Review Article

Describing some behavioural animal models of anxiety and their mechanistics with special reference to oxidative stress and oxytocin relevance

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A R T I C L E   I N F O

A B S T R A C T

It is now generally accepted that animal studies are playing an important role in the understanding of anxiety disorders, since they contribute to the current knowledge regarding the mechanisms and possible therapeutic approaches in anxiety. In the present review we will detail some essential aspects of behavioral animal models of anxiety related to social defeat paradigm, elevated plus maze, elevated zero or T maze, light/dark box, social interaction test or tests based on predator models, considering the latest theories and methodological approaches in this area of research, as well as our previous studies focusing on anxiety manifestations in a variety of species including rats, zebrafish, dogs and pigs. Moreover, in this context, we will focus on the recent theories concerning oxidative stress, as well as importance of oxytocin administration (especially the intranasal route). This could be important considering that these two factors are currently being investigated as possible mechanisms (oxidative stress status) and related therapeutic target (both intranasal oxytocin and antioxidants) in the pathology of the anxiety disorders.
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Contents

1. Introduction

Anxiety disorders are clusters of psychiatric disorders that are mainly described by excessive and unjustified fear, somatic symptoms, and related behavioral responses (escape, avoidance or assurance behaviors) [1]. It is important to mention the fact that animal models will never be perfectly replicating the complex human neuro-psycho-pathology. Still, they have a fundamental relevance in studying neuropsychiatric disorders, considering important physiological and anatomical similarities between humans and animals [2].

Thus, animal models studies are mainly based on the physiological, anatomical, and genetic structural similarities between human and animals [3]. Moreover, animal models have many positive characteristics including, the fact that they are easy to obtain, maintain, handle, relatively inexpensive, and exhibit increased reproducibility, as compared to the clinical studies [4,5].

Considering these advantages, many rodent models of anxiety disorders have been developed and extensively used to study changes in animal behavior after exposing them to various types of stressors. Models of rodents (rat and mouse) [6] are the most commonly used models in neuropsychiatric studies, although other species of animal models have been described in neuropsychiatry research including primates [7], fish [8] or birds [9].

Studying the mechanisms of mental disorders such as anxiety on animal models is often questioned in terms of equivalence. However, as mentioned above, many evidences of similarities between rodents and human exist starting with the organization of nervous and endocrine systems [10,11]. Additionally, aspects regarding equivalences in terms of basic cognitive process and behaviors related to anxiety such as conditional learning, anticipation of danger, avoidance, freezing, and escape are important as survival mechanisms [12,13].

Concerning the validity type of the animal models, there are mainly three classes of models: 1- a correlation model with a predictive validity, implying that the model is sensitive to pharmacological agents [12], 2- an isomorphic model sustained by a face validity that implies an equivalence between behavioral responses in human and animals [13], and 3- a homologous model based on a construct validity that speculate similar etiology for a certain disorder between humans and animals [14]. By detailing, it is generally accepted now that especially for the rodent models of psychiatric and neurological diseases, there should be firstly a clear resemblance to the classical human symptoms (the so-called face validity), an additional resemblance to the main mechanisms of the disease (e.g. construct validity) and an identifiable response to the drugs that are generally working in humans (predictive validity), as described by Crawley in 2004 [15].

Considering all the aforementioned aspects, we will summarize both the current knowledge and our previous experience in animal models of anxiety, by focusing on various species such as rats, zebrafish, dogs, and pigs, as well as describing the relevance of oxidative stress in this context. Additionally, we will briefly describe the possible relevance of the oxytocin administration in this context, considering its increased relevance in this area of research in the last couple of years.

2. Some common tests of anxiety and their associated mechanisms

2.1. Social defeat stress

An important model in studying anxiety disorders relies on social defeat stress. Social defeat paradigm was used in large number of models that studied depressive [16,17] and anxiety disorders [18,19]. In this way, social defeat model is based on an interaction of the experimental animal with specific stimuli that the animal considers dangerous, without the possibility of escaping from the situation [20]. A model of social defeat is thus created by the exposure of the animal to an aggressive-type interaction that the animal considers dangerous. The classical experimental settings involve a short interaction with another larger individual, which is more aggressive, inducing a relation of coordination-submission [21].

Also, the model of social defeat could be a one-time confrontation with the threat or may be designed as chronic repeated exposures. Generally, exposure to repeated obligatory social stress (chronic stress) is fairly used in depression model, while acute exposure (acute stress) is rather investigated for anxiety disturbances [17,22].

In addition, several methods [23–25] have been described and used for study anxiety and stress related disorders that are generally divided into conditioned [26] and unconditioned methods [23,24]. The most common unconditioned tests are exploration-based models including elevated plus-maze, elevated zero maze, elevated T-maze, and the light/dark box or social interaction test [24,25], and also tests based on predator models (e.g. including cat exposure or rat exposure) [23].

2.2. Elevated plus maze

Elevated plus-maze is considered an important test that evaluates anxiety behavior by counting the number of entries in the open-arms [27]. The preference for the closed arms is a sign of need for security and reveals anxiety traits. The measure for anxiety is calculated by counting the number of open-arm entries and the number of closed-arm entries expressed as a percentage of the total number of arm entries and the period of time spent on the open arms [14]. In fact, this behavioral test has more sophisticated concerns, as additional anxiety-related behaviors are evaluated including head dipping in open-arms (sticking the head below the level of the maze and towards the floor), protected stretch-attend postures (stretching) in the closed arms (the animal stretches with the forepaws while maintaining the hind paws in the same place and then retracts to the original position), and grooming (cleaning of any part of the body with the paws and/or the mouth) [27]. Our team was the first; to the best of our knowledge; to show that the intra-cerebro-ventricular administration of a drug such as angiotensin-(1-7) could result in anxiolytic-like behavior, 3 weeks after neurosurgery, when administrated 8 consecutive days, 0.1 mg/kg body weight, as demonstrated by the significant increase in the time spent in the open arms of the elevated plus maze and increased head-dipping behavior in the open arms, as well as decreased stretching in closed arms [27]. Even more, one

important aspect to be studied in the elevated plus-maze test is the so-called "locomotor control" of the data, since it is difficult to differentiate between motor deficiencies and anxiety-induced suppression of exploration [25,27]. That is the reason why the counting of the closed arms entries; which are generally considered as an index of the locomotor activity; is essential in analyzing the behavioral significance of elevated plus maze.

Furthermore, grooming behavior can sometimes be associated with both anxiogenic and anxiolytic behavior, mainly depending on the environment where it is performed (e.g. inside the elevated plus maze - an anxiogenic behavior vs. inside the home cage - anxiolytic) [28,29].

Results from our former laboratory investigations of the anxiety-related functions of the central renin-angiotensin system, showed that blocking angiotensin II, by both its specific receptors antagonists or by blocking the angiotensin converting enzyme, resulted in anxiolytic effect [30,31], in studies involving the administration of intracerebroventricular captopril, as a central angiotensin converting enzyme blocker [30] or losartan and PD-123177, as AT1 and AT2 specific receptors antagonists [31].

Other authors [32,33] as well have used elevated plus-maze to study anxiety in rat models. For instance, Berton group had found that one time social defeat exposure followed by 14–18 h of sensory contact with the aggressor increased anxiety behavior in Lewis rats evaluated by elevated plus-maze [32].

2.3. Other specific behavioral testing for anxiety

Anxiogenic responses were also recorded by Bulos et al., who showed an anxiogenic effect on the rats after 5 min exposure to a predator (cat), but not 30 min after contact, as demonstrated both in elevated plus-maze and in elevated T-maze [23].

Another important test used for investigating the social anxiety is the elevated T-maze test. It is particularly used for panic and generalized anxiety disorders that investigate two types of behaviors related to anxiety (inhibitory avoidance and escape) [32].

Elevated plus-maze was successfully used by our research group in many experiments as follow:

1 Demonstrating an increased anxiety as a result of tartrazine administration, in which we work on to further develop a possible model of Attention Deficit Hyperactivity Disorder (ADHD) in rats (e.g. as a result of various specimens of tartrazine administration) [34], considering also the strong correlations between these manifestation, as sometimes more than half of the patients with ADHD have an co-occurring anxiety pathological manifestation [35].
2 Study the effect of black tea which is protective in both anxiety and fluoride-induced neurobehavioral toxicity in rats [36].
3 Study the anxiolytic-like effect of the deuterium depleted water, which has a concentration of deuterium 6–7 times lower than the normal water, as demonstrated by the increase in the time open arms of the elevated plus maze. In addition, this water generated an increased head-dipping suggesting a possible usage for anxiolytic actions [37].
4 Investigating the lesioning of the hypothalamic paraventricular nucleus through the usage of 5, 7-dihydroxytryptamine resulted in clear anxiolytic effects in rats, as mainly expressed in increased time in the open arms of the elevated maze [6].

3. Animal models commonly used in behavioral tests

3.1. The social defeat rat model

The social defeat rat model is involving a resident-intruder model, where a male rodent that plays the role of the intruder is placed in the cage of another male, which is the resident male. After the fight of the animals, a dominant and a subordinate male are established. In this way, social stress is believed to be experiences by the defeated rat model [38].

3.2. Studies of zebrafish

Another model to study behavioral changes is the zebrafish (Danio rerio) [8]. In fact, lately there is an increased concern in studying anxiety behavior in species such as the zebrafish. This could be extremely relevant, especially considering that zebrafish shares a fundamental pattern of neurodevelopment and functional brain organization with other vertebrates, including humans.

These shared features provide a basis for experimental research, demonstrating the mechanisms of disease-associated brain abnormalities [8], as current rapports are describing sleep related studies in zebrafish, associated or not with specific behavioral tests such as light and dark conditioning [39,40] or how to generate autism models in this specie [41].

Thus, Maximino et al. [42] clearly showed that zebrafish is increasingly being used in behavioral neuroscience, neuropsycho-pharmacology and neuro-toxicology. They showed how the behavioral tests used to model anxiety in rodents were adapted to zebrafish. Other research described how these processes are studied in this specie based on some related and conditional-associated color developed anxiety [43]. Thus, in this study, Blaser and Rosemberg [43] showed that in specific behavioral tasks for zebrafish, such as novel tank diving test (which is developed on the basic response of zebrafish to initially dive to the bottom of a novel experimental tank), anxiety could be modulated by the colors used for the tank construction (e.g. black, white or transparent, with the zebrafish always and naturally preferring the black one). In addition, specific behaviors related to anxiety, such as shuttling and immobility were affected by the aforementioned colors manipulation [43].

3.3. Models related to post-traumatic stress disorder

Another model as shown by Solanki et al. [44] shows how to generate animal models of post-traumatic stress disorder (PTSD). They demonstrated; in an original approach in rats; that witnessing a traumatic event is leading to the development of anxiety-like and depression-like behavior deficits. In this way, the aforementioned authors used their trauma witness model to subsequently interact with a socially defeated rat, generating a PTSD model [44]. Moreover they showed that anxiety and aggression can be associated and linked together by factors such as corticosterone and increased oxidative stress status [45].

3.4. Models related to ADHD and interactions

As previously shown in the case of complex interactions between ADHD and anxiety or aggression and anxiety, many of these behavioral manifestations are in tight correlations with each other, as our group previously described the interactions between anxiety and depression-related behaviors when studied in specific rat tests (Fig. 1). This also occurs when anxiety and depression-related behaviors are studied in rat models [46]. We investigated the hypothesis that oxidative stress is affecting anxiety, depression or PTSD, by mainly referring to their main biomarkers, animal models relevance, genetic perspectives and antioxidant approaches [46].

3.5. Oxidative stress implications

Lately, there is an increased concern in understating the exact relevance of the oxidative stress status in most of the
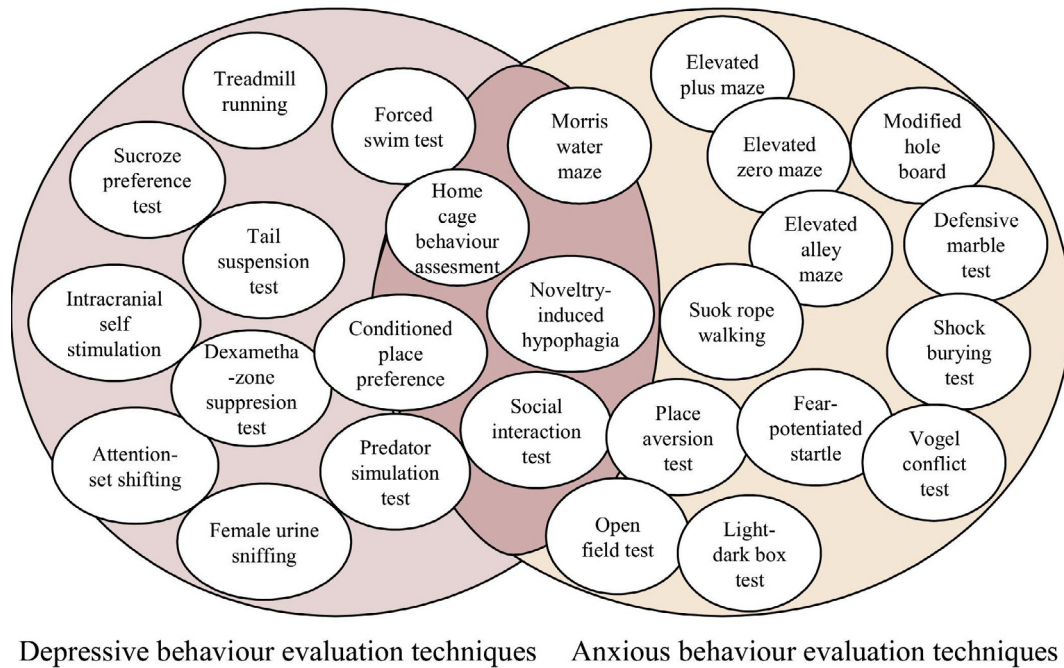
Fig. 1. The interactions between anxiety and depression-related behaviors as studied in various tasks in rat animal models, as described by Balmus et al. [46].

neuropsychiatric disorders [47–53] such as anxiety. Previous reports described for example that anxiety behavior in light/dark box task is clearly related to the oxidative status (as expressed through the ROS concentration) in both neuronal and glial cells of the cerebellum or hippocampus, as well as in the neurons of the cerebral cortex and peripheral leucocytes (monocytes, granulocytes and lymphocytes) [54,55].

Furthermore, Hughes et al. [56] showed that the administration of vitamins C and/ or E, decreases anxiety-related behavior of rats in open-field. Likewise, in the previous studies on anxiety in rats, most of the anxiolytic effects of angiotensin-1-7 or angiotensin II blockers were very well correlated with some antioxidant actions of these drugs [30,31,50].

3.6. Models related to irritable bowel syndrome

There is also an increased interest in the recent literatures on the irritable bowel syndrome (IBS) [57], which besides being a modern disorder clinically characterized by cramping, abdominal pain, bloat, diarrhea, and constipation. It is also a functional gastrointestinal disorder, which means that it is not characterized by any tissue or organ damage [58], exhibiting complex and controversial pathological features. The etio-pathogenic process in IBS is not well elucidated, but stress may be an important factor, as most of the times it is associated with increased levels of anxiety [59].

What are interesting in IBS animal models is their related anxiety manifestations [60]. In this way, the generation of the IBS animal models is mainly based on stress and anxiety, as shown by Mozaffari et al. [61], by using the method of chronic contention stress to induce gastrointestinal disorders where the animals were placed in plastic containers that permit complete immobilization, without any risk of suffocation. Thus, stress exposure was 6 h/day, for one week, which resulted in high levels of anxiety [61].

Also, our research group is now working in generating a model of IBS with greater reliability, which would enable a more relevant therapeutic approach, based on alternating multiple stressors, such as applying a combination of seven persistent, daily, chronic stres-sor: cage tilting, water/food deprivation, pinch tail, predator sound, wet bedding, and injection mimicking. An increased advantage of this method resides in avoiding the progressive habituation that may occur when a homotypic stimulus is repeatedly consistently.

4. Oxytocin relevance

Besides its well-known role in reproduction [62], oxytocin plays a significant role in most of the neuropsychiatric dysfunctions, with increased number of reports discussing especially the intranasal oxytocin management approach in disorders such as autism [63], schizophrenia [64], fronto-temporal dementia [65], Prader Willi syndrome [66], anxiety [67], depression [68], aggression [69] or suicide-related behavior [70].

Some of these results were also similar to results observed in other animal models, in species such as dogs [71,72], rat [73] or pigs [74–76]. In this way, some authors found changes in complex and specific behavioral tasks performed on dogs (such as Threatening Approach Test) when oxytocin was administered [71,72]. The authors observed that after given intranasal oxytocin some modification in the way the animal reacts to several different stimuli, such as the presence or the posture of their owner or an unfamiliar experimenter may occur [71]. Furthermore, in pig models (which are actually considered much more closer resembling to human brain in anatomy, growth and development, as compared to the brains of rodent [74]) that repeated administration of oxytocin postnatally can actually affect the hypothalamo-pituitary-adrenal axis functioning [75], with increased relevance to further anxiety-related manifestations.

Also, it was previously demonstrated in a different report on dogs that the administration of intranasal oxytocin is increasing the appropriate use of human social cues and can result in decreased aversion to gazing cues in these animals [72]. Also, recently Hernadi et al., showed that positive human contact could actually generate a significant increase in cerebrospinal fluid oxytocin levels, which could last for over 120 min, after 15 min interaction [71].
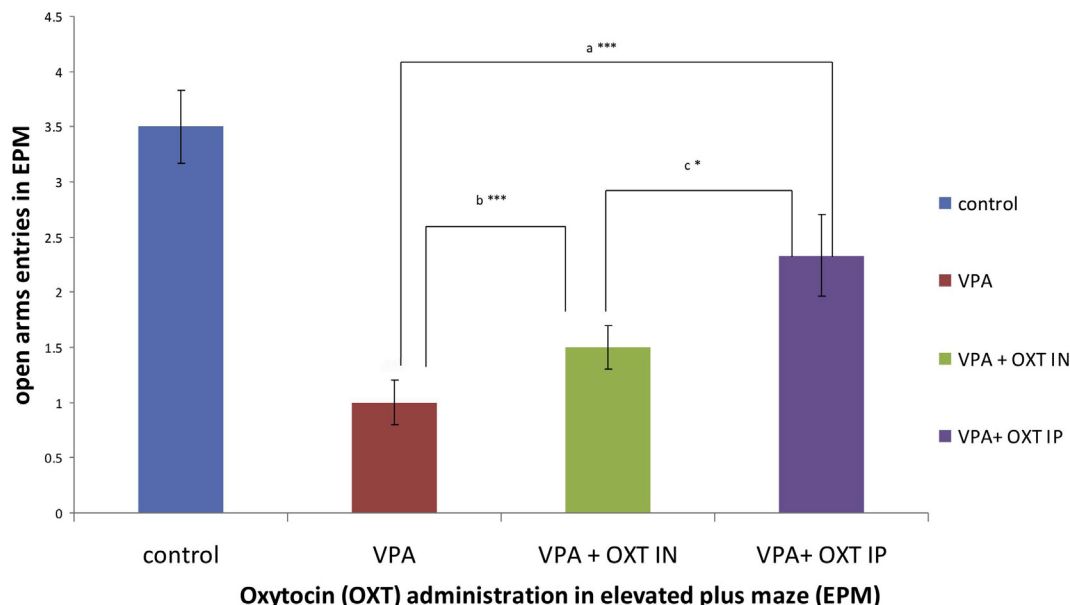
Fig. 2. Preliminary data of the consequences of intranasal (IN) and intraperitoneal (IP) oxytocin (OXT) administration (10 UI, for one week) on the numbers of open arms entries (a parameter of anxiety) in elevated plus maze (EPM) in a rat model of autism, based on Valproic acid (VPA) administration. Post-hoc analysis are equivalent for a, b, and c on the chart (***$P < .01$, *$P < .05$).

All these effects of oxytocin, related to anxiety, were also demonstrated in rats, as showed by Laszlo et al. [73], who reported that 10 ng or 100 ng of oxytocin, injected in volume of 0.4 μL in the central nucleus of the amygdala (an important central area related of course to anxiety behavior [50]) resulted in anxiolytic effects, modulated by the specific oxytocin receptor located at this level. Some effects of oxytocin could be related to its antioxidant ability, considering the current arguments regarding the possible antioxidant actions of this drug in animal models and human studies [77].

Moreover, our research group has some preliminary results (data not shown) on the effects that oxytocin administration could actually exert on the anxiety levels, as studied again in the elevated plus- maze, in an epigenetic schizophrenia rat model generated by the administration of methionine [78]. Even more, we are designing an experimental model to see if intranasal administration of oxytocin could reduce some anxiety manifestations induced in a rat model of autism (e.g. generated by the perinatal administration of valproic acid), with some preliminary positive data (Fig. 2), as seen in elevated plus - maze.

In addition, it seems that the administration of intraperitoneal oxytocin in these rats could also decrease anxiety, as judged by the increased number of open arms entries in the elevated plus maze [79,80] (Fig. 2).

5. Conclusions

We presented here some of our previous experience in working with behavioral animal models of anxiety, as well as some modern theories and methodological approaches regarding anxiety in lab species such as rats, zebrafish, dogs and pigs. Also, in this context, we described some current mechanism regarding oxidative stress and oxytocin administration, keeping in mind that these may represent future therapeutic targets in the pathophysiology of anxiety disorders.

Competing interests

The authors declare that there is no conflict of interest.

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