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Review

Repeated "Day 1" FOB testing in ICH S7A safety assessment protocols: The influence of within- and between-session learning

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ABSTRACT

A large number of CNS safety assessment studies using the standard Functional Observational Battery (FOB) are conducted each year at Contract Research Organizations throughout the globe. Study design characteristics are as varied as the Sponsors for whom they are contracted. Gender inclusion, sample sizes, and timing of the FOBs are generally negotiated during protocol development. The ICH S7A guidelines describe a dose-effect study design for CNS safety assessment to be conducted prior to the first dose administration in man. Additionally, some Sponsors attempt to use the CNS safety FOB to establish both time- and dose-related acute behavioral effects of their compound in this single critical safety study. In this review, we highlight the confounding influences of multiple postdose FOBs (Day 1) versus the more standard, single FOB scheduled near systemic C_{max} of the compound. Within- and between-session learning, combined with changes in vigilance/alertness/fatigue in both the animals and raters, can limit the generalizability of the FOB to accurately assess CNS effects under the current guidelines. Rationale is provided as to the tenuous nature of conducting simultaneous time- and dose-effect behavioral assessments as part of the core safety pharmacology programs.

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1. Introduction

1.1. ICH S7A guideline requirements:

The International Council on Harmonisation (ICH) has established safety testing guidelines for standard assessments of the central nervous

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Table 1
Standard time points at which FOBs are conducted in a typical CNS safety assessment study protocol.

Timeline		
FOB #1 Pre-Dose Day −1, −2, or −3 Baseline Accounts for differences in breeder, generation, age, gender, and strain	FOB #2 Day 1 T_{max}, C_{max} Primary drug effects Acute administration "peak effects" within therapeutic range	FOB #3 Recovery (Day 2) ≥5 half-lives Acute rebound effects Accounts for residual, hangover effects and active metabolites from single dose administration

system (CNS) in laboratory animals. These assessments must be conducted prior to first dose administration of any new molecular entity (NME) in human subjects (ICH, S7A). One commonly used screening method is the functional observational battery (FOB). Under the ICH S7A guidelines, FOB screening may be seen as preliminary research to separate NMEs that have an effect on the CNS from those that do not. Prior to the first dose in man, the ICH S7A guidelines require, at a minimum, tests of: 1) motor activity, 2) behavioral changes, 3) motor coordination, 4) sensory/motor reflex responses, and 5) core body temperature. Additionally, the Food and Drug Administration (FDA) has established that the ICH S7A guidelines are consistent with FDA's Good Guidance Practices regulation (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm074959.pdf). In accepting the ICH S7A guidelines, the FDA acknowledged that these regulations do not create or confer any rights for or on any person, nor do they operate to bind the FDA nor the public to any on specific methodology. An alternative approach may be used if it satisfies the requirements of the applicable statutes and regulations.

The FDA, in accepting the ICH guidelines, has selected the standard purpose-bred laboratory rat as the preferred species for this safety assessment screening and has defined the safety assessment to a single dose administration with the purpose of generating a dose-response function in the selection of doses to be used.

As shown in Table 1, the typical FOB study is conducted using three time points: 1) a predose, baseline FOB to establish characteristics of the rats used on the study (e.g., strain, gender, age, breeder), 2) a FOB scheduled at the T_{max} or C_{max} of systemic exposures, and 3) a recovery FOB, conducted at 5 or more half-lives following the final dose administration. The purpose of the FOB triad is to assess the dose-related short-term and pharmacodynamic effects of the NME within the therapeutic range, and above. But it cautions against the use of "toxic doses" at this stage of analysis.

The S7A guidelines do not specifically include or exclude the use of the FOB screen for time-effect analyses to quantify onset and offset of test article-related effects, the majority of all CNS safety studies conducted in our laboratory are conducted as described in Table 1. However, some Sponsors use the CNS safety assessment protocol to establish both dose and time-effect functions by increasing the Day1 post-dose FOBs to include 2 to 5 other scheduled FOBs to be conducted based on the dose times for each groups test article administrations. This review may be of interest to 1) any laboratory attempting to conduct preclinical safety assessment of the CNS in accordance with the ICH S7A guidelines, or 2) regulatory agencies requesting specific study designs, or Sponsors who attempt to combine toxicology and safety pharmacology endpoints into a single study protocol, in accordance with Section 1.4 of the ICH S7A guidelines, without full realization of the full ramifications to data interpretation.

As reviewed by Hånell and Marklund (2014) rodent behavioral testing in the laboratory setting has proved difficult, with test results varying dependent upon the observer (Chesler, Wison, Lariviere, Rodriguez-Zas, & Mogil, 2002), the particular laboratory the experiments are performed in (Crabbe, Wahlsten, & Dudek, 1999; Lewejohann et al., 2006), and other environmental factors (e.g., animal housing) (Richter, Garner, Auer, Kundet, & Würbel, 2010). As we recently reviewed (Gauvin et al., 2015, 2016), a combination of sound ethological principles and a thorough understanding of rodent biology can yield reliable results in the FOB. However, as noted by Hånell and Marklund (2014) the measure performance in any test will invariably include combinations of both the inherent physical ability and the motivation variables in effect for the rat at the time of testing. A variable level of motivation is a potential problem in many repeated behavioral tests, such as rotarod and grip strength tests (Balkaya, Kröber, Rex, & Endres, 2013). Several tests of spontaneous rodent behavior, such as the exploration of novel environments in the open field (Hall, 1934), rely on previous exposure and current stress levels of the rats being tested. Insufficient motivation to perform tasks is an issue common to all forms of behavioral testing (Hånell & Marklund, 2014).

In virtually all behavioral tests there is a degree of interaction between technician and the animal which potentially influences the obtained results. Alder and Zbinden (1983) have concluded that skilled scientists and dedicated technicians are able to spot a great variety of treatment effects, even those that are novel and unexpected (p. 3). It should be noted, however, that the ability to detect changes in animals' behavior, of their muscle tone, and their reaction to various manipulations, greatly depends on the ability, experience, and devotion of the technicians. As described by Alder and Zbinden (1983),

> An observer used to work with rats can spot even subtle changes and unexpected behavioral patterns, and he can discover minute differences developing in the course of a study (p. 19).

They also admonish that those technicians rating behavioral changes need standard instructions which fit logically and systematically into their work with the animals and which do not burden them with excessive note taking and record keeping. The importance of this interaction was established by the observation that technician identity had a greater influence than genotype on hot plate test results (Chesler et al., 2002). Even more dramatic are the recent reports by Sorge et al. (2014) that the presence of a male technician, but not a female, induced analgesia in rodents. It should always be remembered that rats are able to distinguish between, and the results may be affected by, the level of rodent familiarity with the individual technician conducting the FOBs (McCall, Lester, & Corter, 1969; van Driel & Talling, 2005). Any remaining odor traces from predatory laboratory animals (cats, dogs, etc.) retained on the technicians clothing, skin, or hair from normal feeding, room cleaning, cage changes, etc., will induce stress in rodents (Burn, 2008). Therefore, the level of room cleaning, animal transfers, animal dosing, etc. conducted by the technicians judging the FOBs on the day of the observations have the potential to alter the behavioral response of rats on study (Schallert, Woodlee, & Fleming, 2003; Hurst & West, 2010).

Lack of handling before a series of repeated testing may cause altered results over time as the animals get more and more familiar and accustomed to technician contact. Note, however, that human presence

Table 2
Critical experimental variables that can influence the outcome of behavioral studies conducted in rats.

Type	Examples
Organismic	Species, strain, gender, age
Procedural	Housing conditions, circadian rhythm/light cycle, prior handling and injection experience, exposures to prior stressors, apparatus construction (e.g., floor surface, color, size), illumination level, prior test experience (habituation), behavioral measures scored subjectively and/or objectively, definition/validation of measures, method of scoring, and duration of scoring