ASPECTS OF ANIMAL MODELS FOR MAJOR NEUROPSYCHIATRIC DISORDERS

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Abstract – We will review the main animal models for the major neuropsychiatric disorders, focusing on schizophrenia, Alzheimer’s disease, Parkinson’s disease, depression, anxiety and autism. Although these mental disorders are specifically human pathologies and therefore impossible to perfectly replicate in animals, the use of experimental animals is based on the physiological and anatomical similarities between humans and animals such as the rat, and mouse, and on the fact that 99% of human and murine genomes are shared. Pathological conditions in animals can be assessed by manipulating the metabolism of neurotransmitters, through various behavioral tests, and by determining biochemical parameters that can serve as important markers of disorders.

Key words: animal models; neuropsychiatric disorders.

SCHIZOPHRENIA

Although the complex pathology of schizophrenia cannot be replicated completely in animals, studies conducted on postmortem collected tissues of schizophrenic patients and brain imaging techniques revealed neuroanatomical and neurophysiological changes that could be used to generate animal models of schizophrenia, such as reductions of the cortical volume, in particular the frontal cortex, hippocampus and temporal lobes (Kircher, 2006). An important aspect in this field would also be the fact that these reductions can appear both before the onset of the disease and progressively afterwards, thus indicating a neurodegenerative nature (Webster, 2005).

Regarding changes in neurotransmitter systems which could be used to generate possible animal models of schizophrenia, it has been found that at the level of the central nervous system of schizophrenics, there is an increased quantity of dopamine and a large number of dopamine receptors, in particular D2. The administration of neuroleptics or antipsychotics that have an antagonistic action on dopamine D2 receptors exerts a favorable effect, while amphetamines, which are dopamine agonists, increase the schizophrenic symptoms (Jerlhag, 2008).

Moreover, this disorder is characterized by a hyperactivity of the dopaminergic system, mainly the mesolimbic dopaminergic pathway that connects the midbrain ventral tegmental area to the nucleus accumbens (Marcotte et al., 2011). Given the relevance of this area to the positive symptoms of the disorder (Pakkenberg, 1990), our research group has carried out experiments regarding the significance of lesioning the nucleus accumbens in specific behavioral tests, including changes that this injury exerts on the main markers of the oxidative stress (Lefter et al., 2013). Given the roles that smoking and nico-
tine have in schizophrenia (smoking is traditionally considered self-medication in schizophrenics), the effects of the administration of nicotine to animal models were examined (Ciobica et al., 2012).

Another region of potential significance in animal models of schizophrenia is the prefrontal cortex and in particular its dorsolateral side. In humans, injury to this area produces serious deficiencies in attention and cognitive processes. However, in schizophrenics this area does not display a specific pathology, although some authors speak of a degree of local atrophy and a reduction in the number of neurons (Webster, 2005). It is considered that this nervous structure may explain why the first schizophrenia symptoms occur after adolescence. The myelinization of this area is not complete until the age of 20, and experimental injury of this area in young monkeys does not generate immediate cognitive impairments, instead it decreases the ability to perform some cognitive tests (Kerwin, 1997).

Another important aspect that may be of relevance in generating animal models of schizophrenia is presynaptic glutamate receptor deficiency, which seems to be one of the major causes of schizophrenia, as the administration of metabotropic receptor mGlu2/3 agonists was reported to be effective in the treatment of some schizophrenic symptoms (Patil et al., 2007). Similar symptoms of schizophrenia are also produced by the administration of glutamate antagonists for NMDA receptors, such as ketamine and phencyclidine, which are important indicators of the important role of the glutamatergic system functioning in schizophrenia (Reynolds et al., 2005). Related to glutamatergic transmission, another model of schizophrenia in rats proposes the administration of methionine a glutamate inhibitor, as a blocker for this neurotransmitter. While the administration of phencyclidine and ketamine in rat models was reported to lead to an increased release of dopamine, the administration of antipsychotics attenuates this effect (Jentsch and Roth, 1999). Moreover, the administration of agonists for the clozapine group II metabotropic receptors can block the effects of phencyclidine and ketamine (Deutsch et al., 2002).

Regarding the areas that are relevant in mimicking schizophrenic neuropathology, the glutamatergic receptors are densely distributed, especially in the hippocampus and limbic system, areas that are known to suffer important anatomical and functional alterations in schizophrenia (Webster, 2005).

In postmortem tissue samples of patients with schizophrenia, decreases in the concentrations of aspartic and glutamic acids in the frontal cortex, as well as alterations in the activity of the enzyme that converts N-acetylaspartylglutamate (NAAG) have been observed (Tsai et al., 1995).

In addition, serotonin (5-HT) is considered to be involved in the regulation of the dopaminergic system in the prefrontal cortex, striatum (including the nucleus accumbens) and mesencephalic ventral tegmental area, thereby influencing schizophrenic pathology. This was also shown in postmortem studies that indicated an increased density of 5-HT1 receptors in the prefrontal cortex. 5-HT1 agonists produce an increase in dopamine release from the rat prefrontal cortex, which could favor the negative symptoms of schizophrenia (Bantick et al., 2001).

**Alzheimer’s disease**

Alzheimer’s disease (AD) is the most common form of dementia and at present, the most widespread neurodegenerative disorder (Webster, 2005). AD has a complex pathology, reflecting a variety of connected cellular and molecular abnormalities.

The “cholinergic theory” addresses both aging and AD. It states that cognitive disturbances in are due to a deficit in acetylcholine, which has an established role in learning and memory processes. These issues are particularly important for the generation of animal models mainly based on the administration of specific cholinergic antagonists (e.g. scopolamine, a muscarinic blocker) (Ciobica et al., 2009).

It has been observed that with aging, as well as in the case of some types of dementia, the synthesis of acetylcholine decreases by 45-65%, as enzymes
involved in its metabolism degrade, undergoing isomerization processes that alter their properties and performance. Thus, AD was considered the prototype of cholinergic neurotransmission impairment (Webster, 2005).

Additionally, the cholinergic hypothesis for the etiology of AD grants a primary role to the cholinergic neurons connected with muscarinic receptors situated in the cerebral cortex that degenerate preferentially in this form of presenile dementia. The decline in acetylcholine content is linked to the depletion of choline acetyltransferase, the enzyme responsible for acetylcholine synthesis, whose activity is reduced by 90% in the brains of patients with Alzheimer’s disease. This reduction also occurs in the same brain regions that contain large amounts of amyloid β protein (Artenie et al., 2010).

There is substantial evidence that muscarinic blocking by scopolamine disrupts memory in young volunteers in a reversible manner that was qualitatively similar to that occurring naturally in demented subjects (Liem-Moolenaar et al., 2011). Scopolamine-treated normal young human subjects exhibit memory dysfunctions analogous to those observed in demented patients. These dysfunctions are reversible by physostigmine, but not by d-amphetamine, which suggests that the memory impairment is specifically related to reduced cholinergic transmission caused by scopolamine. In this way, scopolamine-induced amnesia has been proposed as a fundamental model for dementia where reduced cholinergic function is the suspected cause, as well as to potentially test anti-dementia drugs by their ability to protect against the cognitive impairment induced by cholinergic dysfunctions (Lee et al., 2012).

In both cases of these anticholinergic-induced animal models of dementia those based on the knock-out of specific genes implicated in dementia, the detection and confirmation of the cognitive deficiencies in animal models of AD is performed through specific behavioral tests that assess changes in memory and learning performance, such as the Y-maze task (Ciobica et al., 2012), radial 8 arm maze task (Gurzu et al., 2008), passive (Bild et al., 2013) or active avoidance (Hritcu et al., 2009).

**Parkinson’s disease**

Parkinson’s disease (PD) is the second neurodegenerative disorder after Alzheimer’s disease in the number of affected patients (Webster, 2005). Our laboratory has utilized an animal model of PD in rats (Hefco et al., 2003) using stereotaxic neurosurgical techniques in order to perform selective dopaminergic damage to the substantia nigra and ventral area tegmental area (part of the mesotelencephalic central dopaminergic system) (Hritcu et al., 2008). This is done by the administration of 6-OHDA, a toxin specific for catecholaminergic neurons (Ciobica et al., 2009).

These stereotaxic techniques require special atlases that describe in detail the specific areas at the central level of the studied animal, such as the atlas of Paxinos and Watson (2006). According to the atlas for the substantia nigra, with the bregma point (situated at the intersection of the coronal suture with the sagittal suture) as the point of reference, the following stereotaxic coordinates were established: 5.5 mm posterior to the bregma, 2 mm lateral to the sagittal suture and 7.4 mm ventral to the surface of the cortex (Fig. 1).

In most of our experiments, 8 μg of 6-OHDA dissolved in 4 μl physiological saline containing 0.1% ascorbic acid, was administrated with a Hamilton syringe over 4.50 min., and the syringe was left in place for 5 min after injection before being slowly removed (Fig. 1). The catecholaminergic terminations were initially protected through the administration of desipramine.

Despite the fact that PD has traditionally been considered a motor disorder, there has been much recent interest in the nature of cognitive impairment in PD, ranging from minor disturbances in memory to various intellectual functions and dementia (Kramberger et al., 2010). Additionally, there is a growing concern regarding the role of oxidative
Fig. 1. Different steps in obtaining a rat model of Parkinson's disease by neurosurgery.
stress in the pathogenesis of PD (Abeliovich, 2010), and it is now generally accepted that oxidative pathology contributes to the cascade leading to dopamine cell degeneration in PD (Jenner, 2003), although the exact mechanisms are not yet fully understood. For this reason, our research group has examined the relevance of several possible treatments (pergolide/nicotine) in a rat model of PD, to learning and memory processes, using several behavioral tasks such as Y-maze, shuttle-box tasks or radial 8-arm maze, including antioxidant enzyme activities from both the temporal and frontal lobe tissue (Ciobica et al., 2010, 2011), and oxidative stress in different neuropsychiatric disorders (Bild et al., 2013; (Padurariu et al., 2013; Stefanescu et al., 2012).

**Anxiety**

One of the classical approaches to the generation of animal models for the study of anxiety is based on the assumption that depression and anxiety are attenuated by agents that alter noradrenergic transmission, as these disorders are considered a neurodegeneration of the wake reaction (Webster, 2005). Since noradrenalin determines the emotional impact of a given stimulus, depression can be explained by inadequate noradrenergic transmission, which would mean that moderate noradrenergic activity causes attention, which is vital for proper cognitive function, and excessive noradrenergic activation climaxes into anxiety and agitation. In fact, any test that explores the memory and learning processes (such as those mentioned above) is affected by an animal's anxiety state, as the substances that modify this state are implicitly modifying the cognitive response. Thus, the link between anxiety and the formation of a new memory engram is based on the animal's tendency to get to know and explore new stimuli, against its natural tendency to avoid what is new and unknown, this conflict generating the anxiety condition (Bild, 2013).

The associative learning processes are linked to the same issues, the subject avoiding a stimulus that generated on another occasion an unpleasant sensation. These states are in general treated with benzodiazepines (diazepam being the classic example) that have specific binding sites in the brain (Espey et al., 2002). Benzodiazepines alleviate the noradrenaline turnover at the central level, thereby reducing the impact of certain stimuli, which would cause an increase in the release of neurotransmitters (e.g. application of an electrical shock in lower limb area). They would also be involved in decreasing the discharge rate of neurons in the locus coeruleus (Vermotten et al., 2002).

Other noradrenergic-mediated central areas are also relevant in this matter. Thus, the exposure of an animal to a new aversive stimulus, for example a long exposure to intense light, increases the concentration of extracellular norepinephrine in the frontal cortex, as well as in the hypothalamus. If a sound stimulus is activated each time an animal is moved into an intensely illuminated room, after a number of repetitions the animal will make an association between the events, which will change the degree of noradrenergic involvement in this process, the audible stimulus increasing the concentration of noradrenaline in the frontal cortex, but not in the hippocampus (Webster, 2005). Thus, the noradrenergic innervation of these two nervous areas is of particular importance for anxiety, considering the different roles in the generation of the conditioned response that occur because of exposure to various aversive stimuli. The prefrontal cortex would most likely generate responses to conditioned stimuli that are already known to be anxiogenic, while both areas appear to be involved in generating responses to unconditioned stimuli (McQuade et al., 2000). These two regions, along with the amygdala, septo-hippocampal system, hypothalamus and grey periaqueductal substance, are considered to form the so-called neuronal defense system, which is particularly important in the conditioning processes to various noxious stimuli (Bild et al., 2013).

Besides neurosurgical and pharmacological modulation of anatomical areas that serve to generate animal models of anxiety, there are also several specific behavioral tests that help our understanding of disease pathology. The elevated plus maze (EPM) is a well-known behavioral test for studying the anxiety state (Espey et al., 2002). In short, the
The elevated plus maze consists of four arms, elevated off the ground (Fig. 2). Two arms are enclosed by high walls and the other two arms are exposed. Rats are placed at the juncture of the open and closed arms and the amount of time spent in the open arms is recorded during a 5-min test (along with some other specific anxiety-related parameters). The time spent in the open arms is considered an index of anxiety.

Depression

Regarding depression, from a neurophysiological point of view the generation of some animal models of depression could be focused on the main neurotransmitters involved in its neuropathology, considered to be serotonin, noradrenaline and dopamine (Webster, 2005).

The serotonin hypothesis of depression originates in the involvement of serotonin in the regulation of the majority of functions affected by depression, such as motor activity, sexual behavior, sleep, appetite, food intake, anxiety, aggressiveness and impulsivity. Serotonin deficiency is thus considered an immediate cause of depression, and several studies have indicated decreasing plasma levels of tryptophan, the precursor of serotonin, in depressed patients (Almeida-Montes et al., 2000).

The study of serotonin metabolites, and in particular 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid of depressed patients, shows a decrease in its level, although this was connected with suicidal behavior and impulsiveness rather than with depression per se (Sullivan et al., 2006).

Inadequate noradrenergic transmission also seems to be involved in the mechanism of depression, given that the noradrenergic system influences the emotional impact of a given stimulus and participates alongside the dopaminergic mesolimbic system in motivation and pleasure. In this way, the administration of reserpine generates serious behavioral changes in the rat, characterized by the lack of reaction to almost any stimulus (a feasible model of depression may be thus obtained) and the motor idleness. This ‘reserpinic syndrome’ is attributed to the depletion of vesicular deposits of noradrenaline. When these reserpinic animal models were injected with iproniazid, which is an irreversible inhibitor of monoamine oxidases (MAO), the above-mentioned effects disappeared, the animals exhibiting even exaggerated mobility, which demonstrates the involvement of norepinephrine in the pathology of depression (Webster, 2005).

One the best-known processes in this respect is learned helplessness, which comprises behavioral
changes in animals that are unable to escape from a harmful stimulus (for example, an animal in a cage from which it cannot escape and where it is exposed to repeated electrical shocks at short intervals). After a period of time in which the animal attempts to avoid the stimulus, its state changes to a general disinterest and passivity towards most stimuli, refusing to seek an escape even when this becomes once more possible. These animals were found to exhibit a marked reduction in the turnover of central norepinephrine, in particular at the level of the cortex, hypothalamus and hippocampus, areas of critical importance in learning and memory processes. Moreover, the administration of substances involved in the noradrenergic depletion (such as reserpine) further aggravates this condition.

It seems that this process could be explained by the depletion of the available stock of norepinephrine in these areas as a result of repeated exposure to harmful stimuli. It should however be stated that in this process other neurotransmitter systems such as the GABA-ergic, cholinergic or the opioid systems, are also involved, which interact with the noradrenergic neurotransmission that seems to hold the crucial role. A similar phenomenon is observed when a rat is placed in a pool from which it cannot escape and in which there is no platform (Anisman, 1997).

It is generally thought that norepinephrine stimulates behavioral activity and nervous activation, with a beneficial effect on memory and learning processes. Thus, the various promoters of the central nervous system, such as amphetamines, increase the release of noradrenaline, resulting in the intensification of various behavioral processes and of amplitude waves on an EEG, while reserpine, which reduces the stocking and release of norepinephrine, causes a general psychomotor retardation. It was also noted that the discharge rate of the neurons that project from the locus coeruleus gradually increases depending on the complexity of the behavioral act that has been performed. In the same way, stimulation of the locus coeruleus causes the desynchronization of EEG rhythms and increases the neuronal activation status, while neurotoxic lesioning of its local neurons synchronizes the EEG rhythms and reduces the period of REM sleep.

A standard test used to assess the properties of possible antidepressant medication in the rat is represented by the forced-swimming test (Foyet et al., 2011). On the first day of this test that is relevant in generating animal models of depression, the rats are individually placed into cylindrical recipients (diameter 30 cm, height 59 cm) containing 25 cm of water. The animals are left to swim for 15 min before being removed, dried and returned to their cages (pretest session). The procedure is repeated 24 h later, in a 6-min swim session (test session). During the test session, the following behavioral responses are recorded: immobility (time spent floating with minimal movements to keep the head above the water) and swimming (time spent actively swimming). Increased percentages of immobility time will suggest more depression-like manifestations.

**Autism**

Although it is not possible to reproduce all autistic symptoms in animals such as the deficiencies of language or intuitive ability, most animal models of autism developed so far are able to shape a series of typical symptoms through certain tasks that assess sociability, vocalization or the change of routine.

It has been found that all teratogenic agents that can induce this complex disorder act during the first eight weeks after conception (Crawley, 2012). While this is a very important aspect in the generation of potential animal models of autism, it also represents evidence that autism arises very early in development of an individual.

Abnormalities of the serotoninergic system are largely indicated as the main mechanism in the pathogenesis of the autistic spectrum. Moreover, most of the brain regions considered responsible for autism are densely innervated by projections of serotoninergic neurons (cerebral cortex, with the prefrontal lobe and superior temporal gyrus, the brainstem with the dorsal raphe nucleus, upper and lower
olivary nuclei, the nucleus of the facial nerve or the limbic system) (Kinast et al., 2013).

To date there are relatively few models of autism of mice and rats mentioned in the literature. According to Crawley, there are several different approaches regarding the methods for designing autistic animal models. On the one hand, there are the genetic mutations either of genes responsible for neurotransmission, or regulation of social behavior (e.g. autistic models of mice obtained by mutations of genes for oxytocin or vasopressin, (Crawley, 2004, 2012). On the other hand, some models can be obtained by inducing abnormalities in neurotransmitters or certain brain areas that are affected in autistic patients. By this approach and considering the reported autistic effects of early fetal exposure to teratogens, Patricia Rodier developed a model of autism in rodents by prenatal exposure of the animals to valproic acid (VPA). This method consists of injecting pregnant females during neural tube enclosure stage of embryonic development with VPA (Rodier et al., 1996; Crawley, 2004).

Valproic acid is a carboxylic acid and a valeric acid analog, with antidepressant and anticonvulsive actions. It is used for treating epilepsy and various bipolar disorders or depression. It stimulates the GABA neurotransmission by inhibiting GABA transaminase.

Following prenatal exposure to VPA, more brain abnormalities similar to the phenomena in human cases of autism were recorded, such as a decreased number of Purkinje cells, neuronal degeneration, altered distribution of 5-HT neurons in the dorsal raphe nucleus, cell losses in the middle and lower layers of the prefrontal cortex and in the lower layers of the somatosensory cortex. Although behavioral testing is not well established and there is no defined set of tasks relevant to autistic symptoms, these were partially described in previous literature (Crawley et al., 2004). Thus, autistic symptomatology should be assessed in relation to social interactions, sociability, communication and repetitive behavior.

For assessing the sociability parameter and especially for studying an abnormally low level of sociability in very social species such as rat or mouse, Crawley et al. (2004) suggests using a special tricameral device: the animal is placed in the central compartment (1), so that it is given the choice of whether to interact (2) or not (3) with a unknown animal placed into one of the two lateral compartments (Fig. 3).

![Image of a tricameral device for social interaction paradigm]
Repetitive behavior is described as rigidity to change an activity that has become routine. As a common trait in autism, rodents can be trained in repetitive behavior until the adoption of some routines and then tested when changing the conditioning stimulus. As a working method, the use of the T-maze task (alternation task) was proposed. The animal subjects are trained to choose only one of the two opposite arms of the maze, where they would find a food reward. The development of routine is accomplished when the tested animal makes at least 8 out of 10 correct choices for three consecutive days, after which the food pellet is moved to the opposite arm (Fig. 4).

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