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Ethological concepts enhance the translational value of animal models

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ABSTRACT

The translational value of animal models is an issue of ongoing discussion. We argue that 'Refinement' of animal experiments is needed and this can be achieved by exploiting an ethological approach when setting up and conducting experiments. Ethology aims to assess the functional meaning of behavioral changes, due to experimental manipulation or treatment, in animal models. Although the use of ethological concepts is particularly important for studies involving the measurement of animal behavior (as is the case for most studies on neuro-psychiatric conditions), it will also substantially benefit other disciplines, such as those investigating the immune system or inflammatory response. Using an ethological approach also involves using more optimal testing conditions are employed that have a biological relevance to the animal. Moreover, using a more biological relevant analysis of the data will help to clarify the functional meaning of the modeled readout (e.g. whether it is psychopathological or adaptive in nature). We advocate for instance that more behavioral studies should use animals in group-housed conditions, including the recording of their ultrasonic vocalizations, because (1) social behavior is an essential feature of animal models for human 'social' psychopathologies, such as autism and schizophrenia, and (2) social conditions are indispensable conditions for appropriate behavioral studies in social species, such as the rat. Only when taking these elements into account, the validity of animal experiments and, thus, the translation value of animal models can be enhanced.

1. Introduction

Animal models are important sources of information for advancing our understanding of the pathophysiology of human diseases and play a critical role in the efficacy and safety screening of novel treatments for these disorders. The translational value of animal models is one of ongoing discussion, particularly in pre-clinical neuropsychiatric research (see e.g. Braff and Braff, 2013; Kaffman and Krystal, 2012; McGonigle and Ruggeri, 2014). Over the past years this discussion has intensified due to frequent failures in clinical trials of novel psychoactive drugs that initially had good preclinical perspectives (Belzung, 2014). For example, results on the efficacy of corticotropin-releasing factor receptor-1 antagonists and neurokinin-1 receptor antagonists in animal models of depression initially seemed very promising in the preclinical phase, but failed to show significant effects in the following clinical trials (for reviews on this topic see for example (Belzung, 2014; Griebel and Holsboer, 2012; Haller et al., 2013). As a result of these setbacks most pharmaceuticals companies are cutting back on their research activities in the field of neurology and psychiatry, while some are even abandoning them all together (McGonigle and Ruggeri, 2014). Thus, the chances for generating therapeutic advances in psychiatric drug discovery appear to be diminished. This is particularly worrying in view of the high social and economic burden psychiatric illnesses place on our society (Greenberg et al., 2003; Wittchen and Jacobi, 2005).

Especially in the areas of depression and anxiety research, it has been noted repeatedly that the majority of the animal models available for these disorders have limitations, which seriously affect their translation value (e.g. Anisman and Matheson, 2005; Belzung, 2014; McArthur and Borsini, 2006; Willner et al., 1992). For example, in the area of depression the forced swim test or the tail suspension test in rodents are frequently used. Both are short behavioral tests in which usually one readout is taken as a measure of behavioral despair, i.e. the degree of immobility shown by the animal. However, these tests only mimic an acute state and are often combined with the one-time treatment with an antidepressant drug, which obviously is not compatible with the chronic treatment depressed patients require (Frazer and Morilak, 2005; McGonigle, 2014). In addition, these tests provide no measurements of anhedonia, which is one of the most important hallmarks of depression (Hasler et al., 2004).

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Of course the complex and heterogeneous symptomatology of most psychiatric conditions complicates the modeling of these disorders in animals, specifically in rodents. The fact that most of these conditions are described by subjective and sometimes contradictory symptoms in humans clearly does not help. Nonetheless, most animal models will benefit from a required improvement in experimental methodology. This will lead to more reliable data and consequently will increase the validity of animal experiments. This concept is also known as 'Refinement', one of the '3 Rs' (Russell and Burch, 1959). Refinement is typically seen as a tool to improve animal welfare; however, it will enhance the translational value of research as well. Ultimately, it will also help to reduce the number of animals required as the validity of each experiment is increased by improving the models and test procedures used.

Here, we discuss in particular the limited translational value of animal models for 'social' neuropsychiatric disorders, such as autism, with a specific focus on the measurement of social behavior in these models. In order to maximize the translational value of animal studies on these human conditions, investigating behavior under social conditions is a prerequisite. However, it is striking that in most animal studies the 'social element' is minimized or even omitted. We argue that refinement of the animal experiments on social disorders is needed, because too simplistic tests are used that cannot capture the full spectrum of social behaviors.

Moreover, the test situation and housing conditions should represent a more ethological valid environment, allowing the animal to express its 'natural behavior' as much as possible (under relative stress-free conditions). Ethology entails taking the environment (or ecological niche) in which the animal has been optimally adapted to (during evolution), into account in the design of the experimental test conditions. For instance, nocturnal animals should be tested in the dark or under dimmed light conditions. Including this concept will benefit all research disciplines, but it is of utmost importance when studying animal behavior. For example, tests involving maze learning in the water should be conducted with semi-aquatic species (such as the rat, but not the mouse), while studies investigating psychopathology that involves social behavior (such as depression, autism and schizophrenia) should be using social species. Thus, next to the classical validation criteria: predictive, construct and face validity, we would like to add the criterion of ecological validity. Finally, an important methodological improvement should be made by employing advanced automated approaches using ethological procedures. This kind of automation aids the detection of new behavioral elements by the 'computers eye', which is indispensable for assessing the quantitative aspects of movements (i.e. speed, distance, duration) or postures of animals, which cannot be analyzed quantitatively by the human eye. Furthermore, the increasing availability of new analysis algorithms enables the classification of behavior using for example machine learning.

2. Limitations of current behavioral animal models and measurements

2.1. An alarming preference for using easy and quick behavioral tests

There is a strong tendency in behavioral neuroscience to continue to use relatively simple, quick and seemingly easy tests, such as the open field and elevated plus maze, in which behavior of rodents is mostly captured by a single readout parameter, like time spent in a certain area. The popularity of these classical tests is rather striking as their low validity has been addressed repeatedly, (e.g. Crabbe et al., 1999; Fonio et al., 2012; Haller and Alicki, 2012; Kafkafi et al., 2005; Wahlsten, 2001; Walsh and Cummins, 1976) and more advanced alternatives are available. We have recently discussed this curious 'status quo' in behavioral science and proposed several solutions to advance the measurement and analysis of rodent behavior (Spruijt et al., 2014). For instance, one critical point to keep in mind when designing a behavioral experiment is the research context in which the study is performed. Behavioral assays are used typically for two research purposes: either as a simple "one parameter" indicator with limited explanatory power, or in a more hypothesis-free context to obtain information on the functional role of behavior (Spruijt et al., 2014). In studies that assess, for example, the toxicity of a compound or its dose–response properties, the use of a simple behavioral assay can be justified. However, when the biological function of behavior is discussed in view of the results obtained with a similar simple "assay-like" behavioral test, it becomes problematic. The "assay like" test is too limited to yield conclusive answers across the full richness and complexity of the behavior. This especially applies to the investigation of social behavior, which is extremely complex.

An example of a relatively simple test typically used to investigate social behavior, such as sociability or social interest, is the three chambered test. There are several versions of this test available in mice (Moy et al., 2004), including an automated version (Nadler et al., 2004; Yang et al., 2011). In this test a familiar or unfamiliar stimulus animal is confined to a small compartment, typically an inverted wired or plastic pencil cup, within one of the three chambers. The experimental animal is all allowed to explore the three chambers freely and social interest is measured by the time the experimental animal spends sniffing the cup holding the stimulus animal and/or by preference of the experimental animal for the chamber containing the stimulus animal. It can be questioned, whether a reduced approach time to the cup by the experimental animal indeed represents decreased sociality per se. Obviously, there is a strong confounding impact of locomotor activity in these kinds of tests, which in turn can be influenced by e.g. the species used, genetic background of the animal or the experimental manipulation. Indeed, performance in the test appears to be dependent on the background strain of the mice and the contextual novelty of the social stimuli used. Pearson and co-workers showed that C57BL/6J mice failed to exhibit preference for social novelty when the novel stimulus animal (an unfamiliar mouse of the same strain) was presented in a familiar context (Pearson et al., 2010). Moreover, the core feature of social behavior, which renders its sensitive to all kind of manipulations, namely the degrees of freedom two animals have to intertwine action-reaction patterns, is not represented in a test where the social interaction is reduced to inter-individual distance and sniffing.

Nonetheless, these tests are very popular. Most probably also because they are short (5–15 min on average), thus, relatively quick to run, and they can be used as part of a high throughput screening battery. Their short duration is at the same time another of their shortcomings. These tests typically only measure immediate reactions to novelty. We have previously shown that habituation of a mouse to a novel environment encompasses several stages, which can last up to days. Moreover, it is demonstrated that remarkable differences exist in the time course of habituation between different mouse strains (De Visser et al., 2006; Loos et al., 2014; Spruijt et al., 2014). In addition, familiarity to an environment has been demonstrated to affect sensitivity to different drugs, such as amphetamine, apomorphine, cocaine and serotonin (Carey et al., 2005; Dunne et al., 2007; Harkin et al., 2000; Joyce and Mrosovsky, 1964). These findings were substantiated by one of our own drug studies in which we could demonstrate that a prolonged observation time (up to 3 h) reveals more pronounced differences between drug effects compared to a 30 min observation time (Spruijt et al., 2014).