parameters, in order to elucidate the underlying mechanisms of depression in females.

Declaration of Conflicting Interests

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

Funding

This study was supported by the Basque Government IT757-13 Project Grant and by the Basque Government Predoctoral grants (PRE_2017_2_0203 and PRE_2017_2_0096).

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