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CNS safety pharmacology: A focus on cognitive functions

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ABSTRACT

Introduction: The guidelines from different agencies do not include studies on cognitive functions as part of safety pharmacology. This is unfortunate as it seems important to verify that drugs entering into the central nervous system (CNS) are devoid of detrimental effects on cognition. Our aim is to show examples on how an evaluation of unwanted effects of drugs on cognitive functions may be included in preclinical studies. Rather than a review of the scientific context, the present text is an appeal for a wider consideration of cognition as a safety pharmacology endpoint.

Methods: The following procedures provide an index of the ability of substances to induce cognitive deficits in rodents. In the passive avoidance (PA) test, rats receiving an electric shock show on a later occasion an avoidance of the shock-associated environment. In the social recognition (SR) test, rats recognize familiar congeners. In the Morris water maze (MWM) test, rats placed into a tank containing water learn to find an invisible escape platform using extra-maze visual cues. In the delayed alternation (DA) test, rats placed in a Skinner box learn to alternate their pressing behavior between two levers in order to obtain food rewards. In the operant reversal (OR) test, rats adapt their behavior following a change of the reinforcement rule.

Results: Standard reference agents were used to confirm that the different assays were able to detect pharmacologically induced cognitive impairments. Diazepam decreased associative memory performances in the PA test. MK-801-induced memory deficits in SR. Haloperidol increased escape latencies in the MWM test. Scopolamine decreased the number of correct responses in the DA test, and nicotine decreased the number of correct responses in the OR test. The relationship between the doses administered and the effects observed was also evaluated.

Discussion: Cognitive assays may provide utility in determining potential undesirable effects or discharging perceived risks with novel CNS drugs under development.

1. Introduction

A set of guidelines was developed by the European Agency for the Evaluation of Medicinal Products (Anon, 2000). These guidelines were subsequently adopted in both the United States and Japan. The European guidelines, which are less specific than the previous Japanese guidelines (Anon, 1995), include core battery central nervous system (CNS) studies to be conducted before a new chemical entity (NCE) is administered to humans.

A distinction is made in ICH S7A between core battery studies and supplemental or follow-up studies (Bass, Kinter, & Williams, 2004; Pugsley, Authier, & Curtis, 2008). Core battery studies include assessing the effects of the NCE on motor activity, behavior, coordination, sensory/motor reflex responses, and body temperature. Core battery CNS procedures typically entail simple tests using standard techniques that can be conducted rapidly. Such studies are performed almost exclusively in rodents under Good Laboratory Practice (GLP) regulations. Supplemental or follow-up studies include examining the effects of an NCE on cognition, brain electrical activity (EEG), and assessment of its potential to cause abuse and/or dependence. Because of their complexity, there are no standard protocols for follow-up assays, and no protocols are currently required to be conducted in compliance with GLP (Bass et al., 2009). The distinction between core and supplementary studies reflects the notion that the risk for vital functions evaluated in the core battery is particularly concerning before the first in human studies. Nevertheless, we think that supplemental studies are needed to evaluate a more global risk level associated with CNS-penetrating substances for drugs used in large human populations. Substances affecting cognitive functions may generate serious health or societal issues. As a concrete example, a substance-decreasing attention may increase the risk of occurrence of accidents caused by treated drivers. The core battery of preclinical tests only includes the Irwin or similar tests (Irwin, 1968). Although the Irwin test is very useful to detect and characterize the main effects of drugs, it is not specific enough in order to predict their potential effects on cognitive functions. It is thus preferable to include in

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supplemental safety studies different procedures specifically targeting cognitive processes in order to provide a comprehensive characterization of the safety profile of drugs acting on the CNS.

By cognition, we mean the mental processes associated with attention, learning, memory, reasoning, and judgment. Attention consists of distinct but interacting mechanisms including vigilance, sustained, divided, and selective attention, and it results in the capacity to select pertinent aspects of the sensory input (Muir, 1996). Learning is the acquisition of new behavior as a consequence of an experience. Memory is the preservation of the learned behavior over a specified time while retrieval is the reproduction of this learned behavior at the end of a retention period (Millan et al., 2012). Reasoning and judgment enter into the category of executive functions whereby behavior is planned, controlled, and adapted to a constantly changing environment (Logue & Gould, 2014). Evaluating these cognitive functions in rodents can be relatively ambitious, complex, and time consuming. Here, we describe different experimental models that we use in rats to evaluate drug effects on specific cognitive functions and to assess the possible side effects of pharmacological substances (Castagné et al., 2013; Porsolt, Lemaire, Dürmüller, & Roux, 2002).

Due to space limitations, rather than extensively reviewing the field of behavioral testing of cognitive functions, our goal is to provide examples of behavioral testing procedures and pharmacological substances potentially used a positive controls based on our own experience of studies used in safety pharmacology programs.

The one-trial passive avoidance (PA) task is a simple procedure evaluating the ability of animals to remember an aversive stimulus received in a specific environmental context (associative memory) (Bammer, 1982).

The social recognition (SR) test evaluates the ability of a mature adult rat to recognize a previously presented juvenile rat (social working memory) (Sawyer, Hengehold, & Perez, 1984). This test is based on a spontaneous behavioral tendency and does not involve artificial situations such as aversive stimulation or food deprivation (Lemaire, Bohme, Piot, Roques, & Blanchard, 1994).

The Morris water maze (MWM) is the gold standard for the evaluation of the reference memory for spatial environment (Morris, 1981). Rodents placed into a tank of water learn to locate a hidden escape platform that is in a fixed position. Although the maze itself is featureless, prominent visual cues fixed to the walls of the experimental room allow the animal to orient itself during the testing phase (spatial learning).

Operant conditioning and in particular the delayed alternation test are powerful techniques for studying working memory function using automated systems (Roux, Hubert, Lenegre, Milinkevitch, & Porsolt, 1994). In delayed alternation (DA), the animal is required to retain information (which lever or stimulus was previously presented) over a short period of time and then to show, by pressing on an appropriate lever, whether it has correctly remembered the information (Dunnett, Evenden, & Iversen, 1988). Operant delayed matching techniques possess a considerable advantage over most other cognitive models in that parallel procedures can be set up in rodents, primates, and even man, thereby increasing the translational validity of the data obtained (Bartus & Dean, 2009).

Executive functions include higher order cognitive operations such as cognitive flexibility, planning of complex actions, working memory, and problem solving. Operant reversal (OR) learning procedures are useful to investigate cognitive flexibility in the rat. In the operant reversal task, rats are first trained to press on the lever opposite to that previously presented to gain a food reward and are then submitted to reversal sessions where the reinforcement rule is changed (Kosaki & Watanabe, 2012).

The five behavioral testing procedures used in the present text are examples of tests able to evaluate the effects of drugs on cognitive functions. Although it would be nearly impossible to evaluate all cognitive processes, we think that including tests dependent on neuronal networks involving the hippocampus (MWM), the frontal cortex (SR, DA, and OR), or the amygdala (PA) allows a reasonable prediction of the liability of a drug to impair cognition. Even if it remains impossible to totally exclude the possible induction of impairing effects on cognition in humans, the incorporation of cognitive performances in animals as safety endpoint allows risk mitigation for patients.

We show here the capacity of various substances with known pharmacologic profile such as diazepam, haloperidol, scopolamine, dizocilpine (MK-801), and nicotine to induce cognitive impairment in testing procedures evaluating different aspects of cognitive functions in rodents.

2. Materials and methods

As indicated in the introduction, the procedures described below are examples of studies performed in our laboratory showing the ability of different testing procedures to detect pharmacologically induced cognitive deficits. It would be presumptuous to try to provide guidelines in such a limited space. Our goal is rather to show that relatively simple testing procedures can detect adverse effects of pharmacological substances known to affect cognitive performances in humans, thereby supporting the translational value of the approach.

2.1. Animals

Male Wistar (Han) rats (Janvier Labs, Le Genest-Saint-Isle, France) were used for the procedures described below. Rats were 2 months old and weighed 205–290 g at the beginning of the PA, MWM, DA, and OR tests or 3–4 months old and weighed 464–596 g at the beginning of the SR test. Mature male rats display more reliable expression of social investigation in the SR test, as compared with younger animals.

It is possible to use other strains, such as Sprague–Dawley rats, which display behavioral performances globally comparable to Wistar rats. Pigmented strains such as Long–Evans rats usually display better performances in the MWM, as compared with albino rats.

Rats were housed grouped 2 to 5 per cage except for the SR test, where they were housed individually under controlled temperature (21 °C ± 2 °C), humidity (20–80%), and lighting (12-h light–12-h dark cycle; light cycle: 7:00 AM–7:00 PM). They were acclimated to the housing environment for at least 5 days (or 2 weeks for the SR) before the beginning of the experiments. Rats had free access to food and water throughout the experiments, except in the DA and OR where they had free access to water but restricted access to food (15 g/rat/day). Independent cohorts of rats were used for each behavioral testing procedure. Behavioral tests were conducted during the light period of the light/dark cycle.

All experimental procedures were approved by Porsolt's internal ethical review committee and were conducted in compliance with the requirements of French regulatory authorities.

2.2. Behavioral testing procedures

The following procedures are derived from classical studies and have already been adapted in various ways by many different laboratories. Our goal being to give examples based on our own experience, we do not review here the impact of procedural specificities such as the number and duration of acquisition sessions, inter-trial interval, or pretreatment delays with pharmacological substances. In the following sections, we thus tend to refer to classical articles describing the main characteristics of a given behavioral procedure. We apologize for not being able citing the numerous authors of important procedural variations in such a limited space.

2.2.1. Passive avoidance

The method was performed as previously described (Glick & Zimmerberg, 1972). The rat was placed individually into the light