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In vivo methodology in behavioural pharmacology – Where are we now?

In vivo metodologija u bihejvioralnoj farmakologiji – gde smo sada?

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Introduction

Behaviour includes all the actions and responses of an organism, related to the external environment, but also the functioning of internal factors. It includes any activity that can be observed directly, through senses, or indirectly, using technical devices. Central and peripheral nervous system, and also hormones, have an essential role in the control of behaviour. Numerous researches and experts, such as pharmacologists, psychiatrists, neurologists, psychologists, and other scientists, make a huge effort in uncovering all the peculiarities of how drugs affect behaviour. Some authors, accepting the risk to wrong, defined that a drug has an influence on behaviour, if it leads to changes in mood, communication, circadian rhythms, and cognitive processes [1]. However, some external circumstances, situations, and individual characteristics can lead to completely different and paradoxical effects and impacts on behaviour, even when administering agents with well known pharmacological profile. Finally, the characteristics of a drug can affect behaviour, such as drug dosage and routes of administration, frequency of administration and interactions.

Modern medicine has made a great progress in neuroscience, from gene and receptor cloning to the revised classifications and introduction of a new group of drugs. Undoubtedly, research work represents a critical, but necessary component of progress in biomedical sciences, and especially in psychotropic drugs research field, this process is very specific and sensitive. It is the complexity of neurological and psychiatric pathology that requires a special research approach in behavioural pharmacology, as from the aspect of scientific research work methodology, so from the aspect of ethical principles [1]. The methodology of experimental work in neuropsychopharmacology includes the complementary application of many different *in vitro* and *in vivo* techniques, whereas *in vitro* methodology applied techniques of molecular biology and computer technology, while *in vivo* studies involve the use of different preclinical models and tests in the study and monitoring of behaviour.

In vivo methodology is a basis of behavioural pharmacology, which developed as a synthesis of two disciplines, experimental analysis of behaviour and pharmacology. Behaviour represents the entire activity of an individual, especially one segment of activity that is the subject of observation. Behavioural tests and models are based on monitoring of certain parameters/index, that is selected segments of individual activities, implemented by engaging skeletal musculature − somatic manifestations, and/or smooth muscles and glands − vegetative manifestations.

There are two basic types of behavioural research: research based on examination of unconditioned or spontaneous animal behavior, and research based on conditioned behaviour, when establishing a correlation between the percep-

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tion of certain stimuli (conditioned stimulus) and rewards or punishments (unconditioned stimulus).

Behavioural results are usually expressed in terms of motor activity parameters (e.g. locomotor activity and sniffing) and rarely through non-motor parameters (e.g. ultrasonic vocalization). However when possible, non-motor parameters should be monitored, because they increase the quality and specificity of research. Each individual behavioural parameter should theoretically provide information on various aspects of nervous system function, while the research work effectively monitors whether, under the influence of some treatments, it will come to an increase or decrease in the value of a parameter when compared to control values. In addition, the interpretation of the value of the monitored parameter is a particular challenge in behavioural pharmacology. Experimental, i.e. animal models of neuropsychiatric diseases try to include different aspects of human characteristics, from the physiological and behavioural parameters to etiology of disease and therapeutic effects. By definition, animal models are developed in one species with the aim of studying characteristics that occur in other species [2]. When it comes to experimental models for studying human psychophysiology and/or pathology, the essence lies in the development of the syndrome in animals that are, in a way, similar to those developed in humans.

In general, the ideal experimental model for testing any clinical condition must meet three criteria of validity: the possibility of qualitative assessment of therapeutic efficacy of the new treatment (predictive validity), similarity of behavioural signs and symptoms in humans and experimental animals (face validity) and strong correlation between the findings at neural and behavioural level in animal model, compared to the findings in patients (construct validity). It is certainly not easy to meet all three criteria of validity in experimental models, especially in the field of neuropsychopharmacology. Furthermore, a model in behavioural pharmacology is described as an experimental setup developed in a non-human species replicating human features, with the purpose of measuring physiological, pathophysiological and behavioral responses [2]. The protocols applied to record these experimental data and to measure behavioural parameters are often called tests. Here we focus on methodology, animal tests and models, used in our laboratory settings: animal methods for testing anxiolytic and antidepressant effects of drugs (elevated plus maze and forced swimming test); animal methods for testing drug effects on cognitive function (active avoidance test and Morris water maze); animal models of schizophrenia and other neuropsychiatric disorders; and genetic approach in designing psychopharmacological studies.

Animal methods for testing anxiolytic and antidepressant effects of drugs

In recent decades various experimental procedures were developed in order to broaden preclinical studies in behavioural pharmacology of anxiety and depression. Furthermore, the benzodiazepines discovery about 60 years ago strongly stimulated the development of these behavioural procedures. Using the animal models in the study of anxiety- and depression-like behaviour in humans is possible, although there is no complete evidence that rodents, the most commonly used in these experiments, experience anxiety and depression the same way like humans do. However, behavioural and physiological studies show a sufficient analogy, if not homology, between anxiety and depression in humans and rodents [3].

Elevated plus maze

Nowadays most studies use anxiety tests based on unconditioned responses during spontaneous behaviour in animals, and here we report elevated plus maze, as one of the most often used. Elevated plus maze, introduced in 1984, soon became one of the most used animal models of anxiety. A widespread application of this test is primarily for practical reasons, because this method allows a quick search of potential anxiolytic effects of drugs, without animal trainings and complex procedures [4]. This type of behaviour is based on exploration phenomenon − exploration as a behavioural activity encouraged by unknown environment [5]. If unknown environment contains contrast (light/dark, open/closed space), another basic biological process is activated: the separation of attractive and repulsive parts in unfamiliar space. In this situation, the animal is faced with two opposing motivation conflicts: to explore the available parts of space in order to find exit, or to avoid aversive parts of unfamiliar space.

Practically, this model consists of four arms: two open and two closed arms. When an animal is placed into the maze, it starts exploring different areas. It is being measured the number of entrances into the open/closed arms, as well as time spent inside of each arm. Due to thigmotaxis phenomenon (the response of an organism to physical contact or touch), i.e. innate choice of enclosed space, rodents enter more often and spend time in enclosed maze arms. Time spent in open maze arms is followed by increased plasma corticosterone concentrations, more frequent motor activity immobility responses (immobility reaction, freezing), defecation, hormonal and behavioural changes referring to elevated anxiety levels. Anxiolytics help the animal to overcome fear of open maze arms, while aversive agents inhibit the entrance into the open maze arms [5].

Forced swimming test

Forced swimming test (FS) is a standard test used to evaluate antidepressant features of substances. During the test, animal is being placed into enclosed space filled with water, thus making the aversive situation that provokes reactions of despair and hopelessness. If the substance has antidepressant potential, it will extend the time an animal spends in finding the way out of a cylinder, i.e. it will reduce the immobility time [6, 7].

However, in recent years, a more complex psychophysiological basis of forced swim reaction has been more and more pointed out to. Repeated forced swim test becomes a model for studying the process of motivation and contextual memory formation in an aversive situation, analogous to

aversive avoidance reaction, which represents the process of instrumental learning through aversive stimuli. Under the influence of aversive situation, in an early phase of forced swimming, defensive reaction is provoked by which an animal attempts to leave the aversive space. Over time, and after many unsuccessful attempts, animal enters into the stillness mode, i.e. immobility. To what extent an animal will continue to fight mostly depends on the degree of motivation. This way, the reaction of forced swimming allows for monitoring antidepressant effects, which are largely shaped by the degree of motivation, referring to motivation as the point of contact in terms of positive reinforcement factors in learning the reaction of aversive stimuli avoidance and successful conflict resolution followed by depressive symptoms [8, 9].

Animal methods for testing drug effects on cognitive function

When examining the potential effect of substances on cognitive function, one of the main issues is how these drugs influence cognition in healthy subjects, compared to those with experimentally-induced cognitive impairment [10]. There are numerous examples where the use of different substances improved certain types of memory in normal, healthy animals. On the other hand, there are examples where pro-cognitive effects of some drugs were noticed only in animals with low basal performances, while in healthy animals no measurable behavioural effect was noticed. For example, some antipsychotics (clozapine, sulpiride), reduce cognitive impairments that occur after the disruption of prefrontal cortex, and the same drugs impair learning and memory when applied to healthy subjects [11]. In this sense, study of pro-cognitive drugs on healthy animals may be a useful screening, and the lack of effect does not necessarily mean that they will not manifest in any other disturbed system. In support of using healthy subjects in the process of discovering pro-cognitive drugs is the fact that the pathophysiological basis, which would be of great importance for creating animal model of disease, is often not sufficiently known or it is usually difficult to achieve satisfactory level of analogy in animal model. Finally, normal, healthy animals usually manifest characteristic responses qualitatively identical to those manifested by animals with certain disease [11].

Active avoidance test

Active avoidance reaction has been used in behavioural experiments for many years, in order to study different memory processes. Devices that are used are different, but they down to the fact that certain activity of an animal, which can be registered, is a way to stop the aversive stimulus. If a passivity is required from an animal during aversive stimulus, then it is passive avoidance response. If certain animal activity is a way to avoid aversive stimulus, it is the reaction of active avoidance [8]. Passive avoidance test is, at the same time, a test of evaluation of anxiolytic drug effects, and also evaluation of drug effects on cognitive function [12]. Amygdaloid complex is critically involved in modulation of memory with emotionally arousing experiences, such as electrical stimulation of the paws in this test. Animal is placed into the lit compartment of the cage, where the cage has a lit and a dark compartment. During transition to the dark compartment, which is a usual reaction of rodents being animals of the dark, they are given unavoidable electric shock. That is the basis for conflict development in animals, which can be mitigated with anxiolytics, i.e. latency time of reentrance into the dark compartment, in which the animal received unavoidable electric shock the other day, can be reduced. However, drug effects on cognitive function should be always kept in mind.

Active avoidance test represents primarily a test for evaluation of cognitive functions. Active avoidance reaction (AA) is a behaviour that is easily learned, but hard to forget, therefore this way high reproducibility of results is provided when investigating drug effects on learning this reaction. AA is a complex acquired behaviour that manifests itself through aversive stimulus avoidance under strictly controlled conditions of exposing an animal to this stimulus. Fear and escape represent two decisive moments in learning this reaction. In the first learning phase, associating conditioned stimulus (light, sound) and unconditioned stimulus (electric shock), in accordance with classical principles of Pavlovian conditioning, results in conditioned fear response. This kind of fear, in further process of learning AA reaction, causes locomotor escape response from the site of aversive stimulus. Animal does not escape because of aversive stimulus, but because of the tendency to resolve fear. In second learning phase, fear represents psychophysiological precondition for further learning AA reaction. Second phase begins when animal escapes from conditioned stimulus, which means that it avoids unconditioned stimulus. This way, conditioned stimulus is a precondition for fear development, which is then the basic motive for active avoidance reaction occurrence. The disappearance of fear is conditioned by an adequate motor response, i.e. by practical avoidance in occurrence of next conditioned stimuli. This way, fear is essential for active avoidance reaction development, while fear disappearance in this reaction performance is an important precondition for its learning [8, 12].

Morris water maze

Next, a very often used laboratory task in behavioural neuroscience is Morris water maze (MWM). Morris water maze is used for studying neurobiology and neuropharmacology of spatial learning in experimental rats and mice, and there is almost no psychoactive compound that has not been tested in this task. Spatial memory is defined as capacity of orientation learning in a new environment [13]. The basic apparatus consists of a circular pool, 1.5−2 meters in diameter, containing room temperature water, and a platform placed at a pool quadrant, is 1.5 cm below the water surface [14].

Animals learn, over sequence of trials, i.e. training, after being placed into the pool, to find a way to the platform in a more efficient and faster way. Reduction of time and path-length to the platform during training refers to the successful task mastering. It is recommended that animals swim

4−6 times per day, over 5−10 days, or until escape to the platform latency reaches asymptomatic level [15]. Over time, as the popularity of MWM grew, a number of methodological approaches developed, some of which significantly expanded the area of its application [16]. The main advantage of Morris water maze, compared with other tasks used in the study of neurobiology of memory and learning, is its performance simplicity and the ability to separate memory deficits from deficits in motor and sensory processes [17]. Submerging the experimental animal into the water can be unpleasant and trigger certain stress level, but this is how significant aversive stimuli are avoided [18].

MWM does not require previous training, and testing can be performed over a couple of days with a relatively small number of animals. Because of the water environment, the influence of olfactory traces is avoided, and using a visible platform version of the test potential visual impairments can be identified. Memory impairment induced by applying treatment is independent from locomotor effects, since an increase or decrease in locomotor activity does not necessarily affect the swimming speed. Since the success in platform discovering does not depend on entire activity or body weight of an animal, this test is suitable for a number of disease models in which cognitive impairment is present [16].

Animal models of schizophrenia

Schizophrenia is a burning issue in animal modeling, because of its still unknown etiology and unique psychopathology. Attempts to replicate delusions, hallucinations and other mental disorders in an animal model seem to be very challenging. The course of schizophrenia is very variable, and environmental and genetic susceptibility factors must also be considered [19, 20]. Therefore, pharmacological animal models of schizophrenia are basically developed on current knowledge of the alterations in different neurotransmitter systems [21].

The models which mimic positive symptoms of schizophrenia, such as delusion and hallucinations, are dominantly based on dysfunction in central dopamine, serotonin and glutamate neurotransmission. Therapeutically challenging are especially negative symptoms and rodent models of affective flattening, anhedonia and diminished social interaction were already introduced. Cognitive deficits are mainly assessed in rodent models of learning/memory (Morris water maze and object and social recognition) and in rodent models of attention (prepulse inhibition - PPI). At first, the stereotypes seemed to have the most face validity. Now, it appears that these symptoms are more closely linked to drug side effects, so diverse extrapyramidal symptoms were explored, such as acute dystonia (purposeless chewing in rodents, dystonia in monkeys), parkinsonism (catalepsy in rodents), akathisia (defecation in rodents) and tardive dyskinesia (long-term antipsychotic treatment in rodents and monkeys) [22, 23]. The advantages of these models are appropriate predictive validity, with some degree of construct validity, but limited face validity.

Lipska [19] discussed dysfunctional glutamate neurotransmission in schizophrenia [19]. Namely, ketamine and phencyclidine, as non-competitive antagonists of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors, produce symptoms similar to acute schizophrenia. When an acute subanesthetic dose of NMDA antagonists were introduced in rodents, it creates schizophrenic symptoms, such as enhanced stereotyped behaviour, hyperlocomotion, cognitive deficits, and impaired social interactions. Phencyclidine and other NMDA antagonists perform their action in the prefrontal cortex, by acutely increasing extracellular levels of glutamate and dopamine, as well as norepinephrine and acetylcholine, and altering firing patterns of dopaminergic neurons. Taken together, face and predictive validity for this model are satisfying, although construct validity remains limited. However, long-term phencyclidine administration produces different effects compared with those of single injection: decrease in stereotyped locomotion and an increase in exploring behaviour, tolerance, immobility time in forced swim test and depressive symptoms. The pharmacological effects of phencyclidine as well as other NMDA antagonists are mostly not mediated by increased dopamine transmission; therefore their behavioural effects are not blocked by typical antipsychotics but reduced by atypical antipsychotics, such as clozapine [24]. NMDA antagonist model can be convenient for testing the efficacy of novel antipsychotic drugs, and the advantage of this model versus dopamine-based model is its strong construct validity in exploring the cognitive dysfunctions in patients with schizophrenia. Further, targeting the modulatory co-agonist glycine-B site of NMDA receptors has been shown as a promising approach to ameliorate NMDA receptors hypofunction and reduce severe side effects of NMDA receptors agonists [25].

The pharmacological properties of the class of agents, represented by lysergic acid diethylamide (LSD), called psychotomimetics or psychotogens, suggest a hallucinogen model of schizophrenia [26]. It has been showed that LSD, acting through direct activation of serotonin 5-HT_{2A} receptors and antagonism of 5-HT_3 receptors, attenuates the behavioural hyperactivity caused by amphetamine, as well as phencyclidine application. It has to be pointed out, however, that the tolerance was rapidly developed to the effects of LSD, whereas the symptoms of schizophrenia are persistent for a lifetime. Furthermore unlike schizophrenia, hallucinations as a consequence of LSD consumption are typically visual rather than auditory [21]. Therefore, these findings somehow impair predictive validity of the hallucinogen model of schizophrenia.

Finally, injection of picrotoxin, a gamma aminobutyric acid (GABA) receptor antagonist, into the medial prefrontal cortex causes reduction of PPI in rats [20]. Pretreatment with the dopamine antagonist haloperidol antagonized this effect, pointing out that blockade of GABA receptors in prefrontal cortex impairs sensorimotor gating in a dopamine-dependent way. However, other GABA-induced behavioural deficits, linked to schizophrenic symptoms, remain to be further elucidated for the predictive and face validity of this model.

Animal models of other neuropsychiatric disorders

An animal model of neuropsychiatric disorder is a challenging attempt to capture the essence of the condition, but it

does not claim to reproduce the complete human condition in an animal. Animal models of schizophrenia are widely used and evaluated; however, there are numerous attempts to develop animal models of other mental illnesses. The most frequent used and robust rodent models of other neuropsychiatric disorders, such as Parkinson's disease (PD), Alzheimer's disease and obsessive-compulsive disorder (OCD) are presented in Table 1.

Finally, the animal modeling of obsessive compulsive disorder (OCD) is certainly one of the most complex approaches in behavioural pharmacology. Attempts to replicate obsessions (intrusive recurrent thoughts) and compulsions (repetitive aberrant behavior) seem to be very challenging. Research has been focused on developing models for compulsivity, as the obsessions cannot be effectively approximated in animal models [31]. Among a few animal models of

Table 1

The reference animal models of other neuropsychiatric disorders

Disorder	Animal model
Parkinson's disease	Neurotoxin-based model
Alzheimer's disease	Scopalamine-induced amnesia model
Obsessive-compulsive disorder	Quinpirole-induced model

An ideal animal model of PD should consist of pathophysiological and clinical features, involving central and peripheral nervous systems, dopaminergic and non-dopaminergic neurotransmissions, as well as motor and non-motor symptoms. In addition, the progressive characteristics of the disease and age-dependent onset should be reflected [27]. Neurotoxin-based model represents a robust animal model for PD, in which toxic molecules are mainly used to lesion the nigrostriatal dopaminergic pathway. Among the different types of neurotoxin-based models, the 6-hydroxydopamine (6-OHDA) model has been validated and well established in rodents [28]. Basically, 6-OHDA is capable of inducing degeneration of both dopaminergic and noradrenergic neurons in the brain. These neurons are highly sensitive to 6-OHDA, since their plasma membrane transporters possess high affinity for this molecule. Once taken up into neurons, 6-OHDA accumulates in the cytoplasmic matrix, producing oxidative stress-related cytotoxicity. The model scores well in the face and predictive validity; however, its construct validity remains to be further elucidated. Although not ideal, neurotoxin-based animal models have significantly contributed to our understanding of the pathophysiology of PD and potential pharmacological targets.

In the field of behavioural pharmacology scopolamine has been widely used as a reference agent for inducing dementia and age-related decline in cognitive functions in animals. The use of scopolamine, as a pharmacological model of dementia, is based on the hypothesis that the age-related cognitive deficits are predominantly related to a decrease in cholinergic signaling. Since scopolamine-induced amnesia is most likely caused by blocking of the cholinergic neurotransmission, it is used to model cognitive dysfunctions associated with aging and Alzheimer's dementia [29]. Moreover, another important issue in which scopolamine is often used is the preclinical testing of new compounds to treat cognitive deficits. If the substance possesses potential procognitive properties, it will reverse the scopolamine-induced cognitive dysfunctions in animals. Thus, scopolamine-induced model provides a quick and simple way for drug testing on cognition-enhancing properties. On the other hand, this model has a limited predictive validity, since it is associated with a high number of false positive results [30].

OCD, the model induced by quinpirole, the selective dopamine D2/D3 agonist, has been widely used and evaluated [32]. The quinpirole model is based on the hypothesis that dopaminergic system is predominantly involved in controlling compulsive behaviour. Sesia et al. [32] showed that chronic exposure to quinpirole induced the clear increase in compulsive behaviour in rats, which further supported the face validity of this model. Furthermore, they found that dopamine neuron activity corresponded to behavioural outcomes, confirming the findings that compulsive behaviour likely reflects, at least partially, a disruption of dopaminergic pathways. Although with an excellent face validity, the quinpirole-induced model of OCD still lacks pharmacological predictive validity.

Genetic approach in designing psychopharmacological studies

In study of genetic basis of neuropsychiatric diseases, two approaches are primarily used: bidirectional breeding of phenotype extremes principle and genetically engineered models, with a possible combination of these two principles [33, 34]. Bidirectional breeding of phenotype extremes principle is based on determination of variability within wild mice or rats lines, where animals are selected on the basis of specific criteria [1, 34]. By using selection, two extremely different lines in terms of behaviour are produced. Animals produced with predisposition for certain behaviour, are ideal for further genetic research and genetic manipulation. By using this approach, in studying anxiety in elevated plus maze, two lines of Wistar rats are produced: rats with low anxiety-related behaviour and rats with high anxiety-related behaviour [35]. The anxiety phenotype in these two lines is further used in behavioural and pharmacological researches. Genetic engineering produced the models of genetically conditioned anxiety in mice with targeted mutations in certain genes. Discovery of genes, that provide synthesis of key proteins for certain structural and regulatory functions, is the most specific, and at the same time subtlest approach in the study of pathological forms of anxiety. For this purpose in behavioural pharmacology we can analyze natural variations in genotype, elicit ran-

dom mutations using chemical mutagens, and, the most up to date, use the possibility of gene technology.

According to the mentioned approach, genetic modification can be achieved in two ways: by developing transgenic and targeted mutant animals [35]. Transgenic technology is applicable in mice, rats, and other animal species, and consists of microinjection of selected DNA sequence into fertilized egg cell. In accordance with the law of probability principle, DNA is integrated into genome, so that the tissue distribution and expression level of transgenic constructs varies from one to another animal line. The result of such approach is mostly the excessive expression of function determined by foreign gene, but it can also result in the loss of the monitored function. In targeted mutation approach targeted vectors are generated, which are specifically, by homogenic recombination, integrated into a desired location in genome in mice embryonic stem cells. Cells modified in this way are then injected into blastocysts, by which, mutation is transferred into strain line *via* embryo in development. In the case of gene inactivation experiment, targeted gene is inactivated by introducing neomycin resistance markers and/or knocking out a part of gene, which is marked as knockout technology. Knock-out mutations can be monitored in heterozygous state (evaluation of phenotype expression potential) and homozygous state (zero phenotype analysis). Changes noticed in phenotype serve as the basis for conclusion on normal function of the examined genes in the so-called wild, unmodified animals. Targeted mutations are not confined to ablations: essentially every desired change, such as point mutations, gene switching in mice, or switching of a mice gene with homologous human gene can be inferred into genome, and these subtle changes are marked as knock-in technology. Embryonic stem cells technology is currently applicable only in mice.

Several experimental models with genetic mutations on serotonin genes and certain $GABA_A$ receptor complex subunits have been created so far [35, 36]. Serotonin neurotransmitter takes a central place in pathology of many neuropsychiatric diseases. There is a number of pharmacologically different serotonin receptor types involved in a number of central and peripheral functions. Pharmacology and neuroanatomy point to serotonin receptor role in regulation of anxiety-depression expression, especially type 5-HT_{1A}. The results of recent studies in mice with 5-HT_{1A} receptor gene deletion confirm these findings. Several mice lines with single $GABA_A$ subunit inactivated gene have been produced so far. Knock-out of γ subunits had a lethal effect, and the inactivation of α6 subunit did not lead to phenotype manifestations. The lack of β subunit produced mice with epileptic phenotype, while ablation δ subunits reduced the sensitivity to neuroactive steroids. Mice lacking α1 subunit, normally present in about 50% of $GABA_A$ receptors, show intention tremor and increased sensitivity to convulsive action of bicuculline [35]. Still, the biggest drawback of this approach is the possibility of compensatory changes in development and function of the brain, caused by an absolute lack of these genes.

Preclinical/clinical interface

More and more discoveries of psychotropic substances speed up the tempo of clinical researches. However, it is obvious that preclinical studies run faster than clinical researches. Besides, over the last 10−15 years hundreds of compounds, on the basis of promising preclinical data, have been in clinical trial phase, but the majority of them have been dismissed due to lacking clinical efficacy. The main methodological problem in preclinical researches presents inexistence of an adequate animal model of neurological and psychiatric diseases, which could be completely applicable to human. Therefore, in behavioural pharmacological research, because of the different mechanisms of behaviour control and the processes of higher nervous activity regulation, there is no possibility for translating research results from an animal to the human. Special contribution have profiled behavioural research on genetically modified animal strains, as well as the use of the whole battery of tests, which is certainly going to provide the development of new drugs with higher specificity of action. Thus it is necessary to integrate the knowledge from all derived animal models and consider possible drug side effects.

Furthermore, it is always questioned which parameters of neurotransmission one should examine in an animal model to obtain the most appropriate characterization of behavioural, as well as neurochemical changes. There are certainly many biochemical parameters that describe dopaminergic, serotonergic and noradrenergic neurotransmission (synthesis of neurotransmitters, density and functionality of different receptors and transporter, alteration in the second messenger signaling), but only a few of them can be studied at any time. It should be taken into account, however, when compared to alteration of monoaminergic systems, modulation of inhibitory/excitatory amino acid neurotransmission, such as GABA and glutamate, has much more potent and rapid behavioural effects.

Conclusion

The animal preclinical methodology represents the so-called “bottleneck” in psychotropic drugs development. The use of animal tests and models as screening techniques seems to be slow when compared with the chemical synthesis speed and fast techniques of gene sequencing. However, such *in vivo* methodology are certainly necessary in order to obtain the initial evaluation of new compounds pharmacological effects, because it is not realistic nor ethical to go directly from the test tube into the clinic.

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REFERENCES

1. *Samardžić J*. Behavioural effects of the inverse agonists of benzodiazepine receptors. Beograd: Zadužbina Andrejević; 2015. (Serbian)
2. *Mckinney WT*. Animal models of depression: An overview. Psychiatr Dev 1984; 2(2): 77−96.
3. *Fuchs E, Flugge G*. Experimental animal models for the simulation of depression and anxiety. Dialogues Clin Neurosci 2006; 8(3): 323−33.
4. *Wall P*. Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. Neurosci Biobehav Rev 2001; 25(3): 275−86.
5. *Samardžić J, Savić K, Stefanović N, Matunović R, Baltezarević D, Obradović M*, et al. Anxiolytic and antidepressant effect of zinc on rats and its impact on general behavioural parameters. Vojnosanit Pregl 2013; 70(4): 391−5.
6. *Abelaira HM, Réus GZ, Quevedo J*. Animal models as tools to study the pathophysiology of depression. Rev Bras Psiquiatr 2013; 35(Suppl 2): S112−20.
7. *Samardžić J, Jadžić D, Radovanović M, Jančić J, Obradović DI, Gojković-Bukarica LJ*, et al. The effects of resveratrol on rat behaviour in the forced swim test. Srp Arh Celok Lek 2013; 141(9−10): 582−5. (Serbian)
8. *Samardžić J, Švob Štrac DO, Oprić D, Obradović DI*. DMCM, a benzodiazepine site inverse agonist, improves active avoidance and motivation in the rat. Behav Brain Res 2012; 235(2): 195−9.
9. *Samardžić J, Puškaš L, Obradović M, Lazić-Puškaš D, Obradović D*. Antidepressant effects of an inverse agonist selective for α5 GABA-A receptors in the rat forced swim test. Acta Vet (Beograd) 2014; 64(1): 52−60.
10. *Floresco SB, Geyer MA, Gold LH, Grace AA*. Developing Predictive Animal Models and Establishing a Preclinical Trials Network for Assessing Treatment Effects on Cognition in Schizophrenia. Schizoph Bull 2005; 31(4): 888−94.
11. *Floresco SB, Jentsch JD*. Pharmacological enhancement of memory and executive functioning in laboratory animals. Neuropsychopharmacology 2011; 36(1): 227−50.
12. *Savić MM, Clayton T, Furtmüller R, Gavrilović I, Samardžić J, Savić S*, et al. PWZ-029, a compound with moderate inverse agonist functional selectivity at GABAA receptors containing α5 subunits, improves passive, but not active avoidance learning in rats. Brain Res 2008; 1208: 150−9.
13. *Craver C, Darden L*. Discovering mechanisms in neurobiology: The case of spatial memory. In: *Machamer PK, Grush R, Mclaughlin P*, editors. Theory and method in the neurosciences. Pittsburgh: University of Pittsburgh Press; 2001. p. 112−37.
14. *Zhang F, Zhu ZQ, Liu DX, Zhang C, Gong QH, Zhu YH*. Emulsified isoflurane anesthesia decreases brain-derived neurotrophic factor expression and induces cognitive dysfunction in adult rats. Exp Ther Med 2014; 8(2): 471−7.
15. *Paul CM, Magda G, Abel S*. Spatial memory: Theoretical basis and comparative review on experimental methods in rodents. Behav Brain Res 2009; 203(2): 151−64.
16. *Vorhees CV, Williams MT*. Morris water maze: Procedures for assessing spatial and related forms of learning and memory. Nat Protoc 2006; 1(2): 848−58.
17. *McNamara RK, Skelton RW*. The neuropharmacological and neurochemical basis of place learning in the Morris water maze. Brain Res Rev 1993; 18(1): 33−49.
18. *Terry AV Jr*. Spatial Navigation (Water Maze) Tasks. In: *Buccafusco JJ*, editor. Methods of Behavior Analysis in Neuroscience. 2nd ed. Boca Raton (FL): CRC Press; 2009.
19. *Lipska B*. To Model a Psychiatric Disorder in Animals Schizophrenia As a Reality Test. Neuropsychopharmacology 2000; 23(3): 223−39.
20. *Schizophrenia Research Forum*. Animal Models for Schizophrenia Research 2014. [cited 2015 Apr 26]. Available from: http://www.schizophreniaforum.org/res/animal/animal_tables.asp
21. *Marcotte ER, Pearson DM, Srivastava LK*. Animal models of schizophrenia: A critical review. J Psychiatry Neurosci 2001; 26(5): 395−410.
22. *Porsolt RD, Moser PC, Castagné V*. Behavioral indices in antipsychotic drug discovery. J Pharmacol Exp Ther 2010; 333(3): 632−8.
23. *Porsolt RD, Castagné V, Hayes E, Virley D*. Nonhuman primates: translational models for predicting antipsychotic-induced movement disorders. J Pharmacol Exp Ther 2013; 347(3): 542−6.
24. *Jentsch JD*. Enduring Cognitive Deficits and Cortical Dopamine Dysfunction in Monkeys After Long-Term Administration of Phencyclidine. Science 1997; 277(5328): 953−5.
25. *Möhler H, Boison D, Singer P, Feldon J, Pauly-Evers M, Yee BK*. Glycine transporter 1 as a potential therapeutic target for schizophrenia-related symptoms: evidence from genetically modified mouse models and pharmacological inhibition. Biochem Pharmacol 2011; 81(9): 1065−77.
26. *Geyer M, Moghaddamn B*. Animal models relevant to schizophrenia disorders. In: *Davis K, Charney D, Coyle J, Nemeroff C*, editors. Neuropsychopharmacology: The Fifth Generation of Progress. Washington, DC: American College of Neuropsychopharmacology; 2002. p. 689−701.
27. *Jackson-Lewis V, Blesa J, Przedborski S*. Animal models of Parkinson's disease. Parkinsonism Relat Disord 2012; 18(1): 183−5.
28. *Bové J, Perier C*. Neurotoxin-based models of Parkinson's disease. Neuroscience 2012; 211: 51−76.
29. *Klinkenberg I, Blokland A*. The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. Neurosci Biobehav Rev 2010; 34(8): 1307−50.
30. *Sarter M*. Preclinical research into cognition enhancers. Trends Pharmacol Sci 2006; 27(11): 602−8.
31. *Albelda N, Joel D*. Current animal models of obsessive compulsive disorder: An update. Neuroscience 2011; 211: 83−106.
32. *Sesia T, Bizup B, Grace AA*. Evaluation of animal models of obsessive-compulsive disorder: Correlation with phasic dopamine neuron activity. Int J Neuropsychopharmacol 2013; 16(6): 1295−307.
33. *Collins A, Hill LE, Chandramohan Y, Whitcomb D, Droste SK, Reul JM*, et al Exercise Improves Cognitive Responses to Psychological Stress through Enhancement of Epigenetic Mechanisms and Gene Expression in the Dentate Gyrus. PLoS ONE 2009; 4(1): e4330.
34. *Rudolph U, Mohler H*. Genetically modified animals in pharmacological research: Future trends. Eur J Pharmacol 1999; 375(1−3): 327−37.
35. *Obradović D, Savić M, Ugrešić N, Bokonjić D*. GABAA receptors: Molecular substrate for development of new anxiolytics. Vojnosanit Pregl 2003; 60(3): 345−52. (Serbian)
36. *Barnes NM, Sharp T*. A review of central 5-HT receptors and their functions. Neuropharmacology 1999; 38(8): 1083−152.

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