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Behavioral studies on anxiety and depression in a drug discovery environment: Keys to a successful future

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ABSTRACT

The review describes a personal journey through 25 years of animal research with a focus on the contribution of rodent models for anxiety and depression to the development of new medicines in a drug discovery environment. Several classic acute models for mood disorders are briefly described as well as chronic stress and disease-induction models. The paper highlights a variety of factors that influence the quality and consistency of behavioral data in a laboratory setting. The importance of meta-analysis techniques for study validation (tolerance interval) and assay sensitivity (Monte Carlo modeling) are demonstrated by examples that use historic data. It is essential for successful discovery of new potential drugs to maintain a high level of control in animal research and to bridge knowledge across *in silico* modeling, and *in vitro* and *in vivo* assays. Today, drug discovery is a highly dynamic environment in search of new types of treatments and new animal models which should be guided by enhanced two-way translation between bench and bed. Although productivity has been disappointing in the search of new and better medicines in psychiatry over the past decades, there has been and will always be an important role for *in vivo* models in-between preclinical discovery and clinical development. The right balance between good science and proper judgment versus a decent level of innovation, assay development and two-way translation will open the doors to a very bright future.

1. Introduction, background and objective

Observing behavior in animals is important for various reasons. In their natural environment it can be a matter of eating or being eaten. Humans use knowledge on animal behavior while hunting. The other way around, humans should also be able to recognize behavioral signs of threat displayed by animals. Observation of animals by human beings has turned into science in order to understand the world around us, but also to understand ourselves better. In the pharmaceutical industry, observation of animal behavior after treatment with a new potential drug is a key component in the drug discovery process.

The paper is reflecting on a personal career of 25 years in behavioral science. Initially, I've been trained during my early years to observe animals like chickens, mice, rats and monkeys with the intention of understanding their social behavior towards their peers and the behavioral responses towards all kinds of stimuli in their environment. This type of research can teach us, for example, how social systems are built and maintained within and across species. Typically, the role of the observing researcher is to interfere as little as possible with the intention to study spontaneous behavior in the animals. For example, when studying a large colony of monkeys in an open space like a zoo or even in the wild it means that as a researcher, one is dependent on being able to (1) recognize each individual animal and (2) learn about the hierarchy that is typically seen in such groups and (3) understand the behaviors displayed. The behavioral observations are performed from a distance without interfering with the social interactions that spontaneously occur. As a consequence, the researcher has little or no control about what will happen. A basic principle of scientific research is to build and test a hypothesis. However, in purely ethological studies like the example of a group of monkeys, it is not always feasible to drastically change the situation in order to test whether the hypothesis is valid. Such limited control and consequent complexity of building, testing and adjusting hypotheses is a real challenge for scientists who study pure and undisturbed behavior. Still, understanding innate behavior in animals is important for all scientists practicing animal research. For example, it is important to be able to recognize when animals in an experiment show abnormal behavior.

My personal focus shifted from basic behavioral science towards a more controlled hypothesis-driven approach in which animal experiments are better controlled and designed around treatment groups. One of the generally accepted arguments for

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using animals in captivity for research purposes is related to the drug discovery process. The goal is to find new and better treatments and medication for patients. The history of animal testing goes back to the writings of the Greeks in the 4th and 3rd centuries BCE, with Aristotle and Erasistratus among the first to perform experiments on living animals (http://en.wikipedia.org/wiki/History_of_animal_testing). Initial studies on animals were based on dissections in order to learn about the way the body is build and to practice surgical techniques for use in patients. Thus, the link between animal research and treatment of patients goes back a long way.

This paper will focus on animal research for anxiety and depression in a drug discovery environment. Here, animal research still plays an important role in the development of new treatments for patients. Prior to administering any new molecule to humans, various experiments are performed to build confidence that the potential drug is safe. In addition, other animal studies are performed to evaluate whether the treatment has any effect which might predict efficacy in the patient population of interest. Thus, animal research plays an important role in drug discovery in order to predict both safety and efficacy. However, the pharmaceutical industry is subject to change and the role of animal research within the drug discovery process will have to adapt accordingly.

The following sections will cover various factors that are essential for success in optimizing the quality of our animal research in a laboratory setting. There is not a single perfect animal model that is fully predictive for anxiety and/or depression in humans. This paper is not intended to provide the reader with all the answers, but rather to openly discuss several key factors that should be considered while conducting animal research. In 2008, I have published a review paper on potential pitfalls in the interpretation of data from animal models used in a laboratory setting with a focus on anxiety paradigms in rodents (Bouwknecht and Paylor, 2008). The goal of the present paper is to elaborate along the same lines, though with the focus on animal research in a drug discovery environment using anxiety and depression models as examples. Each section will highlight specific points to take into account while the overall approach across sections should provide at least some answers to the challenges that are continuously faced in a changing drug discovery environment. In the end, the ideas expressed in this paper should facilitate proper selection of the most relevant molecule for further development and clinical evaluation.

2. Typical 'acute animal models' for anxiety or depression

Several rodent models have been very popular in drug discovery research and are being used for multiple decades. Typically, these assays are relatively simple to perform in a laboratory with an acceptable through-put such that the turnaround time to get the results is short. While for each of these assays there are many ways of collecting and analyzing different parameters, the intention of this overview is to highlight the most frequently used readouts. Some generally accepted details of the assays are included in the brief description. For most models listed, specific key parameters are used to estimate a level of anxiety-like or depression-like behavior (so-called main effect), but also at least one extra parameter that is indicative of potential side effects or alternative explanation to the findings.

2.1. Anxiety models

The exploration models below are based on the balance between an innate drive to explore new areas versus suppression of risk taking behavior. The outcome of such a balance depends on the state of the animal. A rodent with a low anxiety level will explore more, while anxious animals will take fewer risks. Here, the duration of exposure to an unknown arena is limited since a reduction in exploration is seen over time, which then becomes less informative from an anxiety perspective. In addition to spontaneous exploration models, there are also anxiety assays that are based on conditioned responses. Here, the animal needs to first learn the link between an unpleasant experience (*e.g.* shock exposure) and a particular signal. Once the link is established, the signal predicting the unpleasant experience will elicit an anxiety-like response (typical Pavlovian conditioning approach). Examples of conditioned anxiety models are the conditioned avoidance, conditioned freezing, defensive burying and lick-suppression test. In the present overview, such conditioned anxiety models are not included for simplicity since there is a potential impact of cognitive deficits induced by the treatment.

2.1.1. Open-field test

The open-field test is widely being used in mice and rat (Bolivar et al., 2000; Britton and Britton, 1981; Igarashi and Takeshita, 1995; Lipkind et al., 2004; Prut and Belzung, 2003). The arena can be either circular or a square with vertical walls high enough such that the animals cannot escape. The size of the arena is relatively large compared to the standard housing cage. The lighting conditions used are ranging from complete darkness all the way up to bright intensities as high as 800 lx. The rodent is typically tested for 5–15 min allowing free exploration of the arena. An anxious animal typically displays thigmotaxia (*i.e.* exploring the environment in close vicinity of the walls which they can sense through their whiskers) rather than exploring the center of the arena. As a consequence, the start position of the test animal is important. When the rodent is placed in the center of the arena, there is a risk that in case of high anxiety levels freezing behavior occurs. The key parameters for this test to evaluate anxiety levels are 'percentage time' and 'percentage distance' that is spent in the center of the arena compared to the outer area which is surrounded by walls. In addition to the relative measures for anxiety (see also Section 4.4.2), it is important to also take into account the total distance moved. The latter parameter should be seen as a potential measure for side effects caused by the treatment.

2.1.2. Light–dark test

The light–dark test is primarily based on the fact that rodents are active during the night which is opposite to humans. This means that while humans typically avoid dark areas, a rodent does not like to be in a brightly-lit arena. The light–dark box contains an illuminated (200–800 lx range) section as well as a dark section with a little opening in-between (Bilkei-Gorzo et al., 1998; Hascoet et al., 2001; Maldonado and Navarro, 2000). The size of the dark compartment is in general equal or smaller than the illuminated part and the total surface of the arena is about 2–4 fold the size of the home cage. The rodent is typically tested for 5–15 min allowing free exploration of the two compartments. The start position of the test animal is also important here. When the rodent is placed in the illuminated part of the arena, there is a risk that in case of high anxiety levels the animal freezes on the spot and never enters the 'safer' dark section. The key parameters for this test to evaluate anxiety levels are 'percentage time' and 'percentage distance' that is spend in the illuminated section of the arena compared to the dark area. In addition to the relative measures for anxiety (see also Section 4.4.2), it is important to also take into account again the total distance moved and the number of transitions between the two compartments. The latter parameters can be seen as a potential measure for side effects.

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