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Review

Optimizing laboratory animal stress paradigms: The H-H* experimental design

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ABSTRACT

Major advances in behavioral neuroscience have been facilitated by the development of consistent and highly reproducible experimental paradigms that have been widely adopted. In contrast, many different experimental approaches have been employed to expose laboratory mice and rats to acute versus chronic intermittent stress. An argument is advanced in this review that more consistent approaches to the design of chronic intermittent stress experiments would provide greater reproducibility of results across laboratories and greater reliability relating to various neural, endocrine, immune, genetic, and behavioral adaptations. As an example, the H-H* experimental design incorporates control, homotypic (H), and heterotypic (H*) groups and allows for comparisons across groups, where each animal is exposed to the same stressor, but that stressor has vastly different biological and behavioral effects depending upon each animal's prior stress history. Implementation of the H-H* experimental paradigm makes possible a delineation of transcriptional changes and neural, endocrine, and immune pathways that are activated in precisely defined stressor contexts.

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1. Introduction

Major advances in elucidating brain-behavior relationships have frequently depended upon the development of laboratory experimental approaches and model systems that can be replicated across laboratories. Some examples include studies of the gill withdrawal reflex in *Aplysia californica* (Kandel, 2001), passive avoidance testing to study memory modulation in laboratory rats (McGaugh, 2015), the nictitating membrane response in rabbits to understand the neurobiology of classical conditioning (Berger et al., 1976), the acoustic startle response in rodent models to probe brain mechanisms of sensory processing and associative learning (Koch, 1999), conditioned place preference in laboratory rats and mice to study brain circuits activated by drugs of abuse (Hayes and Greenshaw, 2011), and the Morris water maze to examine spatial navigation and memory (Brandeis et al., 1989). These and other experimental behavioral paradigms have been critical to advances in behavioral neuroscience by permitting comparisons of research findings across laboratories, by facilitating replication of key experimental results, and by accumulating results across investigators to provide a more complete picture of a given brain-behavior relationship.

Consistent with the approaches described above, several laboratory stress paradigms have been developed for use with humans, including the paced auditory serial addition task, the Socially Evaluated Cold Pressor Test, the Trier Social Stress Test (TSST), and the TSST for children (TSST-C) (Allen et al., 2014; Bali and Jaggi, 2015; Boesch et al., 2014; Buske-Kirschbaum et al., 1997; Kirschbaum et al., 1993; Schwabe et al., 2008). Finally, a paradigm for simultaneous exposure of small groups to social stress was developed, the TSST for groups (TSST-G) (Von Dawans et al., 2011).

In a recent comparison of three laboratory stress protocols, the Trier Social Stress Test and the Socially Evaluated Cold Pressor Test, but not a computerized mental math task, produced robust effects on mood and physiological measures, including salivary cortisol and alpha-amylase as well as heart rate (Giles et al., 2014). These two stress protocols have been employed by many investigators in hundreds of studies conducted around the world and lead to significant increases in activity of the sympathetic nervous system and the hypothalamic-pituitary-adrenocortical (HPA) axis. Comparisons across studies and laboratories have been highly reliable given the use of similar stress methodologies (Kudielka et al., 2007).

2. Variability in the design of stress experiments with laboratory animals

2.1. Overview of variability in stressor protocols with laboratory mice and rats

Experimental approaches taken by investigators interested in neural, endocrine, immune, molecular and behavioral aspects of adaptation to chronic intermittent stress in laboratory mice and rats stand in striking contrast to the widely adopted laboratory stress paradigms for humans described above. Some of the variability inherent in basic research on stress can be traced back to the highly cited paper by Selye that was published as a letter to the editor in the international journal, *Nature*, on July 4, 1936. In this brief but influential report, Selye described a variety of "nocuous agents" to which his laboratory rats were exposed either continuously or intermittently, including placement in a cold room, surgical injury, spinal shock, muscular exercise, or sub-lethal doses of a variety of drugs or tissue extracts (Selye, 1936). He concluded that the rats developed a consistent triad of symptoms in three stages that was independent of the nocuous agent employed. He labeled this triad of symptoms the General Adaptation Syndrome (Selye, 1951). He later coined the term, stress, and defined it as the nonspecific response of the body to any demand placed upon it (Selye, 1973). Given the nonspecific nature of the stress response, one could argue there was little reason to focus on the development of specific and consistent stress procedures (McCarty, 2016a).

Over the past 80 years, there has been a proliferation of experimental protocols for the study of stress in laboratory mice and rats. In many ways, this proliferation of laboratory stress protocols has made comparisons of research results across laboratories and stressors challenging. Another issue concerns the varied approaches to repeated stressor exposure. How many times per day and over how many days should an animal be exposed to a given stressor or a variety of stressors as a means of studying adaptive responses to chronic intermittent stress? On a positive note, if consistent findings for a given stress-responsive neural, endocrine, molecular or behavioral measure are reported across laboratories that employ different experimental protocols, then one can have confidence in the generality of the findings.

2.2. Literature review on chronic intermittent stress protocols

To capture the variety of laboratory protocols employed for the study of stress, I assembled and evaluated 178 empirical peer-reviewed articles published from 2000 to 2015 that employed 239 stress protocols. These articles were identified by limiting Medline search terms to "habituation" *or* "sensitization" *and* "stress responses," and from a total of 609 articles retrieved, only studies with adult laboratory animals were considered. This is by no means an exhaustive listing of relevant research articles; however, it does illustrate clearly the variety of approaches taken in the study of stress responses in laboratory mice and rats over a 16-year period.

For the most part, I have excluded from this review any experiments that employed stressors that were continuous in nature over days or weeks, even if the results were consistent with findings from experiments that employed intermittent stressors. Examples include placement of laboratory rats in a cold room continuously for multiple days (e.g. Fukuhara et al., 1996; Vernikos et al., 1982) or housing animals in social groups that experienced changes in dominant animals over time (Uschold-Schmidt et al., 2012). Although these studies are valuable in their own right, they do not include discrete, time-limited exposures to a stressor (usually once per day) followed by stress-free periods during the remainder of each day.

The most frequently employed stressors out of 239 stressors described in the sample of 178 articles included restraint (30%), immobilization (12%), electric shock to the paws or tail (8%), social defeat (8%), chronic variable stress (CVS) that involves exposure of animals to a series of stressors over multiple days (8%), forced swimming (6%), intermittent cold exposure (5%), exposure to a predator or the odor of a predator (5%), or exposure to audiogenic stress (continuous white noise) (4%). Other less frequently utilized stressors (a combined 15% of total) included handling (with or without an injection), formalin injected into the hind leg or exposure to formalin vapor, exposure to ether vapors, or insulin-induced hypoglycemia.