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# Defensive burying as an ethological approach to studying anxiety: Influence of juvenile methamphetamine on adult defensive burying behavior in rats

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### ABSTRACT

The defensive burying test is an ethological approach that has both pharmacological and physiological validity in studying rodent anxiety-like behaviors. The defensive burying test measures the naturally occurring behavior of displacing bedding toward a noxious stimulus. Exposure to psychostimulants can alter anxiety behaviors in children and adults, however few studies have investigated the long-term effects of chronic juvenile stimulant exposure on anxiety behaviors in adulthood. Therefore, the current experiment tested the effects of juvenile methamphetamine exposure on defensive burying behavior in adulthood. Juvenile (PD20–34) male and female rats were either given intraperitoneal injections of 2.0 mg/kg methamphetamine or saline or were not handled, and then all groups were left undisturbed until adulthood. As adults, rats' anxiety-like behavior was assessed after exposure to a single shock probe in a defensive burying paradigm. Adult male rats that were exposed to methamphetamine as juveniles showed a significant increase in the latency to begin burying following the shock, indicative of decreased behavioral reactivity towards the shock. However, there were no other differences between treatment groups. Interestingly, there were no sex differences in any measure. These findings suggest that early exposure to the stimulant drug methamphetamine has limited effects on defensive burying behaviors when measured in adulthood. Defensive burying is an important ethological approach to assess rodent anxiety and can increase our understanding of coping behaviors in rodents following stimulant drug exposure.

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## 1. Introduction

Anxiety disorders are the most common mental illness in the United States, with a lifetime prevalence rate of 29 percent (Substance Abuse and Mental Health Services Administration, 2012). One difficulty in studying complex human disorders, such as anxiety, is the ability to carefully control and manipulate variables to allow for causal conclusions. Animal research can be used to improve our understanding of the mechanisms underlying human behavior, including clinical disorders (Andersen, 2003; Borsini et al., 2002; Bourin, Petit-Demoulière, Dhonnchadha, Hascoët, 2007; Walf & Frye, 2007).

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An experimental design is thought to be ethologically valid if it represents a natural environment and uses environmental stimuli that elicit natural responses (Calatayud, Belzung, & Aubert, 2004; Rodgers, Cao, Dalvi, & Holmes, 1997). Many approaches have been used to study the construct of anxiety in animals, but the ethological validity of these approaches has been debated. In this paper, we will provide a brief review of the various approaches to studying anxiety in rodents, include a more detailed review of defensive burying as an ethological test of anxiety, and then describe a recent study investigating the long-term effects of juvenile stimulant methamphetamine (METH) exposure on adult defensive burying behaviors.

### 1.1. Conditioned and unconditioned tests of anxiety

There are a wide variety of methodologies to assess anxiety-like behavior in rodents; these methodologies have been categorized previously into conditioned and unconditioned tests (for reviews see, Belzung & Griebel, 2001; Steimer, 2011). Conditioned tests include conditioned emotional response and fear potentiated startle, which require repeatedly pairing a noxious unconditioned stimulus, such as a shock, with a neutral stimulus until the neutral stimulus elicits the response in the absence of the unconditioned stimulus (Davis, Falls, Campeau, & Kim, 1993; Millenson & Leslie, 1974). Typically, in conditioned tests, anxiety can be measured through the frequency or duration of freezing (i.e. body becomes immobile) or startle (i.e. fast contraction of muscles, generally in the neck and shoulders) behaviors (for reviews see, Davis, 1992; Fendt & Fanselow, 1999). While these tests provide valuable information on the learning and persistence of anxiety-like behaviors, there are inherent limitations to their use. The conditioned tests require training and the anxiety-like behaviors can be measured only after the association is learned. Although animals develop anxiety surrounding those associations, incorporating learning inherently adds an additional construct, whereby the measures may be influenced by both the learning and anxiety.

Unconditioned tests do not require learning to study anxiety and incorporate stimuli that elicit naturally occurring behaviors. Some of the most commonly used unconditioned behavioral tests to assess anxiety are the elevated plus maze (EPM), open field, and light/dark boxes (for reviews see, Bourin, Petit-Demoulière, Dhonnchadha, & Hascöet, 2007; Walf & Frye, 2007). For the EPM, rodents are placed on a four-arm maze elevated 50 centimeters above the floor, in which two arms have high walls (closed arms) and two do not (open arms; Walf & Frye, 2007). The duration of time the animal spends in the closed arms (more anxiety) compared to the open arms (less anxiety) is typically measured (Walf & Frye, 2007). Another commonly used test is the open field test, which measures the activity of a rodent in a square box with walls. An animal is thought to be less anxious if it spends more time in the center portion of the box (i.e. where it would be prone to predation in a natural environment) than closer to the walls (for review see, Prut & Belzung, 2003). Similar to the above paradigms, the light/dark box test measures the amount of time an animal spends in a closed, dark compartment versus an open, brightly lit compartment, with more anxious animals spending more time in the dark compartment (Bourin & Hascoët, 2003). The use of artificial mazes poses a problem for eliciting natural behaviors because the animal's perception of that environment may be influenced by numerous test variables, such as if the open arms of the EPM have a raised lip (Calatayud et al., 2004). Although these tests of anxiety are based on naturally occurring behaviors, they are still limited by their use of artificial testing environments.

### 1.2. Defensive burying

A test that makes use of both an ethological behavior and a more natural environment to assess anxiety is the shock probe defensive burying test, where an animal encounters a harmful object (i.e. a shock probe) and subsequently engages in behaviors to avoid further interaction with the object (Pinel & Treit, 1978). Rodents innately engage in behaviors to bury harmful, noxious, or novel objects by using their forepaws and heads to shovel bedding material onto or near the potentially noxious object (Cleary, Wallace, & Poling, 1982; Pinel & Treit, 1978; Wilkie, MacLennan, & Pinel, 1979). Burying toward a stimulus has been viewed as a defensive behavior since 1950, when Hudson observed rats covering an electrified food cup with available wood shavings (Hudson, 1950). While some studies have investigated rodents' behaviors toward novel and non-threatening objects, like marbles (Deacon, 2006), most of the defensive burying research has been geared toward harmful or noxious stimuli, such as shock probes (Pinel & Treit, 1978), noxious odors (for review see, Dielenberg & McGregor, 2001), or noxious tastes (Cleary et al., 1982; Poling, Cleary, & Monaghan, 1981; Wilkie et al., 1979). Typically in the defensive burying test, increased time to touch the shock probe and therefore receive the shock is measured as an increase in exploration and a decrease in avoidance behavior. Decreased latency to begin burying the probe following the shock, increased total time spent burying, and increased height of the bedding pile are indicative of heightened levels of anxiety (for review see, De Boer & Koolhaas, 2003).

The shock-probe defensive burying test has several advantages over other rodent tests of anxiety-like behaviors. From an ethological perspective, defensive burying does not require the use of a maze apparatus and measures a repertoire of behavioral responses that an animal may exhibit naturally, without training, after encountering a noxious stimulus. Further, although it is typically used as an unconditioned test of anxiety-like behaviors within a single day of testing, additional testing can be done to measure the conditioned response to the shock probe (Pinel & Treit, 1978; Treit, Pinel, & Fibiger, 1981). In addition, there are an expansive number of behaviors that can be recorded during the defensive burying test, with the most common being the total burying time and pile height. Interestingly, burying makes up only a small percentage of behaviors exhibited in the defensive burying test (for review see, De Boer & Koolhaas, 2003). Additional measures that

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