

Effects of Social Isolation on the Development of Anxiety and Depression-Like Behavior in Model Experiments in Animals

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UDC 612.821

Translated from Zhurnal Vysshei Nervnoi Deyatel'nosti imeni I. P. Pavlova, Vol. 71, No. 6, pp. 760–784, November–December, 2021. Original article submitted January 20, 2021. Accepted March 2, 2021.

This review describes the role of social isolation in the development of anxiety and depression-like behavior in rodents. The duration of social isolation, age from onset of social isolation, sex, species, and strain of animals, the nature of the model used, and other factors have been shown to have influences. The molecular-cellular mechanisms of development of anxiety and depression-like behavior under the influence of social isolation and the roles of the HHAS, oxidative and nitrosative stress, neuroinflammation, BDNF, neurogenesis, synaptic plasticity, as well as monoamines in these mechanisms are discussed. This review presents data on sex differences in the effects of social isolation, along with the effects of interactions with other types of stress, and the roles of an enriched environment and other factors in ameliorating the negative sequelae of social isolation.

Keywords: social isolation, anxiety and depression-like behavior, stress, open field, elevated plus maze, sucrose preference test, forced swimming.

The year 2020 became the year of the severe coronavirus pandemic affecting virtually whole world and killing nearly two million people to date. One current WHO recommendation to prevent infection and disease is social isolation (SI), which consists of minimizing social connections ranging from partially restrictive measures to full lockdown – with closure of industrial trade, transport, and other forms of social connections among people. However, SI is not a harmless situation. Specialists (psychologists, psychiatrists, sociologists, etc.) have long known that social isolation, especially when lasting long periods of time, leads to serious disorders of mental and physical health in humans. Social isolation increases the risk of death no less than smoking or alcohol and more than physical inactivity and obesity [Li and Xia, 2020]. SI not infrequently leads to family strife and within-family acts of aggression, primarily against women [Vieira et al., 2020]. SI activates the hypothalamo-hypophyseal-adrenal system (HHAS), increases sympathetic activity and weakens parasympathetic activity, and increases the

body's proinflammatory immune responses. These processes are linked with the development of cardiovascular diseases. The commonest states in prolonged social isolation are fear, anxiety, and depression. The mechanisms producing these states remain poorly studied. This present review addresses the following aspects of the problem: 1) SI as a model of psychoemotional stress; 2) the molecular-cellular mechanisms of the influences of SI on the formation of anxious and depression-like behavior in animals; 3) sex differences in the effects of SI; 4) the interaction of SI with other types of stress; 5) the roles of factors ameliorating the adverse sequelae of SI.

Social Isolation as a Model of Psychoemotional Stress. There are various models of psychoemotional stress leading to anxious-depressive disorders, phobias, and other serious adverse sequelae (for a detailed review see [Grigoryan and Gulyaeva, 2015]). Models of SI occupy a special place among these, as they are not linked with direct physical or mental traumas applied to individuals, but with influences on another no less important aspect of life – social communication and interaction. As humans and most animals live and develop in a social environment, their ontogeny (development and formation of organs, tissues, behavior, etc.) is directly influenced by this environment. Some ani-

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imals, true, live in solitude, socializing with an individual of the other sex only during the mating period. Lack of social communication at different life stages will lead to impairments to normal ontogeny, which in turn lead to abnormal behavior – aggressivity, anxious-depressive disorders, phobias, etc. The period of life during which SI occurs is very important, as the forms of communication and interaction between subjects typical for each stage of development are modified or completely altered under the influence of SI. For example, SI at early age weakens play behavior in adolescence; it leads to loss or weakening of play components in the form of somersaults, roughhouse, fighting, etc. As social interactions in adolescent rats and adult animals are significantly different behaviorally, these differences are reflected in the various influences of SI [Walker et al., 2019; Hall et al., 1998]. However, before proceeding to analysis of the influences of SI on behavior, we will briefly address the models most frequently used for assessment of anxiety and depression-like behavior.

Behavioral models evaluating anxiety and depression-like behavior. A number of models exist for assessment of anxious and depression-like behavior (for review see [Grigoryan and Gulyaeva, 2015], though the systems most commonly used for this are the open field (OF) and elevated plus maze tests (EPM) (for assessment of anxiety) and the sucrose preference test (SPT) and the forced swimming test (FST) (for assessment of depression-like behavior). Anxiety is assessed in the OF in terms of the number of excursions to the center and the times spent in the center and periphery of the OF. The greater the time spent by rats in the center and the less spent at the periphery of the OF, the less anxious they are and vice versa. Ditto the EPM: the more time spent by the animals in the open arms of the maze and the more frequently they visit these arms, the lower their anxiety level and vice versa. The SPT assesses the ability of animals to perceive the hedonistic properties of sweet sucrose solution. The less sucrose they drink as compared with water, the more they lack these properties (have anhedonia), which is typical of the depression-like state. The FST assesses another property of depression-like behavior – the state of “despair,” in which the animal loses the ability to resist in a difficult (hopeless) situation. At the beginning of the forced swimming test, placing a rat into a cylinder containing water causes it to swim and try to escape from the cylinder, scrabbling up the walls. However, there come a moment at which the animal stops resisting, stops swimming, “hangs” in the water, and carries out only weak movements with the tail and paws to maintain equilibrium. The total duration of immobility and the moment of the first hanging are used to assess the level of the animal’s depression-like behavior. The earlier the animal starts to hang and the more time the animal spends in the immobile state during the test period, the more marked its depression-like behavior. It should be noted that the literature contains contradictory opinions as to the adequacy of the FST for assessing depression-like be-

havior, though these opinions and their critical analysis are beyond the scope of this review.

Effects of SI on levels of anxiety and depression-like behavior. In most studies, SI induced anxiety and depression-like behavior [Du Preez et al., 2020a; Liu et al., 2020; Dhmelova et al., 2019; Zorzo et al., 2019; Ramos-Ortolaza et al., 2017; Todorovic and Filipovic, 2017; Wang et al., 2017; Amiri et al., 2015; Djordjevic et al., 2012; Zhang et al., 2012; Wallace et al., 2009; Weiss et al., 2004]. For example, SI for three weeks decreased the proportion of excursions made by animals into the open arms of the EPM and decreased movement activity, i.e., had an anxiogenic action [Djordjevic et al., 2012]. Two-week isolation of adult rats was followed by a shorter period of time spent in the light sector of the dark–light chamber [Carrier and Kabbah, 2012]. Spasojevich et al. [2007] showed that SI for three weeks induced anxiety behavior in adult male rats, while Zlatković et al., using SI of the same duration, found depression-like behavior in the forced swimming test and sucrose preference test. These results are consistent with data from the forced swimming test [Brenes and Fornaguera, 2009] and results from the sucrose preference test in male rats [Carrier and Kabbaj, 2012]. Chmelova et al. [2019] used male and female rats kept in SI from age 21 days for nine weeks and found that they displayed anxiety-like behavior. Male mice socially isolated for eight weeks showed depression-like behavior in the forced swimming test and the tail suspension test, while females displayed this behavior in the tail suspension test [Liu et al., 2020]. Female mice subjected to seven weeks of SI developed anxiety-like behavior in the EPM and OF [Kumari et al., 2016]. SI induced decreases in sucrose preference and increases in total hanging time in the FST, i.e., had a depressive action [Guarnieri et al., 2020; Holgate et al., 2017; Wang et al., 2017; Mileva and Bielajew, 2015; Takatsu-Coleman et al., 2013]. It should be noted that a number of studies have not seen any influence for SI on the occurrence of anxious and depression-like behavior. Thus, Gorlova et al. [2018] found that SI had no influence on the level of depression-like behavior. The authors’ view was that this occurred because SI was transient (1–3 weeks), and manifestation of these effects required at least three weeks of social isolation. In another study [Alshammari et al., 2020], the OF, sucrose preference, and forced swimming tests after 10 days showed no behavioral differences compared with control rats, though molecular studies showed that isolated rats displayed significantly increased expression of Toll-like receptors and had significantly greater contents of proinflammatory cytokines in the hippocampus. There were also no behavioral differences in the EPM between the control group and animals kept in SI for four weeks from day 24 of life [Dunphy-Doherty et al., 2018]. Brenes et al. [2006] suggested that manifestation of the effects of SI on anxious and depression-like behavior required at least two months of isolation. This suggestion, however, contradicts data from other authors [see above], which demonstrated the negative influences of SI at very low

durations [Hall, 1998]. Thus, for example, rats showed increased corticosterone levels at just three days of SI, and also displayed an appetite for alcohol at one day [Wolffgramm and Heyne, 1991]. The authors linked this latter circumstance with the development of anxiety-like behavior and the desire to relieve it using alcohol. Male mice needed even less time (12 h) in social isolation for them to manifest depression-like behavior and have elevated corticosterone levels [Takatsu-Coleman et al., 2013]. Gong et al. [2018] showed the mice exposed to SI for eight days at early age developed depression-like behavior in the tail suspension, forced swimming, and sucrose preference tests. Thus, both long-lasting (chronic) and short-term (acute, 1–7 days) [Hall, 1998] social isolation could have adverse influences on behavior. However, there are cases where SI has anxiolytic rather than anxiogenic actions [Lukkes et al., 2012; Kuleshkaya et al., 2011; Pietropaolo et al., 2008; Vöikar et al., 2005]. For example, weakening of anxiety behavior was seen in mice exposed to SI at age three weeks and lasting eight weeks, especially when it was combined with depletion of nesting material [Kuleshkaya et al., 2011]. Guo et al. [2004] found that male and female mice in SI for 1–4 months spent less time in the closed arms of the EPM than control animals, i.e., social isolation in these animals produced an anxiolytic action. Isolated males spent less time in the closed arms of the EPM than females and control animals in the second, third, and fourth months of testing. A number of studies have found that SI increased the consumption of sucrose solution [Hong et al., 2012; Yildirim et al., 2012; Brenes et al., 2020] and decreased the total duration of hanging in the FST [Huang et al., 2017; Voikar et al., 2005; Hong et al., 2012], i.e., there was an antidepressant effect. Our recent study [Pavlova et al., 2012] showed that SI starting at age 45 days and lasting two months increased anxiety in rats in the OF and EPM. The greatest changes occurred in isolated rats which during the neonatal period had additionally received s.c. doses of the bacterial toxin lipopolysaccharide (LPS). The sucrose preference test in rats in social isolation produced signs of depression-like behavior only in females, in groups which had and had not received additional neonatal LPS stress, the latter showing a tendency to differences from control animals. In the FST, isolation did not lead to any increase in hanging time in either males or females, i.e., this test revealed no significant differences in the level of depression-like behavior [Pavlova et al., 2021]. Differences in the effects of SI were found depending on test type and mouse strain [Voikar et al., 2005]. Thus, mice of the strains C57BL/6J and DBA/2 subjected to SI for seven weeks starting at age four weeks showed reduced anxiety in the light–dark box and increased anxiety in the EPM. The forced swimming test demonstrated reduced depression-like behavior in response to SI.

Effects of SI on behavioral responses depending on duration and age at beginning of SI. SI in most studies starts at days 21–30 of life and continues for 3–8 weeks (see [Walker et al., 2019] for detail). The first work seeking to find

the “critical window” for the effects of social isolation was reported by Einon and Morgan [1977]. They compared the effects of isolation at different postnatal days (16–25, 25–45, and 90–180) on anxiety levels in the OF. The latencies of excursions to the center of the OF in isolated animals of all groups were longer than those of control animals, though this measure did not show any differences between groups spending different durations in isolation. The most marked and long-lasting effect was produced by isolation on days 25–45. Rats enter the adolescent period on days 21–35, with puberty (sexual maturation) in the period 35–55 days, after which they reach the adult stage [Walker et al., 2019]. During the adolescent period, when maturation of key structures occurs – the hippocampus, amygdala, prefrontal cortex, and their associated neurotransmitter systems – social interactions, play behavior, and exploratory activity play a particularly important role for rat pups. This period is characterized by “social reward,” to which males are especially sensitive [Walker et al., 2019]. During this same period, animals are more sensitive to stress than in adulthood, leading to the future development of anxious and depression-like disorders, degradation of learning and memory, etc. The response to stress in adolescence include a more powerful increase in the corticosterone level and a more responsive HPA axis as compared with reactions to stress in the adult body. In contrast to adult animals, where repeated stress weakens reactions, acclimation did not occur in rats in the adolescent period and responses to repeated stress persisted even longer or even increased [Walker et al., 2019]. Thus, isolation in the adolescent period deprives rats of social communication, interaction, and play behavior, which are prerequisites for development of defensive reactions in future and for copulation in the pubertal period.

In most studies, SI in the early adolescent period (days 21–28) decreased the time spent in the open arms of the EPM in males [Skelly et al., 2015; Karkhnis et al., 2014; Chappell et al., 2013; Pritchard et al., 2013; Yorgason et al., 2013; McCool and Chappell, 2009; Weiss et al., 2004; Wright et al., 1991; Parker, 1986]. This effect persisted after the animals were resocialized [Wright et al., 1991]. Two studies [Weintraub et al., 2010; Thorsell et al., 2006] found that males isolated from day 30 displayed not an anxiogenic, but rather than anxiolytic effect. Rats entered the open arms of the EPM more frequently than control animals. It is interesting that this period coincides with the decline in play behavior in animals kept in groups [Panksepp, 1981]. In females, no anxiogenic effects of social isolation were seen in the EPM in the early adolescent period [Butler et al., 2014; Jahng et al., 2012; Weintraub et al., 2010; Weiss et al., 2004]. Males kept in isolation from day 21 to day 42 entered the center of the OF less frequently (an anxiogenic effect) [Lukkes et al., 2009]. On the other hand, Thorsell et al. [2006] found that males which had been kept in isolation from day 45 to day 130 showed increases in the time spent in the center of the OF [an anxiolytic effect]. Females displayed greater movement activity in the OF, with faster and

more frequent visits to the central area than males [Walker et al., 2019]. Thus, isolation in the early adolescent period in males induced anxiogenic effects in both the EPM and the OF, while in the late adolescent period the same models showed anxiolytic effects [Walker et al., 2019]. However, this assertion can only be made with some reservations, as the comparisons were made in different rat strains and with social isolation of different durations.

SI for six weeks in adolescence and early adulthood (days 28–70) increased social behavior in male and female mice and anxiolytic behavior in females in the EPM. On the contrary, SI of the same duration but in adulthood (after day 77) induced anxious behavior in the light–dark box [Rivera-Irizarry et al., 2010]. Female C57b/129sv mice subjected to SI at later age (18–24 days) showed greater levels of anxiety as compared with control mice kept in groups. These also showed weaker cognitive functions [Arranz et al., 2009]. Begni et al. [2020a] recently studied SI depending on its duration and the age at which it started. In one group of rats, isolation was run from day 21 to day 101 (the prolonged isolation group), while in the second group isolation ran from day 21 to day 58 (the adolescent period) and in the third group it ran from day 58 to day 101 (adult group). Between-group differences were seen clearly in terms of movement activity in the OF. This was most intense in the groups of rats with prolonged isolation and rats with isolation in adulthood. It was weaker in rats isolated in the adolescent period. No differences were seen in measures of anxiety in the open field between groups of rats isolated in different age periods. SI in rats of intermediate age (eight months) for six weeks did not produce signs of depression-like or anxious behavior [Ren et al., 2015]. SI for three months in transgenic APP695/PS1-dE9 mice promoted the development of cognitive dysfunction and amyloid plaque accumulation in the hippocampus of old (17 months) transgenic mice, increasing γ -secretase and decreasing neurilysin expression. Furthermore, transgenic mice which had been kept in isolation showed increased hippocampal atrophy, loss of myelin- and synapse-associated proteins, and increases in neuroinflammatory reactions [Huang et al., 2015].

Thus, analysis of behavioral data on the occurrence of anxious and depression-like behavior in response to social isolation reveals the role of a large number of factors in these influences. Of particular significance among these are the duration of isolation and the age at which it started. Among other causes factors affecting behavior in SI conditions are sex (see below), animal species (results in rats and mice in SI are different), and rat strain [Painsipp et al., 2011], the nature of the models used and the equipment involved (for example, the designs of the OF and EPM) [Pavlova et al., 2021], and a number of others.

Mechanisms of the Influence of Social Isolation on the Development of Anxious and Depression-Like Behavior. *The role of the hypothalamo-hypophyseal-adrenal system.* The HHAS plays an important role in acute

and chronic stresses and the development of anxious-depressive disorders. In normal conditions, secretion of corticotropin-releasing hormone (CRH) and arginine vasopressin in the paraventricular nucleus of the hypothalamus activates ACTH secretion in the hypophysis. ACTH in turn stimulates glucocorticoid secretion (corticosterone in animals) in the adrenal cortex; corticosterone interacts with its receptors in many brain structures, inhibiting CRH and arginine vasopressin release via negative feedback (see review by [Grigoryan et al., 2014] for detail). Glucocorticoids are known to function optimally at moderate concentrations, while hypo- and hypersecretion lead to anomalies. This occurs because of the differences in the properties and levels of binding of the two main types of receptors for these hormones in the brain – glucocorticoid (GC) and mineralocorticoid (MC) receptors. MC receptors bind glucocorticoids at moderate concentrations in the body. These stimulate cell activity in the hippocampus and influence maintenance of the basal daily glucocorticoid level. GC receptors bind glucocorticoids during their daily peaks or in stresses induced by inhibition of hippocampal neurons and regulate HHAS responses to stress via inhibitory feedback mechanisms. Glucocorticoids inhibit CRH corticotropin secretion in the paraventricular nucleus of the hypothalamus and, via local mobilization of endocannabinoids, induce subsequent inhibition of excitatory afferent inputs. Rapid inhibitory feedback terminates CRH and ACTH release for several minutes, decreasing the strength of the stressor. How the HHAS operates in stress and on development of depression has been discussed in detail in a review [Grigoryan et al., 2014]. We will present a number of studies relevant to social isolation, anxious-depressive behavior, and blood corticosterone levels. In particular, Takatsu-Coleman et al. [2013] showed that SI in mice for 12 h induced depression-like behavior in the FST, SPT, and tail suspension test and increased the serum corticosterone level. Another study [Ferland and Schrader, 2011] found that in the case of rats living in pairs, removal of one from the cage left the other animal with a sharp increase in the corticosterone level. In response to acute stress, corticosterone was at the same high level as in rats previously subjected to chronic varying stress. Another study [Stevenson et al., 2019] showed that animals developed depression-like behavior (anhedonia) and increased corticosterone levels after six weeks of chronic isolation.

Ieraci et al. [2016] found that despite the fact that SI induced anxiety (in the OF) and depression-like behavior in the tail suspension test, corticosterone levels did not increase, but could even decrease as compared with those in control mice. Rats kept in SI from day 21 for three weeks developed anxiety behavior assessed in a number of tests. However, it was of note that the corticosterone level in these animals, as in control rats, was elevated after restriction stress, though with the difference that at 2 h the level decreased to below the prestress level [Lukkes et al., 2009]. SI in male but not female rats increased anxiety in the EPM

and increased ACTH and corticosterone levels, in the latter case affecting both baseline value and the response to stress [Weiss et al., 2004]. On the other hand, despite the development of anxious behavior in the light–dark box and OF in response to SI, neither the basal nor the stress-induced corticosterone levels changed [Schrijver et al., 2002]. Isolated female Kyoto–Wistar rats, which are themselves a model of depression, showed decreased corticosterone levels as compared with control Wistar animals [Mileva et al., 2017]. Similar results were reported in [Roekner et al., 2017], where chronic SI induced anxious behavior and simultaneous decreases in serum corticosterone levels in females. These results were explained in terms of the influences of the different durations of isolation and the age at which it started [Serra et al., 2007]. Three-week isolation did not alter basal corticosterone levels from that in adult control rats [Filipović and Pajović, 2009]. Filipovic et al. [2017] showed that SI weakened negative glucocorticoid feedback regulation of corticosterone secretion levels. Boero et al. [2018] found that SI decreased the levels of total corticosterone and its carrier, corticosteroid-binding globulin, but had no effect on the active free corticosterone fraction in the basal state in response to acute stress. In the basal state, SI increased the number of glucocorticoid receptors and decreased the number of mineralocorticoid receptors. In response to acute stress, rats subjected to SI retained elevated corticosterone, ACTH, and corticotropin-releasing hormone levels for long periods. Glucocorticoid receptor expression in the hippocampus and hypothalamus of rats kept in groups increased with time, reaching a peak, and then returned to the basal level, while glucocorticoid receptor expression in socially isolated rats did not change over time [Boero et al., 2018]. Corticosterone levels in response to acute stress were higher in male isolated animals than females [Pisu et al., 2016], though another study [Heck and Handa, 2019] reported a stronger HHAS reaction in females than males, due to modulation by sex hormones. The basal corticosterone level after nine months of SI was no different from the corticosterone level in control animals, though it was higher in females than males regardless of housing conditions [Krupina et al., 2020]. In our study [Pavlova et al., 2021], a significant increase in the corticosterone level was seen in all groups of rats after two months of SI, which is evidence of their greater reactivity to stressors (FST) as compared with the control group.

The role of oxidative stress. Along with impairments to HHAS operation, SI induces oxidative stress in the brain and leads to dysregulation of the antioxidant enzyme [Colaïanna et al., 2013]. The antioxidant enzymes are cytosolic copper-zinc superoxide dismutase CuZnSOD [Chang et al., 1988] and mitochondrial manganese superoxide dismutase (MnSOD), which catalyze conversion of the superoxide anion (O_2^-) to oxygen and hydrogen peroxide (H_2O_2), which are then detoxified by the enzymes catalase (CAT) and glutathione peroxidase (GPx) [Chelikani et al., 2004].

Increases in corticosterone levels during chronic stress decrease the activity of the antioxidant enzymes in rat brains, pointing to a direct involvement of corticosterone in oxidative stress [Zafir and Banu, 2009]. High glucocorticoid levels increase glutamate release, lead to calcium-dependent activation of nitric oxide synthase (NOS) and production of high (toxic) concentrations of nitric oxide (NO), thus inducing impairments to mitochondrial functioning. Increases in the expression of the enzyme NOX2 (the main source of reactive oxygen species, ROS) and oxidative stress in the frontal cortex are the early pathological signs of the effects of SI in rats. NOX2-induced oxidative stress leads to increases in glutamate levels and decreases in the numbers of parvalbumin-positive inhibitory neurons. Administration of the NOX inhibitor apocynin during seven-week SI protected against the adverse sequelae of oxidative stress. Increases in the reactive oxygen species (ROS) content inhibit the antioxidant activity of the enzyme CAT [Spiers et al., 2015], while high levels of hydrogen peroxide H_2O_2 inactivate copper-zinc superoxide dismutase (CuZnSOD) activity [Halliwell and Gutteridge, 1989]. Social isolation for eight weeks was shown to decrease CAT and GPx activities and total antioxidant capacity, at the same time increasing the hydrogen peroxide level in the prefrontal cortex and hippocampus in rats [Shao et al., 2015]. In addition, expression of CuZnSOD is under control of NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells, NF- κ B) [Meyer et al., 1993], which is activated by hydrogen peroxide H_2O_2 [Bowie and O'Neill, 2000]. Hydrogen peroxide triggers feed-forward with NF- κ B, inducing accumulation of toxic hydrogen peroxide, with the result that the antioxidant activity of CuZnSOD decreases further. Glutathione is an important component of the nonenzymatic antioxidant system. Glutathione is the main buffer for the redox system [Giustarini et al., 2004] and a significant cofactor for a number of enzymes protecting cells from oxidative stress. Changes in glutathione levels and glutathione peroxidase activity provide evidence of weakening of antioxidant defense. SI for three weeks in adult male rats significantly decreased glutathione contents in the prefrontal cortex [Zlatković et al., 2014], while three-week SI in adult male mice decreased glutathione content in the hippocampus [Todorović et al., 2014]. This is evidence that SI induces impairments to the glutathione-dependent defense system, provoking prooxidant effects in the hippocampus. Chronic isolation of rats for three weeks, including depression-like and anxious behavior in the FST, SPT, and MB marble burying tests increased markers of oxidative and nitrosative stress (NF- κ B and COX-2) in the prefrontal cortex, and, to a lesser extent, in the hippocampus [Zlatković et al., 2014]. Stevenson et al. [2019] found that six-week chronic SI inducing depression-like behavior in prairie voles in the SPT increased the metabolites of reactive oxygen species, reflecting the occurrence of oxidative stress. Daily administration of oxytocin almost completely protected animals

from the adverse sequelae of SI. Another study in rats subjected to SI for three and eight weeks demonstrated clear signs of depression-like behavior in the FST and tail suspension test as compared with control animals [Mohale and Chandewar, 2012]. Stressed animals showed significant increases in signs of oxidative stress: lipid oxidation increased and the glutathione, GSH, superoxide dismutase, SOD, and CAT levels decreased at eight weeks of SI. For more detailed data on the link between social isolation and anxiety/depression-like behavior and oxidative/nitrosative stress see review by Filipovic et al. [2017].

The role of nitrosative stress. Nitric oxide (NO) is produced by isoforms of nitric oxide synthase (NOS). It plays a key role in synaptic plasticity, neuromodulation, and other physiological functions. However, overexpression of NO as a result of elevated nuclear nNOS and inducible iNOS activities during stress increases glutamate receptor activity [Ridnour et al., 2004]. Furthermore, local selective activation of the microglia by chronic stress in rats leads to release of high nitric oxide concentrations, provoking nitrosative stress [Cassina et al., 2002]. Elevated nNOS and iNOS expression in the prefrontal cortex during three-week SI in adult male rats provided evidence of nitrosative stress [Zlatković and Filipović, 2012]. One factor stimulating nitrosative stress due to chronic SI is activation of nuclear factor κ B (NF- κ B) [Maes et al., 2007]. Nitric oxide induces upregulation of NF- κ B [Connelly et al., 2001], while increased expression of both nitric oxide synthases (nNOS and iNOS) increases NO production, which promotes activation of NF- κ B. Increased NO production due to chronic SI can lead to activation of proapoptotic signaling in the prefrontal cortex in adult male rats [Zlatković and Filipović, 2012]. Filipovic et al. [2017] showed that SI for three weeks increased cyclooxygenase-2 (COX-2, a marker of inflammation) expression in the prefrontal cortex of adult male rats [Zlatković et al., 2014]. Increased COX-2 and iNOS expression is linked with increased NF- κ B production [Maes et al., 2007]. Activation of COX-2 induces release of additional free radicals and proinflammatory cytokines [Arimoto and Bing, 2003] and activates prostaglandin biosynthesis, further boosting the prooxidant state of cells. Prostaglandins themselves can also damage cells, provoking glutamate release from astrocytes or leading to apoptosis [Vesce et al., 2007]. On expression, iNOS and COX-2 can generate large quantities of reactive oxygen species (ROS), promoting oxidation of cell components [Madrigal et al., 2003] and are involved in proapoptotic signaling in the prefrontal cortex. The 5-HT₃ receptor antagonist tropisetron has been shown to decrease the depressive influence of four-week social isolation in adolescent male mice by decreasing nitrosative stress by restoring mitochondrial functioning and decreasing nitric oxide levels in cortical areas [Haj-Mirzaian et al., 2016]. In this study, tropisetron ameliorated the adverse influences of inducible nitric oxide synthase (iNOS) on mitochondrial activity, while combination with the specific

iNOS inhibitor aminoguanidine enhanced the protective effect of tropisetron.

The role of neuroinflammation. One important mechanism of the influence of SI on the development of anxious-depressive states consists of neuroinflammation. Many studies have shown that SI increases proinflammatory cytokine (IL-1 β , IL-6, tumor necrosis factor α) contents in the brain [Du Preez et al., 2020b; Todorovich et al., 2017; Wang et al., 2017] and Toll-like receptors [Alshammari et al., 2020, 2019] in the hippocampus. These latter recognize conserved microbial molecules and trigger cellular immune responses. Males have more Toll-like 4 receptors and females more Toll-like 7 receptors. Different stressors have been shown to induce different changes in behavior and biochemical indicators. In particular, De Preez et al. [2020a] demonstrated differences in the effects of two different stressors in mice – chronic SI from day 42 for 6–9 weeks and daily injections of physiological saline at the same times. Stress due to daily injections induced anxiety behavior in the mice. This was associated with weakening of inflammatory reactions: decreased serum TNF- α and IL-4 levels, increased corticosterone reactivity, increased microglial activation, and reduced neuronal differentiation in the dentate fascia of the hippocampus. Conversely, prolonged social isolation induced depression-like behavior: serum TNF- α increased, IL-10 decreased, corticosterone reactivity was weakened, and microglial density decreased [Du Preez et al., 2020a]. This latter plays a very important role in immune and inflammatory reactions and is one of the main sources of the formation and release of interleukins. Microglial death goes through stages of activation and apoptosis. Administration of minocycline prevented loss of microglia and prevented the development of anxious and depression-like behavior. Gong et al. [2018] found that both microglial activators, LPS, and macrophage colony-stimulating factor (M-CSF) completely protected the body from developing depression-like behavior due to increased microglial proliferation in the hippocampus. SI at early age increased the expression of the microglial activation marker Iba1 in the hippocampus and decreased expression of the microglial receptor C200, which promotes entry of microglia into a quiescent state [Wang et al., 2017]. The involvement of immune and inflammatory reactions in the effects of SI is confirmed by data obtained in *Drosophila*. Adult male *Drosophila* socially isolated for four days displayed changes in the expression of 90 genes, most of which were part of the immune system [Agrawal et al., 2020]. This indirectly indicates a relationship between SI and the immune system and inflammation.

The role of trophic factors, neurogenesis, and synaptic plasticity. BDNF (brain-derived neurotrophic factor) is one of the most important neurotrophins involved in the processes of brain development, including neuron survival, specialization, migration, synaptogenesis, spine density, and dendrite branching. BDNF expression levels influenced

by SI depend on the duration of isolation, the age at which it starts, the sex and species of the animal, the brain structure studied, and other factors. Thus, SI in adolescent (38–51 days old) males increased BDNF levels in the medial prefrontal cortex of adult rats [Shao et al., 2013]. Two-week SI increased BDNF expression in the medial prefrontal cortex, but decreased it in the nucleus accumbens and dentate fascia (DF) of the hippocampus [Han et al., 2011]. Four-week SI in four-week-old rats increased BDNF expression in the medial prefrontal cortex and hippocampal fields CA1 and CA2/3 and the DF [Meng et al., 2011]. Isolation for two months in two-month-old rats decreased BDNF levels in the hippocampus [Scaccianoce et al., 2006]. In C57BL/6 mice, social isolation for three weeks starting at age three months decreased the BDNF level in the hippocampus, frontal cortex, hypothalamus, and midbrain structures [Barry et al., 2012]. In the study cited above [Begni et al., 2020a], rats with different durations of isolation and different ages showed decreases in BDNF levels in isolants of all groups as compared with controls. However, the level of BDNF transcripts containing exons 4 and 6 was lower only in the group of rats with prolonged social isolation. Two other groups, in which isolation was imposed in the adolescent and adult periods, showed no differences in the levels of these transcripts from controls. Chmelova et al. [2019] found that neither males nor females showed any changes in BDNF expression in response to SI. Decreases in BDNF levels in response to chronic SI in male mice, which displayed anxious and depression-like behavior, were seen in the hippocampus and prefrontal cortex [Ieraci et al., 2016]. Mice kept for two or seven weeks in social isolation following stroke displayed depressive behavior and had decreased BDNF levels [O'Keefe et al., 2014]. Evans et al. [2012] found that chronic SI in rodents induced depression-like behavior and anxious behavior, decreased BDNF levels, and reduced neurogenesis and the level of the endogenous neurosteroid allopregnanolone. Administration of exogenous allopregnanolone from onset of chronic SI prevented the development of anxious-depressive behavior and normalized the BDNF level and neurogenesis [Evans et al., 2012]. Female mice exposed to eight-week SI showed, along with signs of an anxiety state, an increase in BDNF expression in the cerebral cortex [Kumari et al., 2016]. There were simultaneous increases in the regulation of CREB-1 (cAMP-responsive element binding protein 1) and CBP (CREB binding protein), which play important roles in BDNF transcription. Conversely, HDAC-2 (histone deacetylase-2) activity, which adversely affects BDNF expression, weakened in isolants. As regulation of BDNF levels is bound to the actions of Limk-1 (LI domain kinase 1), miRNA-132, and miRNA-134, increases in BDNF expression are accompanied by increases in the expression of Limk-1 mRNA and miRNA-132 and decreases in the expression of miRNA-134 (inhibits translation of Limk-1). Thus, the molecular changes in anxiety behavior due to the effects of social isolation are induced via in-

creased expression of BDNF, CBP, CREB-1, Limk-1, and miRNA-132 and weakening of the expression of HDAC-2 and miRNA-134 in the cerebral cortex [Arzate-Mejía et al., 2020; Kumari et al., 2016].

Considerable amounts of data have been obtained providing evidence that SI decreases neurogenesis in the hippocampus [Guarnieri et al., 2020; Liu et al., 2020; Lieberwirth et al., 2012; Ibi et al., 2008; Lu et al., 2003]. For example, isolation of adult mice (males and females) for one week has been shown to weaken neurogenesis in the olfactory bulb and the ventral part of the hippocampus [Guarnieri et al., 2020]. Social isolation for four weeks in male mice aged 2.5 months altered neuroplasticity and the activity of genes associated with it [Ieraci et al., 2016], while SI in rats aged 1–2 months showed decreased neurogenesis in the dentate fascia and decreased long-term post-tetanic potentiation of hippocampal neurons [Lu et al., 2003]. Wang et al. [2019] showed that chronic SI also weakened long-term post-tetanic potentiation of hippocampal field CA1 neurons. The hippocampus showed decreases in the expression of protein PSD-95 (postsynaptic density protein-95) and glutamate receptors (GluA1, NR1, and NR2B) without any changes in extracellular glutamate release or NR2A or GABA_A receptor expression. This is evidence that SI at early age impairs postsynaptic functioning and alters the interaction between AMPA and NMDA receptors, influencing spatial memory and learning [Wang et al., 2019]. Female rats in isolation from day 35 to day 55 of life showed decreased branching of dendritic trees in the radial layer of the dorsal hippocampus [Chen et al., 2018]. Conversely, the ventral part of the hippocampus showed increased dendritic tree branching. Thy-1-GFP knockout mice in SI from age 21 days to adulthood showed fine, immature dendritic spines in the prefrontal cortex, at smaller numbers than in control animals. These same mice showed weaker long-term post-tetanic potentiation [Medendorp et al., 2018]. Decreased branching of apical/basal dendrites and spine density was also seen in hippocampal field CA1 neurons in male and female mice subjected to SI [Liu et al., 2020]. Scala et al. [2018] demonstrated the involvement of glycogen synthase kinase 3 β (GSK3 β) and voltage-gated sodium channels (Nav1.6) in the control of neuroplasticity in chronic SI and an enriched environment. Transcriptome studies in the nucleus accumbens in rats in SI and an enriched environment revealed low levels of GSK3 β and SCN8A (sodium voltage-gated channel α) mRNA, which can be regarded as a sign of the operation of protective mechanisms linked with reductions in the excitability of medium spiny neurons. Levels of GSK3 β activity and the state of Nav1.6 reflect the excitability of medium spiny neurons. Silencing of GSK3 β activity protects neurons from abortive plasticity in SI [Scala et al., 2018].

The role of monoamines. Social isolation decreased transcription of the genes for all postsynaptic 5-HT receptors – 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{3A}, 5-HT₆, and 5-HT₇ – in the prefrontal cortex and 5-HT_{1B}, 5-HT_{2A},

and 5-HT_{2C} receptors in the hypothalamus and midbrain. Only overexpression of 5-HT₆ receptor occurred in the hippocampus. There were no differences in transcription of the 5-HT transporter gene as compared with controls [Bibancos et al., 2007]. SI had no effect on the basal extracellular serotonin level in the nucleus accumbens [Howes et al., 2000], though it decreased basal 5-HT turnover [Heidbreder et al., 2000]. Social isolation also had no effect on the basal extracellular serotonin level in the prefrontal cortex, but decreased its metabolism [Holson et al., 1988]. Krupina et al. [2019] showed that two-month isolation of rats starting at early age decreased the serotonin level in the hippocampus and increased metabolism in terms of the 5-HIAA/5-HT ratio. Three-month SI increased the serotonin level and decreased its turnover in the amygdala. The frontal cortex showed a tendency to a decrease in the serotonin level. Similar data were obtained by Brenes et al. [2020] for the hippocampus, where one-month SI from early age in rats led to a decrease in the serotonin concentration and increases in the 5-HIAA concentration and serotonin turnover. Some investigators link depression-like behavior with decreased serotonin levels in the brain or, more precisely, with changes in the serotonin/kynurenine ratio [Ramírez et al., 2018]. The authors took the view that powerful and/or recurrent stress activates indole-2,3-dioxygenase (IDO) [sic], an enzyme of kynurenine metabolic pathways which increases quinolinic acid synthesis, with an associated adverse influence on serotonin synthesis. Quinolinic acid activates NMDA receptors and stimulates the secretion of proinflammatory interleukins (IL-6 and IL-1 β). HHAAS activity increases and tryptophan metabolism is shifted towards production of quinolinic acid and interleukins, which results in an even greater reduction in serotonin synthesis and consolidates depression-like behavior [Ramírez et al., 2018].

A number of studies have shown that SI increases extracellular dopamine levels [Whitaker et al., 2013; Heidbreder et al., 2009; Hall et al., 1998], though in some cases this did not occur [Brenes and Fornaguera, 2009; Miura et al., 2002]. In male rats, SI had no effect on extracellular dopamine levels in the nucleus accumbens [Hall et al., 1998; Howes et al., 2000; Karkhanis et al., 2014], though basal dopamine turnover increased. In addition, electrical stimulation, electrocutaneous shocks, and systemic administration of amphetamine, ethanol and cocaine increased dopamine release in the nucleus accumbens in male isolants as compared with control animals. The basal dopamine level in the prefrontal cortex did not change in response to SI [Powell et al., 2003; Holson et al., 1998], though dopamine turnover decreased. Social isolation decreased the basal dopamine level in the basolateral amygdala [Karkhanis et al., 2015] but increased its turnover [Heidbreder et al., 2000]. In contrast to changes affecting 5-HT receptors in response to SI, there were no changes in the sensitivity of expression of dopamine receptors [Walker et al., 2019]. Although recent data [Begni et al., 2020a] indicated small changes in the expression of D₁ and

D₂ receptors in response to SI in adolescence with a trend to decreased expression, SI in adulthood increased the level of expression of D₂ receptor mRNA.

Thus, these results provide evidence that SI influences the levels and turnover of serotonin and dopamine in various brain structures. This again confirms their important role in the development of anxious and depressive disorders, while the changes seen in neurotransmitter activity and metabolism evidently reflect one of the many pathological components linking the influences of SI with these disorders.

Sex Differences in the Effects of Social Isolation. At least three factors may influence sex differences in the effects of social isolation. The first consists of the characteristics of the course of the neuroinflammatory process and activation of the immune system.

Social isolation for six weeks in the social vole has been shown to produce anxiety-like behavior, microgliolysis, and specific changes to neurons and neurochemical reactions in individual brain structures depending on the sex of the animal [Donovan et al., 2020]. Furthermore, SI impaired the gut microbiome, which is tightly associated with key brain functions and behavior. The authors suggested that SI alters the gut-immune system-brain axis depending on the animal's sex, and the gut microbes, central glial cells, and neurochemical systems together play a critical integrative role in mediating the adverse fluence of social isolation [Donovan et al., 2020]. Early SI for eight days, apart from depression-like and anxious behavior, caused microglial death and dystrophy in the hippocampus [Gong et al., 2018]. Preservation of the microglia and their proliferation protect against the development of behavioral impairments. Microglial reactions are assessed in terms of the levels of expression of CD200R (a glycoprotein CD200-binding membrane receptor, which suppresses immune activity) and CX3CL1 (chemokine/fractalkine, for detail see review by Villa et al. [2016]) in the hippocampus. CD200R and CX3CL1 are expressed on microglia. Social isolation at early age increases the expression of a microglial activation marker (Iba1 – ionized calcium-binding adapter molecule 1) in the hippocampus and decreases the expression of microglial receptor CD200R, which promotes the quiescent state of microglia [Wang et al., 2017]. The interaction between CD200R and CX3CL1 ligands and the corresponding receptors on microglia leads to inhibition of the inflammatory process. As stress decreases CD200R and CX3CL1, it can sensitize neuroinflammation by decreasing neuronal inhibition of microglia. The CD200/CD200R and CX3CL1/CX3CR1 dyads support normal neuron-glial crosstalk. This interaction is impaired by stress (CD200R and CX3CL1 decrease), with the result that neuronal inhibition of microglia declines, such they cease to be controlled, produced large quantities of proinflammatory cytokines, prolonging and exacerbating the inflammatory process. After stress, males showed increased in vivo microglial activation and potentiation of ex vivo microglial reactions to repeat stress [Frank

et al., 2007]. This is evidence that microglia in males are highly sensitive to neuroinflammation. In females, microglia did not display any sensitization effect. Resistance to the neuroinflammatory process in females in response to repeat stress was mediated not by changes in microglial reactivity but by some other mechanisms [Fonken et al., 2018]. It is interesting that during repeat stress, females showed increases in the anti-inflammatory interleukin IL-10. This provided grounds for the suggestion that the immune system in females may function as a buffer to ease responses to repeat stress by activating the anti-inflammatory pathways [Barrientos et al., 2019].

The second possible cause of sex differences in reactions to social isolation, as already noted, is the life period during which SI is applied, as this influence modifies or completely changes the types of communication and interaction between subjects typical of each stage of development [Shao et al., 2013]. Males are more sensitive to isolation at early age, losing the adolescence-typical forms of social interaction as play behavior, roughhouse, exploratory activity, etc. Females are less sensitive to the influences of SI on their future behavioral reactions in terms of these indicators than males. Finally, the third very important factor in sex differences under the influence of SI probably consist of sex hormones themselves. The effects of sex hormones are often assessed using the ovariectomy (OE) model.

Confirming the above, SI in adolescence, with rare exceptions [Weintraub et al., 2010; Thorsell et al., 2006], induced anxious behavior in the EPM and OF in males but not females. Pisu et al. [2016] also showed that SI induced anxiety-like behavior only in males. This behavior was not seen in females, possibly because of the protective effects of sex hormones. Acute stress increased corticosterone levels in socially isolated males and females, though the rise in males was greater than that in females [Pisu et al., 2016]. At the same time, opposite results were obtained by Bourke and Neigh [2011], who kept rats in isolation during the adolescent period (days 37–49) and then tested their levels of anxious and depression-like behavior in late adolescence (days 48–57) and adulthood (days 96–104). The results showed that only females, both in late adolescence and adulthood, displayed anxious and depression-like behavior in the sucrose preference and forced swimming tests, and only these animals showed anomalous corticosterone reactions to acute stress in forced swimming. Males subjected to SI in adolescence did not display signs of anxious or depression-like behavior at any life stage [Bourke and Neigh, 2011]. True, it has to be said that SI in this study was used not in pure form, but in combination with limited stress and social defeat stress. Similarly, but in somewhat different conditions, Nishinaka et al. [2015], applied SI to mice simultaneously with weaning from mothers at 2–3 weeks. Mouse pups were removed from their mothers on days 15–21 of life and were placed in individual cages for six hours/day; starting from day 21 of life they were kept constantly

in sole-occupancy cages. In other words, social isolation started before weaning from nursing mothers and continued after completion of suckling. At nine weeks, the sciatic nerve was ligated to induce neuropathic pain. Depression-like behavior in the FST at week 12 of life was seen only in females, not in males. In our study [Pavlova et al., 2021], we also observed depression-like behavior in the sucrose preference test only in females exposed to SI from day 45 to day 105 of life, though the effect of isolation was clearly apparent only in combination with early proinflammatory LPS stress. This behavior was not seen in males. We did not see any significant differences in anxiety levels between males and females which had been in SI, though they had higher anxiety indicators than control animals [Pavlova et al., 2021]. Similar results were obtained by Caruso et al. [2017]; in their experiments, repeated episodes of isolation of ALB/cJ mice in adolescence alternating with social novelty for four weeks increased anxiety in males and females, while depression-like behavior was seen only in females.

The literature contains a number of studies demonstrating the role of OE (alone and combined with other stressors) in the development of anxious and depressive disorders and the protective functions of estrogens. Ge et al. [2020] found a significant increase in microglial cells in the prefrontal cortex after OE, with activation of a large number of proinflammatory cytokines and prooxidant genes. Depression-like behavior was found to be induced by OE over long periods of time [Khayum et al., 2020]. Administration of estradiol or ER β -receptors in the hippocampus decreased OE-induced depression-like behavior at 12 weeks [Bastos et al., 2015]. All these data provide evidence of the important protective function of estrogens in relation to the manifestations of anxious-depressive disorders. Unfortunately, our literature search revealed no studies combining the influence of SI with the effects of OE. However, one study [Ramos-Ortolaza et al., 2017] addressed the interaction of chronic SI with the influences of ovarian hormones on anxious and depression-like behavior and the expression of glucocorticoid receptors in the hippocampus and hypothalamus during the normal cycle in female rats. Animals in SI for eight weeks were tested in diestrus (the resting state with minimal estrogen and progesterone levels), estrus, and proestrus (high hormone levels). Levels of depression-like behavior in females in isolation were found to be higher than in control animals regardless of the phase of the cycle. However, levels of anxious behavior were lower in estrus, which was accompanied by decreased expression of GC receptors in the dentate fascia and hippocampal field CA1 [Ramos-Ortolaza et al., 2017].

Interaction of Social Isolation with Other Types of Stress. As the body can be subject to other stresses (for example, intoxication, infection, etc.) throughout life, including during social isolation, it is important to discuss how the effects of different forms of stress interact with each other and what the sequelae of such interactions are. Weak

short-term stresses in some cases can have positive influences on subsequent responses by “hardening” the body. In other cases, the effects of different stresses can accumulate and the overall negative effect increases. For example, in mice kept in SI and in groups, moderate chronic variable stress for six weeks increased corticosterone levels in response to acute restraint stress, though only in the groups of rats which had experienced social isolation [Heck et al., 2020]. When pregnant females were given a toxin inducing an inflammatory reaction and the interaction of the effects of this toxin with the effects of SI were subsequently assessed in the offspring in adolescence, the inflammatory process during pregnancy was found not to increase the effects of isolation, but to decrease them [Goh et al., 2020]. The hippocampus of adolescent rats subjected to dual stress contained more oxytocin than after single stress, which also points to persistence of the influence of the first stress on the second. Chronic unpredictable stress in rats kept in social isolation was found to alter the balance of “arousal-inhibition” processes and made behavior more active than in the control group, which was apparent as an increase in exploratory activity, a focus on positive reinforcement, and weakening of fear reactions [Sequeira-Cordero et al., 2020]. The combination of different stresses in early childhood and adulthood do not necessarily have to lead to strengthening of the second stress, particularly when the adverse nature of the stresses in childhood and adulthood coincide. When the first and repeat stresses are in different directions, results are not always in the same direction [Santarelli et al., 2017]. The authors showed that early intervention of stressful or other nature do not always lead to strengthening of the reaction to the repeated stress, such that enhancement of the effect may be replaced by resistance to the second stress after the experience of stress at young age. Male rats aged three months were kept in SI in standard control conditions [Viana Borges et al., 2019]. At one month, half the rats of each group were subjected to chronic unpredictable stress for 18 days. Social isolation strengthened HDAC5 expression, decreased H3K9 and H4K12 acetylation, decreased the BDNF level, and weakened long-term memory. Social isolation induced anxious behavior. Combination of the two stresses increased only HDAC5 expression [Viana Borges et al., 2019]. The effects of one stress on the other can depend on the strain of mice studied [Painsipp et al., 2011]. Thus, CD1 animals kept in groups and given the bacterial toxin LPS with behavior testing in the FST the next day and at 28 days showed clear depression-like behavior, which was not seen in rats kept in SI. Conversely, C57BL/6 mice kept in SI showed signs of depression-like behavior. These were maintained for four weeks and were additionally apparent in the sucrose preference test. This behavior was also seen in mice kept in groups [Painsipp et al., 2011]. Social isolation for 2–4 weeks (but not in the first week) increased the effects of LPS administration (10 µg/kg) in mice in relation to the “illness state” and increased levels of corticosterone, cytokine IL-6, tumor necrosis factor α , and IL-10.

However, no synergistic effects in relation to other stressors (restraint stress, tail clipping, loud noises) were seen [Gibb et al., 2008]. In mice subjected to SI for two weeks and then placed in groups, administration of the toxin polyI:C increased behavioral effects (the “illness state”) and levels of corticosterone and cytokines IL-6 and IL-10 as compared with animals given polyI:C alone [Gandhi et al., 2007]. Miura et al. [2009] kept mice in SI conditions for four weeks from day 21 of life and gave LPS; tryptophan, serotonin, and kynurenine contents were measured in the prefrontal cortex, hippocampus, amygdala, and dorsal raphe nuclei. Kynurenine (KYN) is induced by the enzyme indoleamine 2,3-dioxygenase (IDO) and is a key product of tryptophan metabolism. Activity in the inflammatory process is shifted towards kynurenine, which leads to the development of depression-like behavior. Social isolation alone decreased the kynurenine/serotonin ratio in the amygdala and raphe nuclei. Dual stress (LPS + SI) increased the kynurenine/serotonin ratio in all structures except the raphe nuclei. That is, SI alone displaced activity towards serotonin and dual stress towards kynurenine [Miura et al., 2009]. At the behavioral level, this partially coincided with our data [Pavlova et al., 2021], where administration of LPS on days 3 and 5 of life to rats kept in social isolation from day 45 to day 105 induced the strongest negative effects. Social isolation for six and 28 days increased the proinflammatory effects of LPS in mice [Peterman et al., 2020]. The combined influences of six-week moderate chronic unpredictable stress in male mice with a further six weeks of social isolation induced clear depression-like behavior with an enhanced microglial reaction in the DF of the ventral hippocampus and strengthened astrocyte responses in the ventral and dorsal parts of the DF of the dorsal hippocampus [Du Preez et al., 2020b].

The Role of Factors Ameliorating the Negative Sequelae of Social Isolation. Many years ago we advanced the hypothesis that depressive disorders are based on impairments to memory mechanisms [Grigoryan, 2005]. Memory is the central nucleus of the functional system closing all its other elements, including the influx of information from the surrounding world, motivation, reinforcement, and action. The normal functioning of memory supports appropriate operation of the functional system and vice versa. “Breakdowns” in any element of the functional system lead to impairment to memory mechanisms and ultimately to the development of a whole series of psychopathologies, including depressive disorders [Grigoryan, 2005; Grigoryan and Gulyaeva, 2015]. From our hypothesis it follows that any influence countering these “breakdowns” must maintain the operation of the functional system and memory at the normal level and protect the body from developing depressive and other mental disorders. This requires firstly an adequate influx of information from the surrounding world, which in rodent experiments is achieved using an environment enriched with various stimuli and objects. Secondly,

motivation for the animal's behavior must be increased, which is achieved by exploratory activity (novelty), play behavior (social contacts), and other types of social interaction. The animal's activity overall and movement activity in particular is of no little importance. Activity not only maintains body tone at a high level, but also promotes solution of tasks leading to useful adaptive results and positive emotions. Movement activity is supported in animal experiments by physical training, running in a wheel, and a great variety of objects within the cage (ladders, cardboard boxes, etc.), facilitating and motivating movement. We will now consider published data on the influences of an enriched environment, physical exercise, and resocialization on animal behavior in tests assessing anxious and depression-like behavior as compared with animals kept in standard conditions and social isolation.

Brenes et al. [2020] subjected rats from day 21 of life to social isolation for a month. The animals were then divided into groups which were kept for a further month in conditions of performing physical exercise (running in a wheel) with an enriched environment (EE) and treated with fluoxetine (a serotonin reuptake inhibitor). Social isolation induced anxious-depressive behavior in the OF, anhedonia in the SPT, and depression-like behavior in the FST. Only EE induced anxiolytic effects and decreased anhedonia. Administration of fluoxetine decreased depression-like behavior. Physical exercise occupied an intermediate position in terms of the strength of its influence on the behavioral reactions of interest. The authors took the view that physical exercise and environmental enrichment were more effective factors in eliminating the effects of SI than medication with fluoxetine [Brenes et al., 2020]. Park et al. [2020] showed that rats in SI from day 21 of life for six weeks responded to vigorous swimming exercise (60 min/day, six days/week, six weeks in a row) with weakening of anxious and depression-like behavior. Swimming exercise suppressed apoptosis, enhanced neurogenesis, and increased serotonin [Park et al., 2020]. The possibility of preventing the negative effects of social isolation using an enriched environment simultaneously with SI and subsequently – first SI and then the EE – was explored in social voles. In both cases, the EE prevented the development of anxiety behavior in the OF and EPM and depression-like behavior in the FST. The EE was more effective than physical exercise and handling in decreasing the negative effects of SI [Grippio et al., 2014; Cirulli et al., 2010]. Anxious behavior in the OF and EPM in rats subjected to SI for three months combined with physical exercise (running in a wheel) was weaker than in rats with SI without physical exercise. The latter partially restored hippocampus BDNF and NGF levels which had decreased in response to SI [Okudan and Belvirani, 2017]. Another study addressed the influences of different accommodation conditions on anxious and depression-like behavior in response to the serotonin reuptake inhibitors serotonin and sertraline [Yildirim et al., 2016]. The authors took the

view that one characteristic of this work was that rats were housed in different-sized cages and in different numbers for social isolation (one rat per cage), standard housing (four rats/cage), and an enriched environment (12 rats/cage). In other words, the term EE refers not to an increase in the number and diversity of objects in the environment but to more opportunities for social contacts and interactions due to an increased number of animals. At six weeks, anxious and depression-like behavior was assessed in rats of different groups, and the same tests were run after a further week but with sertraline treatment. Different accommodation of animals was found to have an effect on the level of depression-like behavior but did not alter the level of anxious behavior. Sertraline reduced the level of depression-like behavior in rats housed in EE and standard conditions but not in conditions of social isolation [Yildirim et al., 2016]. Furthermore, rats of the SI group consumed more sucrose in the SPT, while sertraline produced a further increase in sucrose consumption, suggesting that sertraline had an antidepressant effect, with the same behavior in isolated rats [Yildirim et al., 2016]. Mora-Callegos and Fornaguera [2019] studied the effects of SI, EE and control conditions for one month starting on day 21 of life on anxiety behavior in the OF and EPM. Animals kept in the EE showed lower levels of anxiety than other groups of rats. In adulthood, the animals' housing conditions were changed to the opposite and they were again tested in the OF. Behavior in the new housing conditions corresponded more to the new conditions, i.e., rats of the EE group, previously kept in SI, now started to show the effects of the EE rather than the effects of SI [Mora-Callegos and Fornaguera, 2019]. Combined use of handling (in this context, taking in the hands to relieve anxiety, rather than taming) and the EE on the one hand and handling and SI on the other had opposite influences on the separate effects of the EE and SI in the EPM and OF [Pritchard et al., 2013]. Thus, SI starting from day 21 and continuing to adulthood increased anxiety levels in terms of the time spent by the animals in the open arms of the maze; the EE at the same times and to the same age decreased anxiety in rats. Addition of short episodes of daily handling to EE conditions for four days increased anxiety levels in rats of the EE group and marginally decreased anxiety in rats of the SI group [Pritchard et al., 2013]. In fairness, it must be said that the EE did not always produce anxiolytic effects [Mileva and Bielajew, 2015; Yildirim et al., 2012], and in some cases led to increased anxiety [Pietro Paolo et al., 2006]. In our experiments [Pavlova et al., 2021], the EE had no significant influence on anxiety levels in control rats but induced a minor increase in movement activity and exploratory behavior. In males of the LPS group, these changes were not seen with the EE, while females showed an unexpected increase in the anxiety level in the open field. Similarly, the EE acted on females of the LPS group as a stressing factor, with effects resembling those of SI. Most studies of the effects of the EE on behavior used animals

placed in an enriched environment as permanent housing, while in our studies rats were kept in the EE for only 20 min every other day, which evidently produced a weaker effect and no influence on anxiety was seen even in the control groups. Nonetheless, an effect was still seen, as the animals of all groups kept in the EE had greater weight than rats kept in standard conditions, which may be associated with an increase in muscle mass. Resocialization of animals had positive effects on the negative sequelae of SI, but without affecting all the adverse actions of SI. Particularly stable were SI-induced aggressivity, though the deficit in social communication under the influence of resocialization returned to the normal level [Tulogdi et al., 2014].

Conclusions. Thus, animal experiments have shown that social isolation is a strong stressor and that the intensity of its influences depends on numerous factors, especially the duration of isolation and the period of life at which it acted. The period most sensitive to social isolation is early adolescence, when formation of the main structures and functions remains incomplete, including those directly related to the stress system, immune and neuroinflammatory reactions, and hormones, neurotransmitters, intracellular biochemical processes. Social communication and interactions between peers promote the development of normal ontogeny and appropriate behavior in adulthood, while the lack of these interactions leads not only to the abortive development of the corresponding structures and functions but also to abnormal behavior, which in its extreme forms is apparent as anxious-depressive disorders, aggressivity, memory loss, and other behavioral deviations. However, SI also has adverse sequelae in adulthood, while in old age it simply shortens life.

The development of anxious-depressive disorders in response to social isolation is not enormously different from other disorders induced by other stressors [Grigoryan et al., 2014]. Differences start with the impairment to HHAS operation. Specifically, at the level of this system there is increased synthesis and release of HVA, ACTH, and corticosterone and reduced negative feedback from the effects of corticosterone on neurons in the paraventricular nucleus of the hypothalamus. Furthermore, a neuroinflammatory process is triggered in the brain, associated with increased formation and hyperfunction of proinflammatory cytokines IL-1, IL-6, TNF- α , and other molecules. Cytokines stimulate quinolinic acid formation from tryptophan. Along with reactive nitrogen and oxygen species, quinolinic acid promotes the development of oxidative stress. Oxidative stress leads to dysregulation of the antioxidant enzymes – cytosolic copper-zinc superoxide dismutase (CuZnSOD) and mitochondrial manganese superoxide dismutase (MnSOD), both catalyzing conversion of the superoxide anion (O_2^-) to oxygen and hydrogen peroxide (H_2O_2). The latter loses its toxicity due to the activity of the enzymes catalase (CAT) and glutathione peroxidase (GPx). Increases in the corticosterone level during chronic stress decrease brain an-

tioxidant enzyme activities in rats. There is a simultaneous increase in extracellular glutamate release, weakening of its reuptake, and a sharp increase in NMDA receptor activity. Ultimately, this all leads to the development of toxic overarousal, apoptosis, demyelination, and cell death. Nitrosative stress also makes its contribution to the pathological process. Overexpression of nitric oxide NO as a result of increased nuclear nNOS and inducible iNOS synthase activities in stress increases glutamate receptor activity. In addition, selective microglial activation due to chronic stress leads to the release of high nitric oxide concentrations, provoking nitrosative stress. There are parallel reductions in the quantity and functions of trophic factors, particularly BDNF. Along with insufficient neurotrophic function of growth factors, neurogenesis is significantly weakened. BDNF deficit and reduced neurogenesis promote cell death in the hippocampus. This produces the typical plastic rearrangements in the form of decreases in the extent of the spiny apparatus and the length, branching, and extent of the dendritic tree and reductions in hippocampus volume.

Social isolation produces significant sex differences in behavior and biochemical markers. These are due to the ambiguous sensitization of the neuroinflammatory system in response to early (primary) stress in males and females. After stress, males show increased *in vivo* microglial activation and potentiation of *ex vivo* microglial reactions to repeated stress. In females, the microglia do not develop a sensitization effect. It has been suggested that the immune system in females can operate as a buffer to ameliorate reactions to repeat stress via activation of the proinflammatory pathways. Sex hormones and the age at which SI occurs play important roles in sex differences in conditions of social isolation. The combination of social isolation with other stresses leads to both increases and decreases of the effects of one stress on another. These differences in the actions of simultaneous stresses are explained by the characteristics of animal species and strain, and the effects of stresses on the different components of the pathogenesis of anxious-depressive disorders or on the same components but in opposite ways.

Factors ameliorating the adverse sequelae of social isolation and the effects of other stresses follow from the integrity of the functional system organizing the behavior [Grigoryan et al., 2005]. As anxious-depressive disorders are based on dysregulation of this system, whose central component is the apparatus of memory, correction of behavior (memory) and, thus, minimization of disorders, is achieved by normalizing the operation of this functional system. This requires: first, an adequate influx of information from the external world by means of an environment enriched with various stimuli and objects; secondly, increased motivation of the animal's behavior by means of exploratory activity (novelty) and play behavior (social contacts), and other forms of social interaction. Of no little value is the animals' movement activity. Activity not only maintains body tone at a high level, but also promotes solution of tasks leading to

useful adaptive results and positive emotions. Animal experiments use physical training (running in a wheel, swimming, etc.) to support movement activity, along with a great variety of objects in the cages (ladders, cardboard boxes, etc.) to facilitate and motivate movement. We have presented several examples of the ameliorating influence of various of these factors on the adverse effects of social isolation.

In conclusion, we would like to note that we still know little of which stresses of different types, especially at early age, act on normally developing brain structures and functions, modifying and transforming their operation into pathological processes in different directions – from anxious-depressive disorders, schizophrenia, and autism to Alzheimer's disease and Parkinson's disease. Although all these cases generally involve the same mechanisms, there are reasons for which these mechanisms are tuned to developing concrete and specific pathologies. The type of stress clearly has a significant role among these reasons. In particular, lack of maternal care leads to impairments to behavioral reactions (latent inhibition, prepulse inhibition) which are typical of manifestations in schizophrenia patients. Depression, aggressivity, and Alzheimer's disease are more associated with the influences of social isolation. The pathogenesis of many diseases induced by early stress is known to be based on impairments to normal HPA operation and the development of the neuroinflammatory process. The multicolored picture of this process, which involves tens of proinflammatory and anti-inflammatory interleukins, along with the specific features of the operation of the immune, hormonal, neurochemical, and other systems in each concrete case, support channeling of the pathological process into a specific disease. The present time is a period of painstaking collection of information on the specific concrete pathology of the adult body imposed by early stress, with serious analytical studies of the data collected. We are currently only at the beginning of this journey, though we can hope that future studies will provide answers to many questions which presently remain questions.

This work was funded by a grant from the Russian Foundation for Basic Research (project EXPANSION No. 20-115-50032).

REFERENCES

- Agrawal, P., Kao, D., Chung, P., and Looger, L. L., "The neuropeptide drosulfakinin regulates social isolation-induced aggression in *Drosophila*," *J. Exp. Biol.*, **223**, No. 2, jeb207407 (2020).
- Alshammari, T. K., Alghamdi, H., Alkhader, L. F., et al., "Analysis of the molecular and behavioral effects of acute social isolation on rats," *Behav. Brain Res.*, **377**, 112–191 (2020).
- Alshammari, T. K., Alghamdi, H., Green, T. A., et al., "Assessing the role of toll-like receptor in isolated, standard and enriched housing conditions," *PLoS One*, **14**, No. 10, e0222818 (2019).
- Amiri, S., Haj-Mirzaian, A., Rahimi-Balaei, M., et al., "Co-occurrence of anxiety and depressive-like behaviors following adolescent social isolation in male mice; possible role of nitrenergic system," *Physiol. Behav.*, **145**, 38–44 (2015).
- Arimoto, T. and Bing, G., "Up-regulation of inducible nitric oxide synthase in the substantia nigra by lipopolysaccharide causes microglial activation and neurodegeneration," *Neurobiol. Dis.*, **12**, 35–45 (2003).
- Arranz, L., Giménez-Llort, L., De Castro, N. M., et al., "Social isolation during old age worsens cognitive, behavioral and immune impairment," *Rev. Esp. Geriatr. Gerontol.*, **44**, No. 3, 137–142 (2009).
- Arzate-Mejía, R. G., Lottenbach, Z., Schindler, V., et al., "Long-term impact of social isolation and molecular underpinnings," *Front. Genet.*, **11**, 589–621 (2020).
- Barrientos, R. M., Brunton, P. J., Lenz, K. M., et al., "Neuroimmunology of the female brain across the lifespan: Plasticity to psychopathology," *Brain Behav. Immun.*, **79**, 39–55 (2019).
- Bastos, C. P., Pereira, L. M., Ferreira-Vieira, T. H., et al., "Object recognition memory deficit and depressive-like behavior caused by chronic ovariectomy can be transiently recovered by the acute activation of hippocampal estrogen receptors," *Psychoneuroendocrinology*, **57**, 14–25 (2015).
- Begni, V., Sanson, A., Pfeiffer, N., et al., "Social isolation in rats: Effects on animal welfare and molecular markers for neuroplasticity," *PLoS One*, **15**, No. 10, e0240439 (2020a).
- Berry, A., Bellisario, V., Capoccia, S., et al., "Social deprivation stress is a triggering factor for the emergence of anxiety- and depression-like behaviours and leads to reduced brain BDNF levels in C57BL/6J mice," *Psychoneuroendocrinology*, **37**, 762–772 (2012).
- Bibancos, T., Jardim, D. L., Aneas, I., and Chiavegatto, S., "Social isolation and expression of serotonergic neurotransmission-related genes in several brain areas of male mice," *Genes Brain Behav.*, **6**, No. 6, 529– (2007).
- Boero, G., Pisu, M. G., Biggio, F., et al., "Impaired glucocorticoid-mediated HPA axis negative feedback induced by juvenile social isolation in male rats," *Neuropharmacology*, **133**, 242–253 (2018).
- Bourke, C. H. and Neigh, G. N., "Behavioral effects of chronic adolescent stress are sustained and sexually dimorphic," *Horm. Behav.*, **60**, No. 1, 112–20 (2011).
- Bowie, A. and O'Neill, L. A., "Oxidative stress and nuclear factor kappaB activation: a reassessment of the evidence in the light of recent discoveries," *Biochem. Pharmacol.*, **59**, 13–23 (2000).
- Brenes, J. C. and Fornaguera, J., "The effect of chronic fluoxetine on social isolation-induced changes on sucrose consumption, immobility behavior, and on serotonin and dopamine function in hippocampus and ventral striatum," *Behav. Brain Res.*, **198**, 199–205 (2009).
- Brenes, J. C., Fornaguera, J., and Sequeira-Cordero, A., "Environmental enrichment and physical exercise attenuate the depressive-like effects induced by social isolation stress in rats," *Front. Pharmacol.*, **11**, 804 (2020).
- Brenes, J. C., Villagra, O. R., and Fornaguera, J., "Factor analysis of forced swimming test, sucrose preference test and open field test on enriched, social and isolated reared rats," *Behav. Brain Res.*, **169**, No. 1, 57–65 (2006).
- Butler, T. R., Carter, E., and Weiner, J. L., "Adolescent social isolation does not lead to persistent increases in anxiety-like behavior or ethanol intake in female Long-Evans rats," *Alcohol Clin. Exp. Res.*, **38**, 2199–2207 (2014).
- Carrier, N. and Kabbaj, M., "Testosterone and imipramine have antidepressant effects in socially isolated male but not female rats," *Horm. Behav.*, **61**, 678–685 (2012).
- Caruso, M. J., Kamens, H. M., and Cavigelli, S. A., "Exposure to chronic variable social stress during adolescence alters affect-related behaviors and adrenocortical activity in adult male and female inbred mice," *Dev. Psychobiol.*, **59**, No. 6, 679–687 (2017).
- Cassina, P., Peluffo, H., Pehar, M., et al., "Peroxynitrite triggers a phenotypic transformation in spinal cord astrocytes that induces motor neuron apoptosis," *J. Neurosci. Res.*, **67**, 21–29 (2002).
- Chang, L. Y., Slot, J. W., Geuze, H. J., and Crapo, J. D., "Molecular immunocytochemistry of the CuZn superoxide dismutase in rat hepatocytes," *J. Cell Biol.*, **107**, 2169–2179 (1988).

- Chappell, A. M., Carter, E., McCool, B. A., and Weiner, J. L., "Adolescent rearing conditions influence the relationship between initial anxiety-like behavior and ethanol drinking in male Long Evans rats," *Alcohol Clin. Exp. Res.*, **37**, Suppl. 1, E394–403 (2013).
- Chelikani, P., Fita, I., and Loewen, P. C., "Diversity of structures and properties among catalases," *Cell. Mol. Life Sci.*, **61**, 192–208 (2004).
- Chen, Y. W., Akad, A., Aderogba, R., et al., "Dendrites of the dorsal and ventral hippocampal CA1 pyramidal neurons of singly housed female rats exhibit lamina-specific growths and retractions during adolescence that are responsive to pair housing," *Synapse*, **72**, No. 7, e22034 (2018).
- Chmelova, M., Balagova, L., Marko, M., et al., "Behavioral alterations induced by post-weaning isolation rearing of rats are accompanied by reduced VGF/BDNF/TrkB signaling in the hippocampus," *Neurochem. Int.*, 104473 (2019).
- Cirulli, F., Berry, A., Bonsignore, L. T., et al., "Early life influences on emotional reactivity: evidence that social enrichment has greater effects than handling on anxiety-like behaviors, neuroendocrine responses to stress and central BDNF levels," *Neurosci. Biobehav. Rev.*, **34**, No. 6, 808–820 (2010).
- Colaïanna, M., Schiavone, S., and Zotti, M., et al., "Neuroendocrine profile in a rat model of psychosocial stress: relation to oxidative stress," *Antioxid. Redox. Signal.*, **18**, 1385–1399 (2013).
- Connelly, L., Palacios-Callender, M., Ameixa, C., et al., "Biphasic regulation of NF-kappa B activity underlies the pro- and antiinflammatory actions of nitric oxide," *J. Immunol.*, **166**, 3873–3881 (2001).
- Djordjevic, J., Djordjevic, A., Adzic, M., and Radojic, M. B., "Effects of chronic social isolation on Wistar rat behavior and brain plasticity markers," *Neuropsychobiology*, **66**, No. 2, 112–119 (2012).
- Donovan, M., Mackey, C. S., Platt, G. N., et al., "Social isolation alters behavior, the gut-immune-brain axis, and neurochemical circuits in male and female prairie voles," *Neurobiol. Stress*, **13**, 100278 (2020).
- Du Preez, A., Law, T., Onorato, D., et al., "The type of stress matters: repeated injection and permanent social isolation stress in male mice have a differential effect on anxiety- and depressive-like behaviours, and associated biological alterations," *Transl. Psychiatry*, **10**, No. 1, 325 (2020a).
- Du Preez, A., Onorato, D., Eiben, I., et al., "Chronic stress followed by social isolation promotes depressive-like behaviour, alters microglial and astrocyte biology and reduces hippocampal neurogenesis in male mice," *Brain Behav. Immun.*, S0889-1591(20)30126-4 (2020b).
- Dunphy-Doherty, F., O'Mahony, S. M., Peterson, V. L., et al., "Post-weaning social isolation of rats leads to long-term disruption of the gut microbiota-immune-brain axis," *Brain Behav. Immun.*, 261–273 (2018).
- Einon, D. F. and Morgan, M. J., "A critical period for social isolation in the rat," *Dev. Psychobiol.*, **10**, 123–132 (1977).
- Evans, J., Sun, Y., McGregor, A., and Connor, B., "Alloprengnanolone regulates neurogenesis and depressive/anxiety-like behaviour in a social isolation rodent model of chronic stress," *Neuropharmacology*, **63**, No. 8, 1315–1326 (2012).
- Ferland, C. L. and Schrader, L. A., "Cage mate separation in pair-housed male rats evokes an acute stress corticosterone response," *Neurosci. Lett.*, **489**, 154–158 (2011).
- Filipović, D. and Pajović, S. B., "Differential regulation of CuZnSOD expression in rat brain by acute and/or chronic stress," *Cell. Mol. Neurobiol.*, **29**, 673–681 (2009).
- Filipović, D., Todorović, N., Bernardi, R. E., and Gass, P., "Oxidative and nitrosative stress pathways in the brain of socially isolated adult male rats demonstrating depressive- and anxiety-like symptoms," *Brain Struct. Funct.*, **222**, No. 1, 1–20 (2017).
- Fonken, L. K., Frank, M. G., Gaudet, A. D., et al., "Neuroinflammatory priming to stress is differentially regulated in male and female rats," *Brain Behav. Immun.*, **70**, 257–267 (2018).
- Frank, M. G., Baratta, M. V., Sprunger, D. B., et al., "Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses," *Brain Behav. Immun.*, **21**, No. 1, 47–59 (2007).
- Gandhi, R., Hayley, S., Gibb, J., et al., "Influence of poly I:C on sickness behaviors, plasma cytokines, corticosterone and central monoamine activity: moderation by social stressors," *Brain Behav. Immun.*, **21**, No. 4, 477–89 (2007).
- Ge, F., Yang, H., Lu, W., et al., "Ovariectomy induces microglial cell activation and inflammatory response in rat prefrontal cortices to accelerate the chronic unpredictable stress-mediated anxiety and depression," *Biomed. Res. Int.*, **2020**, 3609758 (2020).
- Gibb, J., Hayley, S., Gandhi, R., et al., "Synergistic and additive actions of a psychosocial stressor and endotoxin challenge: Circulating and brain cytokines, plasma corticosterone and behavioral changes in mice," *Brain Behav. Immun.*, **22**, No. 4, 573–589 (2008).
- Giustarini, D., Rossi, R., Milzani, A., et al., "S-glutathionylation: from redox regulation of protein functions to human diseases," *J. Cell. Mol. Med.*, **8**, 201–212 (2004).
- Goh, J. Y., O'Sullivan, S. E., Shortall, S. E., et al., "Gestational poly(I:C) attenuates, not exacerbates, the behavioral, cytokine and mTOR changes caused by isolation rearing in a rat 'dual-hit' model for neurodevelopmental disorders," *Brain Behav. Immun.*, **89**, 100–117 (2020).
- Gong, Y., Tong, L., Yang, R., et al., "Dynamic changes in hippocampal microglia contribute to depressive-like behavior induced by early social isolation," *Neuropharmacology*, **135**, 223–233 (2018).
- Gorlova, A. V., Pavlov, D. A., Zubkov, E. A., et al., "Three-week isolation does not lead to depressive-like disorders in rats," *Bull. Exp. Biol. Med.*, **165**, No. 2, 181–183 (2018).
- Grigoryan, G. A. and Gulyaeva, N. V., "Modeling of depression in animals: behavior as a methodological basis, assessment criteria, and classification," *Zh. Vyssh. Nerv. Deyat.*, **65**, No. 6, 643–660 (2015).
- Grigoryan, G. A., "Memory and depression," *Zh. Vyssh. Nerv. Deyat.*, **56**, No. 4, 556–570 (2005).
- Grigoryan, G. A., Dygalo, N. N., Gekht, A. B., et al., "Molecular and cellular mechanisms of depression. The role of glucocorticoids, cytokines, and neurotrophic factors in the genesis of depressive disorders," *Usp. Fiz. Nauk*, **44**, No. 2, 3–20 (2014).
- Grippo, A. J., Ihm, E., Wardwell, J., et al., "The effects of environmental enrichment on depressive and anxiety-relevant behaviors in socially isolated prairie voles," *Psychosom. Med.*, **76**, No. 4, 277–284 (2014).
- Guarnieri, L. O., Pereira-Caxeta, A. R., Medeiros, D. C., et al., "Pro-neurogenic effect of fluoxetine in the olfactory bulb is concomitant to improvements in social memory and depressive-like behavior of socially isolated mice," *Transl. Psychiatry*, **10**, No. 1, 33 (2020).
- Guo, M., Wu, C. F., Liu, W., et al., "Sex difference in psychological behavior changes induced by long-term social isolation in mice" *Prog. Neuropsychopharm. Biol. Psychiatry*, **28**, No. 1, 115–121 (2004).
- Haj-Mirzaian, A., Amiri, S., Amini-Khoei, H., et al., "Attenuation of oxidative and nitrosative stress in cortical area associates with antidepressant-like effects of tropisetron in male mice following social isolation stress," *Brain Res. Bull.*, **124**, 150–63 (2016).
- Hall, F. S., "Social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioral consequences," *Crit. Rev. Neurobiol.*, **12**, No. 1–2, 129–162 (1998).
- Hall, F. S., Huang, S., Fong, G. F., and Pert, A. J., "The effects of social isolation on the forced swim test in Fawn hooded and Wistar rats," *Neurosci. Methods*, **79**, No. 1, 47–51 (1998).
- Halliwell, B. and Gutteridge, J. M. C., *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford (1989), 2nd ed.
- Han, X., Wang, W., Xue, X., et al., "Brief social isolation in early adolescence affects reversal learning and forebrain BDNF expression in adult rats," *Brain Res. Bull.*, **86**, No. 3–4, 173–178 (2011).
- Heck, A. L. and Handa, R. J., "Sex differences in the hypothalamic-pituitary-adrenal axis' response to stress: an important role for gonadal hormones" *Neuropsychopharmacology*, **44**, No. 1, 45–58 (2019).

- Heck, A. L., Sheng, J. A., Miller, A. M., et al., "Social isolation alters hypothalamic pituitary adrenal axis activity after chronic variable stress in male C57BL/6 mice," *Stress*, **23**, No. 4, 457–465 (2020).
- Heidbreder, C. A., Weiss, I. C., Domeney, A. M., et al., "Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome," *Neuroscience*, **100**, 749–768 (2000).
- Holgate, J. Y., Garcia, H., Chatterjee, S., and Bartlett, S. E., "Social and environmental enrichment has different effects on ethanol and sucrose consumption in mice," *Brain Behav.*, **7**, No. 8, e00767 (2017).
- Holson, R. R., Scallet, A. C., Ali, S. F., et al., "Adrenocortical, beta-endorphin and behavioral responses to graded stressors in differentially reared rats," *Physiol. Behav.*, **42**, 125–130 (1988).
- Hong, S., Flashner, B., Chiu, M., et al., "Social isolation in adolescence alters behaviors in the forced swim and sucrose preference tests in female but not in male rats," *Physiol. Behav.*, **105**, No. 2, 269–275 (2012).
- Howes, S. R., Dalley, J. W., Morrison, C. H., et al., "Leftward shift in the acquisition of cocaine self-administration in isolation-reared rats: relationship to extracellular levels of dopamine, serotonin and glutamate in the nucleus accumbens and amygdala-striatal FOS expression," *Psychopharmacology*, **151**, 55–63 (2000).
- Huang, H., Wang, L., Cao, M., et al., "Isolation housing exacerbates Alzheimer's disease-like pathophysiology in aged APP/PS1 mice," *Int. J. Neuropsychopharmacol.*, **18**, No. 7, pyu116 (2015).
- Huang, Q., Zhou, Y., and Liu, L. Y., "Effect of post-weaning isolation on anxiety- and depressive-like behaviors of C57BL/6J mice," *Exp. Brain Res.*, **235**, No. 9, 2893–2899 (2017).
- Ibi, D., Takuma, K., Koike, H., et al., "Social isolation rearing-induced impairment of the hippocampal neurogenesis is associated with deficits in spatial memory and emotion-related behaviors in juvenile mice," *J. Neurochem.*, **105**, No. 3, 921–932 (2008).
- Ieraci, A., Mallei, A., and Popoli, M., "Social isolation stress induces anxious-depressive-like behavior and alterations of neuroplasticity-related genes in adult male mice," *Neural Plast.*, 6212983 (2016).
- Jahng, J. W., Yoo, S. B., Ryu, V., and Lee, J. H., "Hyperphagia and depression-like behavior by adolescence social isolation in female rats," *Int. J. Dev. Neurosci.*, **30**, 47–53 (2012).
- Karkhanis, A. N., Alexander, N. J., McCool, B. A., et al., "Chronic social isolation during adolescence augments catecholamine response to acute ethanol in the basolateral amygdala," *Synapse*, **69**, No. 8, 385–395 (2015).
- Karkhanis, A. N., Locke, J. L., McCool, B. A., et al., "Social isolation rearing increases nucleus accumbens dopamine and norepinephrine responses to acute ethanol in adulthood," *Alcohol Clin. Exp. Res.*, **38**, 2770–2779 (2014).
- Khayum, M. A., Moraga-Amaro, R., Buwalda, B., et al., "Ovariectomy-induced depressive-like behavior and brain glucose metabolism changes in female rats are not affected by chronic mild stress," *Psychoneuroendocrinology*, **115**, 104610 (2020).
- Krupina, N. A., Khlebnikova, N. N., Narkevich, V. B., et al., "Levels of monoamines and their metabolites in brain structures in rats in social isolation for two and three months," *Bull. Exp. Biol. Med.*, **168**, No. 11, 540–545 (2019).
- Krupina, N. A., Shirenova, S. D., and Khlebnikova, N. N., "Prolonged social isolation, started early in life, impairs cognitive abilities in rats depending on sex," *Brain Sci.*, **10**, No. 11, 799 (2020), <https://doi.org/10.3390/brainsci10110799>.
- Kuleshkaya, N., Rauvala, H., and Voikar, V., "Evaluation of social and physical enrichment in modulation of behavioural phenotype in C57BL/6J female mice," *PLoS One*, **6**, No. 9, e24755 (2011).
- Kumari, A., Singh, P., Baghel, M. S., and Thakur, M. K., "Social isolation mediated anxiety like behavior is associated with enhanced expression and regulation of BDNF in the female mouse brain," *Physiol. Behav.*, **158**, 34–42 (2016).
- Li, H. and Xia, N., "The role of oxidative stress in cardiovascular disease caused by social isolation and loneliness," *Redox Biol.*, 101585 (2020).
- Lieberwirth, C., Liu, Y., Jia, X., and Wang, Z., "Social isolation impairs adult neurogenesis in the limbic system and alters behaviors in female prairie voles," *Horm. Behav.*, **62**, No. 4, 357–366 (2012).
- Liu, N., Wang, Y., An, A. Y., et al., "Single housing-induced effects on cognitive impairment and depression-like behavior in male and female mice involve neuroplasticity-related signaling," *Eur. J. Neurosci.*, **52**, No. 1, 2694–2704 (2020).
- Lu, L., Bao, G., Chen, H., et al., "Modification of hippocampal neurogenesis and neuroplasticity by social environments," *Exp. Neurol.*, **183**, 600–609 (2003).
- Lukkes, J. L., Engelman, G. H., Zelin, N. S., et al., "Post-weaning social isolation of female rats, anxiety-related behavior, and serotonergic systems," *Brain Res.*, **1443**, 1–17 (2012).
- Lukkes, J. L., Summers, C. H., Scholl, J. L., et al., "Early life social isolation alters corticotropin-releasing factor responses in adult rats," *Neuroscience*, **158**, 845–855 (2009).
- Madrigal, J. L. M., García-Bueno, B., Caso, J. R., et al., "Stress-induced oxidative changes in brain," *CNS Neurol. Disord. Drug Targets*, **5**, 561–568 (2006).
- Maes, M., Mihaylova, I., and Bosmans, E., "Not in the mind of neurasthenic lazybones but in the cell nucleus: patients with chronic fatigue syndrome have increased production of nuclear factor kappa beta," *Neuro Endocrinol. Lett.*, **28**, 456–462 (2007).
- Medendorp, W. E., Petersen, E. D., Pal, A., et al., "Altered behavior in mice socially isolated during adolescence corresponds with immature dendritic spine morphology and impaired plasticity in the prefrontal cortex," *Front. Behav. Neurosci.*, **12**, 87 (2018).
- Meng, Q., Li, N., Han, X., et al., "Effects of adolescent social isolation on the expression of brain-derived neurotrophic factors in the forebrain," *Eur. J. Pharmacol.*, **650**, No. 1, 229–232 (2011).
- Meyer, M., Schreck, R., and Baeuerle, P., "AH2O2 and antioxidants have opposite effects on activation of NF-kappa B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor," *EMBO J.*, **12**, 2005–2015 (1993).
- Mileva, G. R. and Bielajew, C., "Environmental manipulation affects depressive-like behaviours in female Wistar-Kyoto rats," *Behav. Brain Res.*, **293**, 208–216 (2015).
- Mileva, G. R., Rooke, J., Ismail, N., and Bielajew, C., "Corticosterone and immune cytokine characterization following environmental manipulation in female WKY rats," *Behav. Brain Res.*, **316**, 197–204 (2017).
- Miura, H., Qiao, H., and Ohta, T., "Attenuating effects of the isolated rearing condition on increased brain serotonin and dopamine turnover elicited by novelty stress," *Brain Res.*, **926**, No. 1–2, 10–17 (2002).
- Miura, H., Shirokawa, T., Isobe, K., and Ozaki, N., "Shifting the balance of brain tryptophan metabolism elicited by isolation housing and systemic administration of lipopolysaccharide in mice," *Stress*, **12**, No. 3, 206–14 (2009).
- Mohale, D. S. and Chandewar, A. V., "Effect of social isolation on oxidative stress and transaminase level," *Asian J. Biomed. Pharmaceut. Sci.*, **2**, No. 15, 41–44 (2012).
- Mora-Gallegos, A. and Fornaguera, J., "The effects of environmental enrichment and social isolation and their reversion on anxiety and fear conditioning," *Behav. Proc.*, **158**, 59–69 (2019).
- Nishinaka, T., Kinoshita, M., Nakamoto, K., and Tokuyama, S., "Sex differences in depression-like behavior after nerve injury are associated with differential changes in brain-derived neurotrophic factor levels in mice subjected to early life stress," *Neurosci. Lett.*, **592**, 32–36 (2015).
- O'Keefe, L. M., Doran, S. J., Mwilambwe-Tshilobo, L., et al., "Social isolation after stroke leads to depressive-like behavior and decreased BDNF levels in mice," *Behav. Brain Res.*, **260**, 162–70 (2014).
- Okudan, N. and Belviranlı, M., "Long-term voluntary exercise prevents post-weaning social isolation-induced cognitive impairment in rats," *Neuroscience*, **60**, 1–8 (2017).

- Painsipp, E., Köfer, M. J., Sinner, F., and Holzer, P., "Prolonged depression-like behavior caused by immune challenge: influence of mouse strain and social environment," *PLoS One*, **6**, No. 6, e20719 (2011).
- Panksepp, J., "The ontogeny of play in rats," *Dev. Psychobiol.*, **14**, 327–332 (1981).
- Park, H. S., Kim, T. W., Park, S. S., and Lee, S. J. J., "Swimming exercise ameliorates mood disorder and memory impairment by enhancing neurogenesis, serotonin expression, and inhibiting apoptosis in social isolation rats during adolescence," *J. Exerc. Rehabil.*, **16**, No. 2, 132–140 (2020).
- Parker, V. M. A., "The socially isolated rat as a model of anxiety," *Neuropharmacology*, **25**, 663–664 (1986).
- Pavlova, I. V., Broshevitckaya, N. D., Zaichenko, M. I., and Grigoryan, G. A., "The effects of social isolation and an enriched environment on anxious-depressive behavior in rats in health and after early proinflammatory stress," *Zh. Vyssh. Nerv. Deyat.*, **71**, No. 5, 690–709 (2021).
- Peterman, J. L., White, J. D., Calcagno, A., et al., "Prolonged isolation stress accelerates the onset of Alzheimer's disease-related pathology in 5xFAD mice despite running wheels and environmental enrichment," *Behav. Brain Res.*, **379**, 112366 (2020).
- Pietro Paolo, S., Feldon, J., Alleca, E., et al., "The role of voluntary exercise in enriched rearing: a behavioral analysis," *Behav. Neurosci.*, **4**, 787–803 (2006).
- Pietro Paolo, S., Singer, P., Feldon, J., and Yee, B. K., "The postweaning social isolation in C57BL/6 mice: preferential vulnerability in the male sex," *Psychopharmacology*, **197**, 613–628 (2008).
- Pisu, M. G., Garau, A., Boero, G., et al., "Sex differences in the outcome of juvenile social isolation on HPA axis function in rats," *Neuroscience*, **320**, 172–82 (2016).
- Powell, S. B., Geyer, M. A., Preece, M. A., et al., "Dopamine depletion of the nucleus accumbens reverses isolation-induced deficits in pre-pulse inhibition in rats," *Neuroscience*, **119**, 233–240 (2003).
- Pritchard, L. M., Van Kempen, T. A., and Zimmerberg, B., "Behavioral effects of repeated handling differ in rats reared in social isolation and environmental enrichment," *Neurosci. Lett.*, **536**, 47–51 (2013).
- Ramírez, L. A., Pérez-Padilla, E. A., García-Oscos, F., et al., "A new theory of depression based on the serotonin/kynurenine relationship and the hypothalamic pituitary-adrenal axis," *Biomedica*, **38**, No. 3, 437–450 (2018).
- Ramos-Ortolaza, D. L., Doreste-Mendez, R. J., Alvarado-Torres, J. K., and Torres-Reveron, A., "Ovarian hormones modify anxiety behavior and glucocorticoid receptors after chronic social isolation stress," *Behav. Brain Res.*, **328**, 115–122 (2017).
- Ren, Q. G., Gong, W. G., Wang, Y. J., et al., "Citalopram attenuates tau hyperphosphorylation and spatial memory deficit induced by social isolation rearing in middle-aged rats," *J. Mol. Neurosci.*, **56**, No. 1, 145–153 (2015).
- Ridnour, L. A., Thomas, D. D., and Mancardi, D., "Espes, M. G., Miranda, K. M., Paolucci, N., Feelisch, M., Fukuto, J., and Wink, D. A., "The chemistry of nitrosative stress induced by nitric oxide and reactive nitrogen oxide species. Putting perspective on stressful biological situations," *Biol. Chem.*, **385**, 1–10 (2004).
- Rivera-Irizarry, J. K., Skelly, M. J., and Pleil, K. E., "Social isolation stress in adolescence, but not adulthood, produces hypersocial behavior in adult male and female C57BL/6J Mice," *Front. Behav. Neurosci.*, **14**, 129 (2020).
- Roeckner, A. R., Bowling, A., and Butler, T. R., "Chronic social instability increases anxiety-like behavior and ethanol preference in male Long Evans rats," *Physiol. Behav.*, **73**, 179–187 (2017).
- Santarelli, S., Zimmermann, C., Kalideris, G., et al., "An adverse early life environment can enhance stress resilience in adulthood," *Psychoneuroendocrinology*, **78**, 213–221 (2017).
- Scaccianoce, S., Del Bianco, P., Paolone, G., et al., "Social isolation selectively reduces hippocampal brain-derived neurotrophic factor without altering plasma corticosterone," *Behav. Brain Res.*, **68**, 323–325 (2006).
- Scala, F., Nenov, M. N., Crofton, E. J., et al., "Environmental enrichment and social isolation mediate neuroplasticity of medium spiny neurons through the GSK3 pathway," *Cell Rep.*, **23**, No. 2, 555–567 (2018).
- Schrijver, N. C., Bahr, N. I., Weiss, I. C., and Würbel, H., "Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats," *Pharmacol. Biochem. Behav.*, **73**, No. 1, 209–224 (2002).
- Sequeira-Cordero, A., Salas-Bastos, A., Fornaguera, J., and Brenes, J. C., "Behavioural characterisation of chronic unpredictable stress based on ethologically relevant paradigms in rats," *Sci. Rep.*, **9**, No. 1, 17403 (2019).
- Serra, M., Sanna, E., Mostallino, M. C., and Biggio, G., "Social isolation stress and neuroactive steroids," *Eur. Neuropsychopharmacol.*, **17**, 1–11 (2007).
- Shao, F., Han, X., Shao, S., and Wang, W., "Adolescent social isolation influences cognitive function in adult rats," *Neural Regen. Res.*, **8**, No. 11, 1025–1030 (2013).
- Shao, Y., Yan, G., Xuan, Y., et al., "Chronic social isolation decreases glutamate and glutamine levels and induces oxidative stress in the rat hippocampus," *Behav. Brain Res.*, **282**, 201–208 (2015).
- Skelly, M. J., Chappell, A. E., Carter, E., and Weiner, J. L., "Adolescent social isolation increases anxiety-like behavior and ethanol intake and impairs fear extinction in adulthood: Possible role of disrupted noradrenergic signaling," *Neuropharmacology*, **97**, 149–159 (2015).
- Spasojevic, N., Gavrilovic, L., Varagic, V. V., and Dronjak, S., "Effects of chronic diazepam treatments on behavior on individually housed rats," *Arch. Biol. Sci.*, **59**, 113–117 (2007).
- Spiers, J. G., Chen, H.-J. C., Sernia, C., and Lavidis, N. A., "Activation of the hypothalamic-pituitary-adrenal stress axis induces cellular oxidative stress," *Front. Neurosci.*, **8**, 456 (2015).
- Stevenson, J. R., McMahon, E. K., Boner, W., and Haussmann, M. F., "Oxytocin administration prevents cellular aging caused by social isolation," *Psychoneuroendocrinology*, **103**, 52–60 (2019).
- Takatsu-Coleman, A. L., Patti, C. L., Zanin, K. A., et al., "Short-term social isolation induces depressive-like behaviour and reinstates the retrieval of an aversive task: mood-congruent memory in male mice?" *J. Psychiatry Neurosci.*, **38**, No. 4, 259–268 (2013).
- Thorsell, A., Slawewski, C. J., El Khoury, A., et al., "The effects of social isolation on neuropeptide Y levels, exploratory and anxiety related behaviors in rats," *Pharmacol. Biochem. Behav.*, **83**, 28–34 (2006).
- Todorović, N. and Filipović, D., "The antidepressant- and anxiolytic-like effects of fluoxetine and clozapine in chronically isolated rats involve inhibition of hippocampal TNF- α ," *Pharmac. Biochem. Behav.*, **163**, 57– (2017).
- Todorović, N., Bošković, M., and Filipović, D., "Fluoxetine failed to prevent isolation-induced changes of glutathione-dependent defense in rat hippocampus," *Physical Chemistry. 2014. 12th Int. Conf. on Fundamental And Applied Aspects of Physical Chemistry* (2014), Vol. 3, pp. 1121–1124.
- Tulogdi, A., Tóth, M., Barsvári, B., et al., "Effects of resocialization on post-weaning social isolation-induced abnormal aggression and social deficits in rats," *Dev. Psychobiol.*, **56**, No. 1, 49–57 (2014).
- Vesce, S., Rossi, D., Brambilla, L., and Volterra, A., "Glutamate release from astrocytes in physiological conditions and in neurodegenerative disorders characterized by neuroinflammation," *Int. Rev. Neurobiol.*, **82**, 57–71 (2007).
- Viana Borges, J., Souza de Freitas, B., Antoniazzi, V., et al., "Social isolation and social support at adulthood affect epigenetic mechanisms, brain-derived neurotrophic factor levels and behavior of chronically stressed rats," *Behav. Brain Res.*, **366**, 36–44 (2019).
- Vieira, P. R., Garcia, L. P., and Maciel, E. L. N., "The increase in domestic violence during the social isolation: what does it reveals?" *Rev. Bras. Epidemiol.*, **22**, No. 23: e200033 (2020).
- Villa, A., Vegeto, E., Poletti, A., and Maggi, A., "Estrogens, neuroinflammation, and neurodegeneration," *Endocrine Rev.*, **37**, No. 4, 372–402 (2016).

- Võikar, V., Polus, A., Vasar, E., and Rauvala, H., "Long-term individual housing in C57BL/6J and DBA/2 mice: assessment of behavioral consequences," *Genes Brain Behav.*, **4**, No. 4, 240–52 (2005).
- Walker, D. M., Cunningham, A. M., Gregory, J. K., and Nestler, E. J., "Long-term behavioral effects of post-weaning social isolation in males and females," *Front. Behav. Neurosci.*, **13**, 66 (2019).
- Wallace, D. L., Han, M.-H., Graham, D. L., et al., "CREB regulation of nucleus accumbens excitability mediates social isolation-induced behavioral deficits," *Nat. Neurosci.*, (2009) **12**: 200–209.
- Wang, B., Wu, Q., Lei, L., et al., "Long-term social isolation inhibits autophagy activation, induces postsynaptic dysfunctions and impairs spatial memory," *Exp. Neurol.*, **311**, 213–224 (2019).
- Wang, H. T., Huang, F. L., Hu, Z. L., et al., "Early-life social isolation-induced depressive-like behavior in rats results in microglial activation and neuronal histone methylation that are mitigated by minocycline," *Neurotox. Res.*, **31**, No. 4, 505–520 (2017).
- Weintraub, A., Singaravelu, J., and Bhatnagar, S., "Enduring and sex-specific effects of adolescent social isolation in rats on adult stress reactivity," *Brain Res.*, **1343**, 83–92 (2010).
- Weiss, I. C., Pryce, C. R., Jongen-Rêlo, A. L., et al., "Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat," *J. Behav. Brain Res.*, **152**, No. 2, 279–295 (2004).
- Whitaker, L. R., Degoulet, M., and Morikawa, H., "Social deprivation enhances VTA synaptic plasticity and drug-induced contextual learning," *Neuron*, **77**, No. 2, 335–345 (2013).
- Wolffgramm, J. and Heyne, A., "Social behavior, dominance, and social deprivation of rats determine drug choice," *Pharmacol. Biochem. Behav.*, **38**, No. 2, 389–399 (1991).
- Wright, I. K., Upton, N., and Marsden, C. A., "Resocialisation of isolation-reared rats does not alter their anxiogenic profile on the elevated X-maze model of anxiety," *Physiol. Behav.*, **50**, 1129–1132 (1991).
- Yildirim, E., Erol, K., and Ulupinar, E., "Effects of sertraline on behavioral alterations caused by environmental enrichment and social isolation," *Pharmacol. Biochem. Behav.*, **101**, No. 2, 278–87 (2012).
- Zafir, A. and Banu, N., and "Induction of oxidative stress by restraint stress and corticosterone treatments in rats," *Ind. J. Biochem. Biophys.*, **46**, 53–58 (2009).
- Zhang, Y., Zu, X., Luo, W., et al., "Social isolation produces anxiety-like behaviors and changes PSD-95 levels in the forebrain," *Neurosci. Lett.*, **514**, 27–30 (2012).
- Zlatković, J. and Filipović, D., "Bax and B-cell-lymphoma 2 mediate proapoptotic signaling following chronic isolation stress in rat brain," *Neuroscience*, **223**, 238–245 (2012).
- Zlatković, J., Todorović, N., Bošković, M., et al., "Different susceptibility of prefrontal cortex and hippocampus to oxidative stress following chronic social isolation stress," *Mol. Cell Biochem.*, **393**, 43–57 (2014).
- Zorzo, C., Méndez-López, M., Méndez, M., and Arias, J. L., "Adult social isolation leads to anxiety and spatial memory impairment: Brain activity pattern of COX and c-Fos," *Behav. Brain Res.*, **365**, 170 (2019).