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Chapter

Neuropharmacology of Anxiety Disorders at Young Age: A Perspective from Preclinical Research

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Abstract

Anxiety is one of the most common psychopathologies in the general population that often begin early in life; however, research on this disorder during early developmental stages has been poorly explored compared to adults. A better understanding of the anxiety disorder through childhood is essential to develop more effective treatments. This chapter provides a general overview of the usefulness of animal models of childhood anxiety and its neurobiological bases to discuss how the studies on animals meet the several criteria of validity to discover pathophysiological mechanisms of human disorders and new treatments for these conditions. The research methodology for this chapter consisted in using a thesaurus system such as Medical Subject Headings (MeSH) terms of the National Library of Medicine to find original articles in databases as PubMed or Web of Science about preclinical findings related to the neuropharmacology of anxiety before adulthood. The contribution of this chapter is to provide data from preclinical studies which are encouraged to a better comprehension of anxiety at young age.

Keywords: adolescent, anxiety, anxiolytics, animal model, child, rats

1. Introduction

Anxiety is a disorder that can be developed in offspring as a result of aversive life conditions. Some factors in the childhood and adolescence that predispose the development of anxiety disorders include sexual abuse [1], social isolation [2], maternal separation [3], physical abuse, emotional abuse, negligence, and exposure to partner violence [4]. In addition, children who experience multiple types of abuse can suffer exacerbate symptoms of anxiety and comorbidity with depression compared to those who are only exposed to one type of abuse [1, 5, 6].

Although researchers have tried to probe the heritability of anxiety with studies of twin pairs, first-degree relatives, or big samples of anxiety-diagnosed patients,
findings are inconsistent and could not be replicated [7–9], so there is no clear evidence to suggest a genetic component in the development of anxiety.

The assessment and treatment of childhood disorders are challenging because this population should not be compared to adults. Children and adolescent have their own features (e.g., difficulty to concentrate in school tasks, decreased academic or athletic performance, avoidance, “clinging” behavior, and tantrum [10]) which are in complex interaction with social and physiological environment.

In the similar way in young rats, anxiety has particular characteristics, and manifestation differs with age, e.g., in the elevated plus maze, young rodents (males and females) have high anxiety levels that increase with age [11]. However, adolescent females with food deprivation have lower anxiety level compared to adult rats [12]; these findings suggest that infantile and juvenile stages constitute a period of transition toward adulthood.

Therefore, the objective of this chapter is to review preclinical findings of experimental anxiety with pharmacological manipulations in young rats. This chapter will provide data from preclinical studies which are encouraged to a better comprehension of anxiety before adulthood.

2. Neurobiology of anxiety

Anxiety is a disorder of complex etiology, which includes stressful, environmental, epigenetic, social, and psychological factors that modify neurotransmission systems such as serotonergic, noradrenergic, dopaminergic, and glutamatergic [13–16]. The most studied neurobiological mechanism is the monoaminergic hypothesis, since clinically effective anxiolytic drugs have their place of action on various monoamines, such as serotonin (5-HT), noradrenaline (NE), and dopamine (DA), neurotransmitters involved in the pathogenesis of anxiety [13, 17]. However, in recent years attention has focused on alterations of the hypothalamic–pituitary–adrenal axis (HPA), neuroplasticity, neurogenesis, and inflammatory response [18], opening a new paradigm for the study of the biological bases of anxiety.

The amygdala is the main brain region involved in the processing of fear information by integrating prior learning and incoming sensory information from cortical and subcortical regions [19]. In anxiety disorders it is common to observe a decreased inhibitory neurotransmission mediated by gamma-aminobutyric acid (GABA), an increase in excitatory neurotransmission mediated by glutamate [20], as well as the interruption of fight-or-flight response mechanism regulated by the HPA axis with participation of emotional processing structures including the amygdala, hypothalamus, periaqueductal gray substance, and hippocampus and chemical mediators such as corticotropin-releasing factor, glutamate, and neuropeptides (substance P, neuropeptide Y, oxytocin, orexin, and galanin) [18, 20].

Unpredictable chronic mild stress increases glutamatergic neurotransmission and decreases prefrontal cortex (PFC) function in rats which display anxiety-like behavior [20], and the imbalance between neuronal excitation and inhibition in the medial prefrontal cortex, hippocampus, and amygdala contributes to the development of emotional disorders such as anxiety [21]. Chronic dexamethasone produces deficient learning and decreased pyramidal neurons in CA3 of the hippocampus in rats [22]. These findings lead to improper process of the cognitive responses to face aversive situations.

Glucocorticoids like corticosterone in rats can also alter the functional brain connections responsible for the emotional processing; for example, chronic stress decreases cognitive function due to loss of projections from the basolateral amygdala to the medial prefrontal cortex [23]. These data together indicate that if the
organism remains in a state that deteriorates its homeostasis with alterations in the functionality of the HPA axis, responsible for regulating the response to stress, it leads to the development of diseases such as anxiety [21]. Thus, the secretion of hormones, such as glucocorticoids, catecholamines, growth hormone, and prolactin, promotes adaptive responses, but physiopathological processes are triggered when the response is excessive [24, 25].

On the other hand, glutamate is an excitatory neurotransmitter that acts through different types of N-methyl-D-aspartate (NMDA) and non-NMDA receptors. This neurotransmitter has been associated with anxiety since the increase of brain glutamate/glutamine levels induced by monosodium glutamate produces anxiety-like behavior measured in two models of anxiety, the open field test and the elevated plus maze [26]. In consistency the antagonism of NMDA receptors promotes anxiolytic-like behavior in experimental animal models of open field and marble burying [16].

3. Anxiety animal models

Animal models help to understand the physiopathology of some human diseases, the development of new therapeutic options, as well as the evaluation of the existing ones to identify other relevant effects [27]. Additionally, animals are relatively easy to obtain, maintain, and manipulate. They have broad reproducibility and involve less investment compared with clinical studies.

Our interest is situated in animal models of mental disorders associated to altered emotions. In the book *The Expression of the Emotions in Man and Animals*, Darwin makes it clear that through behavioral patterns, animals have the capacity to express their emotions [28]. Based on this capacity, a wide range of animal models have been developed, which allow us to understand some aspects of various psychiatric disorders as anxiety. Although it is not possible to fully model the complexity of human psychopathology, the physiological, anatomical, and genetic similarities allow us to understand, with limitations, the neurobiological basis of human diseases, as anxiety.

Animal models are very useful approaches at preclinical research to study anxiety and the closest possible to the anxiety disorders described in the DSM-5 which could occur at childhood and adolescence and not only in adults. Table 1 shows some human anxiety disorders that can be studied in laboratory rats.

The animal models mentioned in Table 1 are used to study anxiety disorders and the effectiveness of several pharmacological treatments. These models evaluate conditioned or unconditioned responses to novel or stressful stimuli, measuring

| Human condition                    | Rodent model                                                                                  | Reference |
|------------------------------------|------------------------------------------------------------------------------------------------|-----------|
| Generalized anxiety, posttraumatic stress | Elevated plus maze, defensive burying test, marble burying, open field test, T-maze        | [29–33]  |
| Specific phobia: Photophobia, Social phobia, Agoraphobia | Light–dark box, social interaction test, hole board                                             | [33]      |
| Separation anxiety disorder        | Maternal separation                                                                           | [34]      |
| Panic disorder                     | T-maze                                                                                         | [31–33]  |
| Selective mutism                   | Social interaction test (with measure of pup ultrasound vocalizations during the test)         | [35, 36] |

Table 1.
Anxiety disorders and their experimental model used at young age.
behavioral or physiological responses in accordance to international laws that regulate the use of laboratory animals, with the aim of minimizing their use, pain, and stress [37].

Animal models are accepted as useful tools for studying human pathologies if they meet the criteria proposed by Willner [38] which include (i) predictive validity consisting of the similarity in the production of alterations of the human pathological state with the model and based on the sensitivity and specificity of the drugs used to reverse them; (ii) nominal or appearance validity, consisting of the similarity between the phenomena observed in the modeled and the human disorder; and (iii) construct validity which is the evaluation of the theoretical state in the condition under study, which should resemble the theoretical symptomatology of the human disorder in the animal model used for its study [38, 39]. These criteria continue evolving to have a more relevant approach to the human condition; Table 2 summarizes the proposal of Belzung and Lemoine [40] that reformulates the classical criteria.

| Kind of validity | Aspect of validity | Object of validity (animal/human similarity of...) |
|------------------|--------------------|--------------------------------------------------|
| Homological validity | Species validity | Species |
|                   | Strain validity    | Strain |
| Pathogenic validity | Ontopathogenic validity | Interaction transforming an initial organism into a vulnerable organism |
|                   | Triggering validity | Interaction transforming an initial or a vulnerable organism into a pathological organism |
| Mechanistic validity | Theoretical cognitive or neurobiological mechanisms producing the observable effects of the disease |
| Face validity | Ethological validity | Behavioral symptoms of the disease |
| Biomarker validity | Biomarkers associated with the disease |
| Predictive validity | Induction validity | Relation between the triggering factor and the observable effects of the disease |
| Remission validity | Relation between the therapeutic agent and the observable effects of the disease |

Source: Belzung and Lemoine [40].

Table 2. Update of validity criteria for animal models.

The young age for the purpose of this chapter means the first period of life, from 0 to 8 weeks in rats, since offspring depends from the dams to get nutrition and physical, intellectual, and social growth. While in humans there are already six age stages of development (neonatal, infant, childhood, juvenile, adulthood, and elderly), similarly the same can be identified in laboratory rats to research clinical conditions at preclinical level. In consistency with the validity criteria to study anxiety in childhood and adolescence at preclinical level, researchers should employ animal subjects at similar stages of development that can be observed in Table 3.

Behavioral and physiological responses activated by stress are similar in animals and humans. Thus, stress as a predisposing factor of anxiety can be experienced in several forms and produce nonadaptive responses depending on duration and intensity in animals. It is well-known that adverse experiences during sensitive developmental periods such as childhood and adolescence increase the predisposition to the development of neuropsychiatric disorders at the same age and later in adulthood [44]. In this sense, the preclinical study of anxiety involves experimental
manipulations that generate stress responses during human childhood- and adolescence-like ages and allow us to observe some features of the disorders.

Some stressors used in infant and juvenile rats are chronic and unpredictable mild stress, space restriction, forced swimming, and maternal separation, among others. For example, 60 min of space restriction stress in rats at 30, 45, and 60 postnatal days (PND) increases plasma corticosterone levels and c-Fos protein expression in the amygdala and brain stem, suggesting a greater predisposition to the development of anxiety disorders [45]. Social stress in juvenile rats produces anorexic-like behavior in female mice [46].

Rats of 28 PND display behavioral responses suggestive of anxiety in defensive burying test (increase in burying time), an effect that is reversed by the administration of 1 mg/kg diazepam [30]. Stress by swimming produces a state of anxiety in 21 PND rats evaluated in the elevated plus maze (lower time spent in open arms and higher anxiety index) which is reversed by half of the adult effective dose (0.5 mg/kg diazepam), further suggesting that the infant rats are seemingly more sensitive to low dose of diazepam than adult rats, which is relevant for clinical applications [29].

The underlying mechanisms of anxiety disorders associated with the disruption of the mother-child relations at early stages are still unknown, but animal models of maternal separation can help to reproduce the molecular changes at the central nervous system responsible for anxiety-like behavior. For example, maternal separation in rodents has been shown to induce hyperactivity of the HPA increasing plasma corticosterone concentrations [47], where maternal deprivation for 15 and 180 min from 2 to 14 PND alters the mRNA mineralocorticoid and glucocorticoid receptors in the dentate gyrus of the hippocampus which is accompanied by hypersecretion of adrenocorticotropic hormone and corticosterone in plasma [48]. This is important because at least in animal models, an increase in plasma corticosterone concentrations is related to anxiety-like behaviors [49–51].

Thus, dysregulation of the HPA axis may be a marker of vulnerability to anxiety [48], where the HPA axis may be affected by the postnatal adversity induced by maternal separation [52]. Furthermore, 4–8 h maternal separation from 2 to 21 PND in male C57BL/6 mice increases anxiety-like behaviors in social preference test and in the elevated plus maze (reduction of time spent into open arms), which was related to an increase in IL-1β in the hippocampus, PFC, and serum [53]. Therefore, the inflammatory process induced by maternal separation affects two brain structures related to the pathophysiology of anxiety, i.e., the hippocampus and prefrontal cortex [53].

Maternal deprivation can also affect the brain development of rats, because 24 h of maternal deprivation increases the rate of cell death by labeling the 3′ end of DNA fragments using terminal transferase in the cerebral cortex and hippocampus in 12 PND rats, in addition to an increase in apoptosis-related proteins such as Bax and Bcl-x in the frontal cortex. However, at 20 PND cell death is not as marked as
in PND 12; therefore, maternal deprivation exerts a greater effect on immature neurons which are more vulnerable [47].

Similarly, in male Sprague Dawley rat pups, maternal separation from 2 to 21 PND for 3 h each day affects the serotonergic system, decreasing the number of positive cells to the expression of tryptophan hydroxylase (TPH) and 5-HT, identified with immunohistochemistry in the dorsal raphe nucleus, in addition to increasing pro-apoptotic proteins (cytochrome c, Bax, and caspase-3) and reactive oxygen species (H$_2$O$_2$) in the same brain nucleus, where these changes were again related to an increase in anxiety-like behaviors in the elevated plus maze and the open field test [54].

It should be noted that the dorsal raphe nucleus is a structure that participates in stress processes and mood disorders [55] and the induction of adverse effects in early life could indirectly generate a malfunction of the dorsal raphe nucleus and the serotonergic system which in the long term induces anxiety-like behaviors. Thus, maternal deprivation during critical periods of development will alter the functioning and brain wiring of infants exerting a risk factor for psychiatric disorders.

Social isolation is another factor of adversity in early stage of development that has been studied in basic research. Six hours of social isolation each day from 21 to 30 PND or from 21 to 40 PND in Wistar Kyoto rats reduced time and open arm entries and increased anxiety index in the elevated plus maze with respect to the subjects that remained in a group [56]. These findings could be related to a reduction of neurotrophic factors (BDNF, NGF, Arc), neurogenesis markers (Ki-67, BrdU), and the loss of density of dendritic spines in the hippocampus of the rat exposed to social isolation from 21 to 49 PND which can be reversed after the resocialization of experimental subjects [57]. The above shows how social isolation can affect neurotrophic processes and therefore impact the neuronal plasticity of the hippocampus, which could be indirectly generating negative effects on mood.

4. Experimental pharmacology of anxiety

Anxiety disorders in children and adolescents include symptoms which are similar to adults such as headache, fatigue, muscle tension, shortness of breath, and gastrointestinal problems, among others, as well as typical manifestations of the scholar child such as difficulty to concentrate in school tasks and decreased academic or athletic performance, accompanied by fear, avoidance, “clinging” behavior, and tantrum [10].

Research about the treatment of anxiety disorders during the first 18 years of life continues growing. The pharmacological approach to reverse anxiety disorders includes the selective serotonin reuptake inhibitors (SSRIs) like fluoxetine, fluvoxamine, and sertraline and serotonin norepinephrine reuptake inhibitors (SNRIs) like duloxetine as first-line treatment [58, 59], tricyclic antidepressants like clomipramine as second option [58], alpha-2A-adrenergic receptor agonist like guanfacine [60, 61], and benzodiazepines like diazepam as alternative treatment lines [62], drugs that have been evaluated with the support of animal models. Table 4 resumes the results of some studies that evaluate the anxiolytic potential of substances evaluated using animal models of experimental anxiety at young age submitted to some stressors.

Interesting findings show that the pharmacological approaches in infant and adolescent rats are different from those of adults. The result is that specific adjustments should be applied if hypothesis are made to prove in young rats. Finally, all the attempts to increase the literature of anxiety in young subjects are useful
to extend the comprehension of this clinical condition in order to dedicate higher attention to stress factors associated with it.

5. Discussion

Anxiety disorders can appear from early life stages and have its own characteristics, so diagnosis and treatment require the same particularity. Despite this, the scientific literature on anxiety disorders at young stages of life is less abundant than studies in adults. Infant and juvenile population is heterogeneous and complex, so the experimental characteristics under which the studies are developed are determinants for the results obtained. Naturally, young experimental subjects show anxiety levels that could increase with age, regardless of gender [11]. Some examples of the lower anxiety observed in young rats compared to adults are observed in adolescent animals with food deprivation which display lower levels of anxiety than adults evaluated in the elevated plus maze [12]. Juvenile male and female rats coming from pregnant dams exposed to unescapable low-intensity foot electric shocks remained more time in the open arms and in the elevated plus maze compared to their adult age [67], while adolescent rats exposed to maternal separation have higher levels of exploration in a novel environment and lower levels of corticosterone after exposure to that environment, showing lower levels of anxiety, while these effects are not observed in rats evaluated in the adult stage [68].

Table 4. Effects of anxiolytic drugs evaluated in young animal subjected to behavioral test.
Regarding the response to pharmacological treatments, our group reported that the minimum anxiolytic effective dose of diazepam for animals of 21 PND is 0.5 mg/kg, being half of the minimum effective dose for adults [29]. While the use of selective serotonin reuptake inhibitors (SSRIs) like fluoxetine (first-line treatment in clinical practice [62]) shows diverse results, ranging from the absence of anxiolytic effects of fluoxetine with acute and chronic treatment [69] until paradoxical anxiogenic effects [70], possible explanation for diverse unexpected fluoxetine effects on infant and juvenile anxiety is based on mechanism of action of fluoxetine and the treatment duration. At first, on acute treatment fluoxetine increases extracellular serotonin levels for inhibition of reuptake, and the neurotransmitter remains free to stimulate postsynaptic receptors explaining the transitory increase in anxiety levels when fluoxetine treatment begins [71]. Later with chronic fluoxetine, high concentrations of serotonin inhibit serotonergic neurons in the dorsal raphe nucleus, reducing serotonin production and anxiety [71].

Some limitations that researchers face are the differences in experimental conditions, age, and even strains, which can explain the variability of the results obtained by diverse research groups. Of course the transition of preclinical findings to human condition should be modest and responsible, so generalizations should be avoided. With this brief review, it is clear that the expression of anxiety depends on age and represents a challenge but also an opportunity to generate knowledge that increases the scope of preclinical research. The advantages to study preclinical anxiety may consist in the opportunity to know the neurobiology of the developing brain under stress and pharmacological conditions. These manipulations on an organ with a great plasticity at early stages would lead to better results with potential reversible effects before reaching adulthood. Future studies must be encouraged to extend literature of infant and juvenile anxiety from preclinical to clinical approach, which could prevent adult high incidence of this clinical and disabling condition.

6. Conclusion

Based on preclinical findings, stressors produce human-like alterations before adulthood. The consequences are brain changes that impact behavioral performance generating anxiety. These effects are studied in the field of the experimental anxiety to probe pharmacological substances in order to extend knowledge of mechanism of action of new molecules or their combination with other drugs. This chapter described some aspects of brain function during early postnatal development which involves a critical period of vulnerability to psychiatric conditions. Child and adolescent anxiety preclinical research must be extended in order to improve the knowledge of this clinical condition.

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Conflict of interest

The authors declare no conflict of interest.
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References

[1] Coles J, Lee A, Taft A, Mazza D, Loxton D. Childhood sexual abuse and its association with adult physical and mental health: Results from a national cohort of young Australian women. Journal of Interpersonal Violence. 2015;30(11):1929-1944. DOI: 10.1177/0886260514555270

[2] Simonds LM, Pons RA, Stone NJ, Warren F, John M. Adolescents with anxiety and depression: Is social recovery relevant? Clinical Psychology and Psychotherapy. 2014;21(4):289-298. DOI: 10.1002/cpp.1841

[3] Battaglia M, Bertella S, Politi E, Bernardeschi L, Perna G, Gabriele A, et al. Age at onset of panic disorder: Influence of familial liability to the disease and of childhood separation anxiety disorder. The American Journal of Psychiatry. 1995;152(9):1362-1364. DOI: 10.1176/ajp.152.9.1362

[4] Dhakal S, Niraula S, Sharma NP, Shapit S, Bennett E, Vaswani A, et al. History of abuse and neglect and their associations with mental health in rescued child labourers in Nepal. The Australian and New Zealand Journal of Psychiatry. 2019;4867419853882. DOI: 10.1177/0004867419853882

[5] Berzenski SR, Yates TM. Classes and consequences of multiple maltreatment: A person-centered analysis. Child Maltreatment. 2011;16(4):250-261. DOI: 10.1177/1077559511428353

[6] Moore SE, Scott JG, Ferrari AJ, Mills R, Dunne MP, Erskine HE, et al. Burden attributable to child maltreatment in Australia. Child Abuse & Neglect. 2015;48:208-220. DOI: 10.1016/j.chiabu.2015.05.006

[7] Stein MB, Gelernter J. Genetic factors in social anxiety disorder. In: Weeks JW, editor. The Wiley Handbook of Social Anxiety Disorder. Chichester: John Wiley & Sons; 2014

[8] Shimada-Sugimoto M, Otowa T, Hettema JM. Genetics of anxiety disorders: Genetic epidemiological and molecular studies in humans. Psychiatry & Clinical Neurosciences. 2015;69(7):388-401. DOI: 10.1111/pcn.12291

[9] Dunn EC, Sofer T, Gallo LC, Gogarten SM, Kerr KF, Chen CY, et al. Genome-wide association study of generalized anxiety symptoms in the hispanic community health study/study of latinos. American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics. 2017;174(2):132-143. DOI: 10.1002/ajmg.b.32448

[10] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA: American Psychiatric Association; 2013

[11] Lynn DA, Brown GR. The ontogeny of anxiety-like behavior in rats from adolescence to adulthood. Developmental Psychobiology. 2010;52(8):731-739. DOI: 10.1002/dev.20468

[12] Genn RF, Tucci SA, Thomas A, Edwards JE, File SE. Age associated sex differences in response to food deprivation in two animal tests of anxiety. Neuroscience & Biobehavioral Reviews. 2003;27:155-161. DOI: 10.1016/s0149-7634(03)00017-4

[13] Keck ME, Sartori SB, Welt T, Müller MB, Ohl F, Holsboer F, et al. Differences in serotonergic neurotransmission between rats displaying high or low anxiety/depression-like behaviour: Effects of chronic paroxetine treatment. Journal of Neurochemistry. 2005;92(5):1170-1179. DOI: 10.1111/j.1471-4159.2004.02953.x
[14] Borodovitsyna O, Flamini MD, Chandler DJ. Acute stress persistently alters locus coeruleus function and anxiety-like behavior in adolescent rats. Neuroscience. 2018;373:7-19. DOI: 10.1016/j.neuroscience.2018.01.020

[15] Malikowska-Racia N, Salat K, Nowaczyk A, Fijalkowski L, Popik P. Dopamine D2/D3 receptor agonists attenuate PTSD-like symptoms in mice exposed to single prolonged stress. Neuropharmacology. 2019;155:1-9. DOI: 10.1016/j.neuropharm.2019.05.012

[16] Zhan Y, Xia J, Wang X. Effects of glutamate-related drugs on anxiety and compulsive behavior in rats with obsessive-compulsive disorder. International Journal of Neuroscience. 2019:1-13. DOI: 10.1080/00207454.2019.1684276

[17] McElligott ZA, Fox ME, Walsh PL, Urban DJ, Ferrel MS, Roth BL, et al. Noradrenergic synaptic function in the bed nucleus of the stria terminalis varies in animal models of anxiety and addiction. Neuropsychopharmacology. 2013;38(9):1665-1673. DOI: 10.1038/npp.2013.63

[18] Haj-Mirzaian A, Amiri S, Kordjazy N, Momeny M, Razmi A, Rahimi-Balaei M, et al. Lithium attenuated the depressant and anxiogenic effect of juvenile social stress through mitigating the negative impact of interleukin-1β and nitric oxide on hypothalamic-pituitary-adrenal axis function. Neuroscience. 2016;315:271-285. DOI: 10.1016/j.neuroscience.2015.12.024

[19] Selleck RA, Zhang W, Samberg HD, Padival M, Rosenkranz JA. Limited prefrontal cortical regulation over the basolateral amygdala in adolescent rats. Scientific Reports. 2018;8(1):17171. DOI: 10.1038/s41598-018-35649-0

[20] Shepard R, Coutellier L. Changes in the prefrontal glutamatergic and parvalbumin systems of mice exposed to unpredictable chronic stress. Molecular Neurobiology. 2018;55(3):2591-2602. DOI: 10.1007/s12035-017-0528-0

[21] Lovelock DF, Deak T. Acute stress imposed during adolescence yields heightened anxiety in Sprague Dawley rats that persists into adulthood: Sex differences and potential involvement of the medial amygdala. Brain Research. 2019;1723:146392. DOI: 10.1016/j.brainres.2019.146392

[22] Cerqueira JJ, Pêgo JM, Taipa R, Bessa JM, Almeida OF, Sousa N. Morphological correlates of corticosterone-induced changes in prefrontal cortex-dependent behaviors. The Journal of Neuroscience. 2005;25:7792-7800. DOI: 10.1523/JNEUROSCI.1598-05.2005

[23] Duvarc S, Paré D. Glucocorticoids enhance the excitability of principal basolateral amygdala neurons. The Journal of Neuroscience. 2007;27:4482-4491. DOI: 10.1523/JNEUROSCI.0680-07.2007

[24] Telegdy G, Schally AV. Neurotransmitter-mediated action of an antagonist of growth hormone-releasing hormone on anxiolysis in mice. Behavioural Brain Research. 2012;233(1):232-236. DOI: 10.1016/j.bbr.2012.04.011

[25] Zamorano M, Ledesma-Colunga MG, Adán N, Vera-Massieu C, Lemini M, Méndez I, et al. Prolactin-derived vasoinhibins increase anxiety- and depression-related behaviors. Psychoneuroendocrinology. 2014;44:123-132. DOI: 10.1016/j.psyneuen.2014.03.006

[26] Onaolapo OJ, Aremu OS, Onaolapo AY. Monosodium glutamate-associated alterations in open field, anxiety-related and conditioned place preference behaviours in mice.
Naunyn-Schmiedeberg's Archives of Pharmacology. 2017;390(7):677-689. DOI: 10.1007/s00210-017-1371-6

[27] Valdés SM, Álvarez AL, Del Barrio G. Los modelos animales en la evaluación preclínica de antivirales contra los virus del herpes simple [Animal models in preclinical evaluation of antiviral against herpes virus]. Revista de Salud Animal. 2009;31(2):86-92

[28] Darwin C. The Expression of the Emotions in Man and Animals. London: John Murray; 1872

[29] Bernal-Morales B, Guillén-Ruiz G, Cueto-Escobedo J, Rodríguez-Landa JF, Contreras CM. Sensitivity to diazepam after a single session of forced swim stress in weaning Wistar rats. Acta Pharmaceutica (Zagreb, Croatia). 2018;68(3):381-388. DOI: 10.2478/acph-2018-0027

[30] Cueto-Escobedo J, Contreras CM, Bernal-Morales B, Guillén-Ruiz G, Rodríguez-Landa JF. Defensive burying test in postweaning rats: Use of a small round chamber. Behavioural Pharmacology. 2013;24(8):693-698. DOI: 10.1097/FBP.0000000000000008

[31] Baker S, Chebli M, Rees S, Lemarec N, Godbout R, Bielajew C. Effects of gestational stress: 1. Evaluation of maternal and juvenile offspring behavior. Brain Research. 2008;1213:98-110. DOI: 10.1016/j.brainres.2008.03.035

[32] Campos AC, Fogaça MV, Aguiar DC, Guimarães FS. Animal models of anxiety disorders and stress. Brazilian Journal of Psychiatry. 2013;35(2):S101-S111. DOI: 10.1590/1516-4446-2013-1139

[33] Balemans MC, Huibers MM, Eikelenboom NW, Kuipers AJ, van Summeren RC, Piipers MM, et al. Reduced exploration, increased anxiety, and altered social behavior: Autistic-like features of euchromatin histone methyltransferase 1 heterozygous knockout mice. Behavioural Brain Research. 2010;208(1):47-55. DOI: 10.1016/j.bbr.2009.11.008

[34] Isobe A, Kawaguchi M. Relationship between motor function and ultrasonic vocalizations induced by maternal separation in rat pups. The Journal of Veterinary Medical Science. 2019;81(2):287-293. DOI: 10.1292/jvms.18-0604

[35] Al-Afif S, Staden M, Krauss JK, Schwabe K, Hermann EJ. Splitting of the cerebellar vermis in juvenile rats–effects on social behavior, vocalization and motor activity. Behavioural Brain Research. 2013;250:293-298. DOI: 10.1016/j.bbr.2013.05.013

[36] Al-Afif S, Krauss JK, Helms F, Angelov S, John N, Schwabe K, et al. Long-term impairment of social behavior, vocalizations and motor activity induced by bilateral lesions of the fastigial nucleus in juvenile rats. Brain Structure & Function. 2019;224(5):1739-1751. DOI: 10.1007/s00429-019-01871-3

[37] Hernández S. El modelo animal en las investigaciones biomédicas. Biomedicina. 2006;2(3):252-256

[38] Willner P. Methods for assessing the validity of animal models of human psychopathology. In: Boulton A et al., editors. Animal Models in Psychiatry I. USA: Humana Press; 1991

[39] Morrice JR, Gregory-Evans CY, Shaw CA. Animal models of amyotrophic lateral sclerosis: A comparison of model validity. Neural Regeneration Research. 2018;13(12):2050-2054. DOI: 10.4103/1673-5374.241445

[40] Belzung C, Lemoine M. Criteria of validity for animal models of psychiatric disorders: Focus on anxiety disorders
and depression. Biology of Mood & Anxiety Disorders. 2011;1(1):9. DOI: 10.1186/2045-5380-1-9

[41] Becú-Villalobos D, Lacau-Menguindo IM. Control hormonal del desarrollo puberal en la rata hembra. Acta Physiologica et Pharmacologica Latino americana. 1990;40(1):1-17

[42] Spear LP. The adolescent brain and age-related manifestations. Neuroscience & Biobehavioral Reviews. 2000;24:417-463. DOI: 10.1016/s0149-7634(00)00014-2

[43] World Health Organization. e-Library of Evidence for Nutrition Actions (eLENA): life course [Internet]. 2019. Available from: https://www.who.int/elena/life_course/en/ [Accessed: 13 November 2019]

[44] Lenze SN, Xiong C, Sheline YI. Childhood adversity predicts earlier onset of major depression but not reduced hippocampal volume. Psychiatry Research. 2008;162(1):39-49. DOI: 10.1016/j.psychres.2007.04.004

[45] Kovács LÁ, Schiessl JA, Nafz AE, Csernus V, Gaszner B. Both basal and acute restraint stress-induced c-Fos expression is influenced by age in the extended amygdala and brainstem stress centers in male rats. Frontiers in Aging Neuroscience. 2018;10:248. DOI: 10.3389/fnagi.2018.00248

[46] Madra M, Zeltser LM. BDNF-Val66Met variant and adolescent stress interact to promote susceptibility to anorexic behavior in mice. Translational Psychiatry. 2016;6:e776. DOI: 10.1038/tp.2016.35

[47] Zhang LX, Levine S, Dent G, Zhan Y, Xing G, Okimoto D, et al. Maternal deprivation increases cell death in the infant rat brain. Developmental Brain Research. 2002;133:1-11. DOI: 10.1016/s0926-6410(01)00118-5

[48] Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB, Plotsky PM. Long-term adaptations in glucocorticoid receptor and mineralocorticoid receptor mRNA and negative feedback on the hypothalamo-pituitary-adrenal axis following neonatal maternal separation. Biological Psychiatry. 2004;55(4):367-375. DOI: 10.1016/j.biopsych.2003.10.007

[49] Vega-Rivera NM, Fernández-Guasti A, Ramírez-Rodríguez G, Estrada-Camarena E. Acute stress further decreases the effect of ovarietomy on immobility behavior and hippocampal cell survival in rats. Psychoneuroendocrinology. 2013;38:1407-1417. DOI: 10.1016/j.psyneuen.2012.12.008

[50] Wang Z, Gu J, Wang X, Xie K, Luan Q, Wan N, et al. Antidepressant-like activity of resveratrol treatment in the forced swim test and tail suspension test in mice: The HPA axis, BDNF expression and phosphorylation of ERK. Pharmacology, Biochemistry & Behavior. 2013;112:104-110. DOI: 10.1016/j.pbb.2013.10.007

[51] Vega-Rivera NM, Fernández-Guasti A, Ramírez-Rodríguez G, Estrada-Camarena E. Forced swim and chronic variable stress reduced hippocampal cell survival in OVX female rats. Behavioural Brain Research. 2014;270:48-55. DOI: 10.1016/j.bbr.2014.05.033

[52] Houwing DJ, Ramsteijn AS, Riemersma IW, Olivier JDA. Maternal separation induces anhedonia in female heterozygous serotonin transporter knockout rats. Behavioural Brain Research. 2019;356:204-207. DOI: 10.1016/j.bbr.2018.08.031

[53] Wang Q, Dong X, Wang Y, Liu M, Sun A, Li N, et al. Adolescent escitalopram prevents the effects of maternal separation on depression- and anxiety-like behaviours and regulates
the levels of inflammatory cytokines in adult male mice. International Journal of Developmental Neuroscience. 2017;62:37-45. DOI: 10.1016/j.ijdevneu.2017.07.007

[54] Park SS, Park HS, Kim CJ, Baek SS, Kim TW. Exercise attenuates maternal separation-induced mood disorder-like behaviors by enhancing mitochondrial functions and neuroplasticity in the dorsal raphe. Behavioural Brain Research. 2019;372:112049. DOI: 10.1016/j.bbr.2019.112049

[55] Donner NC, Handa RJ. Estrogen receptor beta regulates the expression of tryptophanhydroxylase 2 mRNA within serotonergic neurons of the rat dorsal raphe nuclei. Neuroscience. 2009;163(2):705-718. DOI: 10.1016/j.neuroscience.2009.06.046

[56] Shetty RA, Sadananda M. Immediate and delayed anxiety- and depression-like profiles in the adolescent Wistar-Kyoto rat model of endogenous depression following postweaning social isolation. Behavioural Brain Research. 2017;320:323-332. DOI: 10.1016/j.bbr.2016.12.030

[57] Biggio F, Mostallino MC, Talani G, Locci V, Mostallino R, Calandra G, et al. Social enrichment reverses the isolation-induced deficits of neuronal plasticity in the hippocampus of male rats. Neuropharmacology. 2019;151:45-54. DOI: 10.1016/j.neuropharm.2019.03.030

[58] da Costa CZ, de Morais RM, Zanetta DM, Turkiewicz G, Lotufo Neto F, Morikawa M, et al. Comparison among ceftriaxone, fluoxetine, and placebo for the treatment of anxiety disorders in children and adolescents. Journal of Child & Adolescent Psychopharmacology. 2013;23(10):687-692. DOI: 10.1089/cap.2012.0110

[59] Locher C, Koechlin H, Zion SR, Werner C, Pine DS, Kirsch I, et al. Efficacy and safety of selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, and placebo for common psychiatric disorders among children and adolescents: A systematic review and meta-analysis. JAMA Psychiatry. 2017;74(10):1011-1020. DOI: 10.1001/jamapsychiatry.2017.2432

[60] Dobson ET, Bloch MH, Strawn JR. Efficacy and tolerability of pharmacotherapy for pediatric anxiety disorders: A network meta-analysis. Journal of Clinical Psychiatry. 2019;80(1). pii: 17r12064. DOI: 10.4088/JCP.17r12064

[61] Strawn JR, Compton SN, Robertson B, Albano AM, Hamdani M, Rynn MA. Extended release guanfacine in pediatric anxiety disorders: A pilot, randomized, placebo-controlled trial. Journal of Child & Adolescent Psychopharmacology. 2017;27(1):29-37. DOI: 10.1089/cap.2016.0132

[62] Rynn M, Puliafico A, Heleniak C, Rikhi P, Ghali K, Vidair H. Advances in pharmacotherapy for pediatric anxiety disorders. Depression and Anxiety. 2011;28(1):76-87. DOI: 10.1002/da.20769

[63] Balaszczuk V, Salguero JA, Villarreal RN, Scaramuzza RG, Mendez S, Abate P. Hyperlocomotion and anxiety-like behavior induced by binge ethanol exposure in rat neonates. Possible ameliorative effects of omega 3. Behavioural Brain Research. 2019;372:112022. DOI: 10.1016/j.bbr.2019.112022

[64] Peng HH, Tsai TC, Huang WY, Wu HM, Hsu KS. Probiotic treatment restores normal developmental trajectories of fear memory retention in maternally separated infant rats. Neuropharmacology. 2019;153:53-62. DOI: 10.1016/j.neuropharm.2019.04.026

[65] Bernal-Morales B, Cueto-Escobedo J, Guillén-Ruiz G, Rodríguez-Landa JF, Contreras CM. A fatty acids mixture reduces anxiety-like
behaviors in infant rats mediated by GABA<sub>A</sub> receptors. BioMed Research International. 2017;2017:8798546. DOI: 10.1155/2017/8798546

[66] Guillén-Ruiz G, Bernal-Morales B, Contreras CM, Cueto-Escobedo J, Rodríguez-Landa JF. Oleic acid produces motor incoordination and hypoactivity in infant Wistar rats through GABA<sub>A</sub> receptors. Journal of Psychiatry & Neuroscience. 2016;4:18-25. DOI: 10.11648/j.jpnm.20160402.11

[67] Estanislau C, Morato S. Behavior ontogeny in the elevated plus-maze: Prenatal stress effects. International Journal of Developmental Neuroscience. 2006;24(4):255-262. DOI: 10.1016/j.ijdevneu.2006.03.001

[68] Yoo SB, Kim BT, Kim JY, Ryu V, Kang DW, Lee JH, et al. Adolescence fluoxetine increases serotonergic activity in the raphe-hippocampus axis and improves depression-like behaviors in female rats that experienced neonatal maternal separation. Psychoneuroendocrinology. 2013;38(6):777-788. DOI: 10.1016/j.psyneuen.2012.08.013

[69] Lapmanee S, Charoenphandhu J, Charoenphandhu N. Beneficial effects of fluoxetine, reboxetine, venlafaxine, and voluntary running exercise in stressed male rats with anxiety- and depression-like behaviors. Behavioural Brain Research. 2013;250:316-325. DOI: 10.1016/j.bbr.2013.05.018

[70] Handley SL, McBlane JW. Opposite effects of fluoxetine in two animal models of anxiety. British Journal of Pharmacology. 1992;107:446

[71] Farhan M, Haleem DJ. Anxiolytic profile of fluoxetine as monitored following repeated administration in animal rat model of chronic mild stress. Saudi Pharmaceutical Journal. 2016;24(5):571-578. DOI: 10.1016/j.jsps.2015.03.006