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Intrasession and intersession habituation in mice: From inbred strain variability to linkage analysis

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ABSTRACT

When placed in a novel environment, mice tend to explore for a period of time, and then reduce the level of exploration. This reduction in locomotor or exploratory behavior is known as habituation and can occur within a single session or across sessions, respectively, termed intrasession and intersession habituation. Recent research indicates that there is a genetic component to habituation behavior and that some of the genes involved differ between the two types of habituation. The genetic evidence also suggests that intrasession habituation and intersession habituation are measuring somewhat different conceptual entities and with more such evidence may eventually help us understand the different pathways involved. Some of the genetic methods and tools used to unravel the roles of specific genes in both types of habituation are outlined here, with examples from the literature, as well as new data, to illustrate that this seemingly simple behavior is actually very complicated in terms of genetics. Evidence to date suggests that a number of genetic regions play roles in one or both types of habituation, and further research will be necessary to determine the specific genes involved.

1. Introduction

Habituation, the waning of a response after repeated exposures to the same stimulus, aids organisms in selectively responding to biologically significant stimuli, while ignoring less relevant ones. It is considered one of the simplest forms of learning (Groves & Thompson, 1970; Harris, 1943; Thompson & Spencer, 1966) and is found in all phyla of the animal kingdom (Bailey & Kandel, 2008; Colombo & Mitchell, 2009; Engel & Wu, 2009; Giles & Rankin, 2009; Leussis & Bolivar, 2006). Habituation may appear relatively simple; however, neurobiological, biochemical and genetic studies indicate that the process is very complicated. The level of complexity is also a function of the type of habituation being investigated, and there is much variability throughout the animal kingdom (e.g., the gill-withdrawal reflex in sea slugs, changes in exploratory behavior in a stimulus rich novel environment in rodents). Furthermore, a host of factors, both internal and external to the organism of study, can influence the habituation response, thereby introducing further variability both within and across species. A set of 10 characteristics of habituation were updated and revised at the August 2007 workshop in Vancouver, Canada. These characteristics illustrate the complexity of habituation and the factors influencing it (Rankin et al., 2009).

One relatively complicated type of habituation is the change in behavior in response to a novel environment over time, a phenomenon that has been studied in rodents for decades. According to cognitive map theory (O'Keefe & Nadel, 1978), whenever an organism explores a novel environment, it constructs an internal representation of that environment in the hippocampus; as the map becomes increasingly complete, exploration is reduced. We say that the organism has habituated to the environment. The level of habituation has been measured in a number of ways; in rodent studies some of the most common measures are distance traveled, number of horizontal beam breaks, number of line crosses and number of vertical beam breaks. The change over time can be measured within a single session (intrasession habituation) or across sessions (intersession). Fig. 1 illustrates four different situations, entailing varying degrees of intrasession and intersession habituation. An animal can display no habituation, or intrasession but not intersession habituation, or intersession but not intrasession habituation, or both types of habituation (see Fig. 1, Panels A–D). Generally, when repeatedly placed in the same environment, rodents over time will display both intrasession and intersession habituation. There will be a general decrease in activity over time within a single session and across sessions. However, this general pattern can be modulated by various factors, such as genotype (see below for detailed discussion), sex and age.

Several types of evidence indicate that intrasession habituation and intersession habituation measure somewhat different constructs (Peeler, 1990). This may be as simple as short-term and

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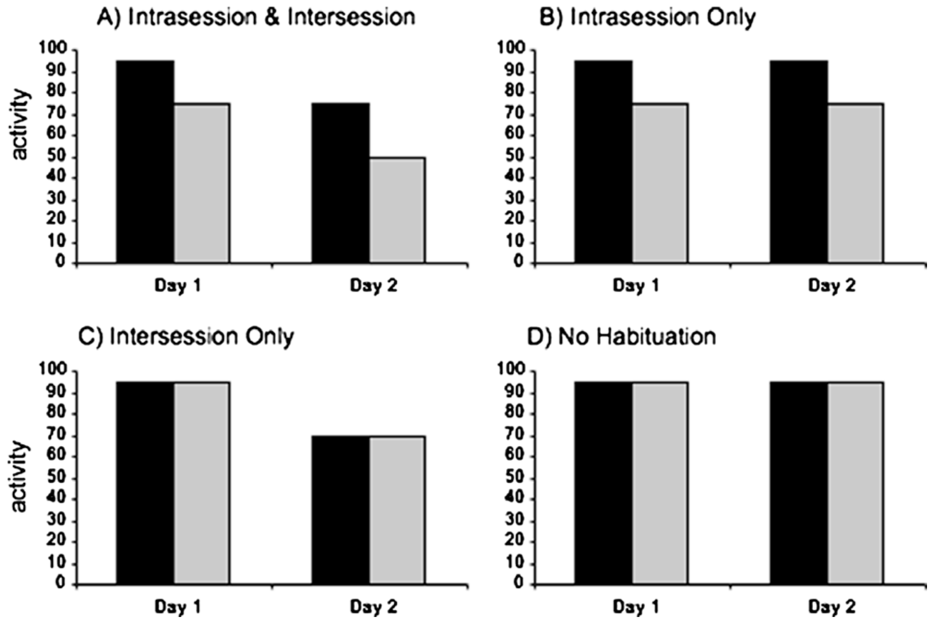

Fig. 1. Graphic representations of various combinations of intrasession and intersession habituation. Graphs represent activity over two consecutive days of testing, for a 10-min period each day. Dark and light bars represent the first five-min block and last five-min block of the session, respectively. Four combinations are illustrated: both intersession and intrasession habituation with activity decreasing across days and within each session (Panel A), intrasession but not intersession habituation, activity decreases within a single session but not across days (Panel B), intersession but not intrasession habituation, activity decreases across days but not within a session (Panel C), and neither intrasession or intersession habituation (Panel D).

long-term memory, respectively. It has been suggested that intrasession habituation measures adaptivity to the environment, whereas intersession habituation measures long-term memory of previous exposure (Muller et al., 1994). However, this distinction does not preclude a role for memory in intrasession habituation. Simply, more studies have focused on the memory component in intersession habituation. For instance, both duration of exposure and retention period between exposures have been found to influence intersession habituation (Fraley & Springer, 1981a, 1981b; Terry, 1979). Furthermore, any number of factors that interfere with formation or retention of a cognitive map (O'Keefe & Nadel, 1978) of the environment can modulate the habituation process.

It is important to remember that a behavior as complicated as habituation will be influenced by many factors. For instance, initial exploratory activity level during an encounter with the novel environment can influence the level of habituation, especially across sessions. An animal that does not explore the novel environment very quickly may not be able to form a complete cognitive map of the area in the allotted time. During a later session, when the animal's performance is not optimal, the behavior may not be the result of memory deficits, but due to incomplete initial exploration of the entire area. This type of situation can result from high levels of anxiety, low levels of locomotor activity, or any number of sensory deficits. Thus, in evaluations of habituation behavior, there must be a consideration of baseline levels of activity, and correct for these differences. A number of methodologies have been developed to take baseline differences in activity levels into consideration, among these are calculation of percentage of baseline, percent change and activity change ratio (Anisman, Kokkinidis, Glazier, & Remington, 1976; Fraley & Springer, 1981b; Nadel, 1968). Although it is less critical to make these sorts of corrections when differences in baseline levels of activity do not exist between groups, as much of our laboratory's research involves hypoactive and/or high-anxiety mouse strains, making this a correction becomes very important.

We have used the activity change ratio developed by Nadel (1968) extensively in our research on habituation (Bolivar, Manley, & Messer, 2004; Bolivar, Ganus, & Messer, 2002; Bothe, Bolivar, Vedder, & Geistfeld, 2004, 2005; Cook, Bolivar, McFadyen, & Flaherty, 2002). The activity change ratio score is used to compare activity levels during initial and final periods in the environment. If one is measuring intersession habituation across 3 days it is calculated as follows: Day 3 activity/(Day 1 activity + Day 3 activity). To examine intrasession habituation in a single 15-min session, it would be calculated as follows: activity during final 5 min/(activity during initial 5 min + activity during final 5 min). Thus, the ratio score will approach 0.5 if no change in activity has occurred, i.e., no habituation. If the ratio approaches 0, there is evidence of habituation. If the ratio approaches 1.0 activity has actually increased from the initial to final time periods.

The biological underpinnings of habituation to a novel environment are numerous and links have been made to a variety of neurotransmitters, including serotonin, acetylcholine, dopamine and glutamate (for a recent review, see Leussis & Bolivar, 2006). Furthermore, a plethora of studies, many of which have addressed the roles of specific neurotransmitters, affirm that genetics plays a role in habituation. The existing body of work has been obtained via manipulations of single genes, so as to determine the effects on habituation behavior (for reviews see Bolivar, Cook, & Flaherty, 2000; Leussis & Bolivar, 2006). However, habituation is a complicated phenotype, likely under polygenic control. Traditional transgenic and knockout technologies do not generally allow the study of multiple genes at the same time and interactions among these genes, i.e., epistasis. In contrast, the linkage methods outlined below allow researchers to study gene–gene interactions.

Accordingly, other behavioral genetics researchers have taken a different approach to the evaluation of the role of genetics in habituation. The mouse has become the model of choice for many researchers, due to the vast knowledge of its genetics, and also to technological advances in the genetic manipulation of murine embryonic stem cells. However, the foundation upon which all mouse genetics rests, and the principle reason for the ascendancy of the mouse in the field of genetics, is undoubtedly the availability of large numbers of inbred mouse strains. This resource is unparal-