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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.

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.

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.

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 nonadaptative 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

Human age	Rat age	Development stage
0–24 months	0–28 days	Neonatal, lactating, infant
2–11 years	—	Childhood
12–18 years	29–55 days	Peripuberal, juvenile, adolescent
19–64 years	56 days–10 months	Adulthood
65 years	11 months	Older adult, elderly, aged

Table 3.
Comparison of the developmental stages between human and rat [41–43].

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 Blc-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_2O_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

Stressor	Result	Drug	Reference
Ethanol acute administration at 7–30 PND	Open field test: hyperlocomotion at 14 PND and reduced time spent in the center of the open field, suggesting a state of anxiety, both effects reversed with omega 3	Omega-3 (720 mg/kg)	[63]
Maternal separation at 0–27 PND	Elevated plus maze: probiotic treatment increased time spent in open arms Light–dark box: probiotics reduced latency to lighted compartment	*Lactobacillus rhamnosus* strain R0011 (95%) and *Lactobacillus helveticus* R0052 (5%)	[64]
Single stress session of forced swim for 15 min at 21 PND	Elevated plus maze: stress reduced open arm time spent and increased anxiety index, reversed with diazepam	Diazepam (0.5 mg/kg)	[29]
Open spaces and height; low-intensity electric shock at 28 PND	Elevated plus maze: A fatty acid mixture (FAM) increased time, entries, and percentage in open arms and reduced the anxiety index similar to diazepam Defensive burying test: FAM reduced burying time	Diazepam 1 mg/kg; FAM 1 ml/rat	[65]
Open spaces and height at 28 PND	Elevated plus maze: diazepam but no oleic acid increased time spent on open arms and reduced the anxiety index	Oleic acid (10, 20, 40, 60, and 80 µg/rat) Diazepam (1 mg/kg)	[66]
Low-intensity electric shock at 21 PND	Defensive burying test: diazepam produced the anxiolytic effect only in a modified smaller round device	Diazepam (1 mg/kg)	[30]

Table 4.
Effects of anxiolytic drugs evaluated in young animal subjected to behavioral test.

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].

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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