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Research report

Behavioral and pharmacological validation of an integrated fear-potentiated startle and prepulse inhibition paradigm

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HIGHLIGHTS

- Fear-potentiated startle and prepulse inhibition were shown in a single paradigm.
- Prepulse inhibition of acoustic startle was lower in the presence of a light.
- This integrated paradigm was validated by diazepam and phencyclidine.

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ABSTRACT

Fear-potentiated startle (FPS) and prepulse inhibition (PPI) of acoustic startle are two widely used paradigms specifically designed to capture the impact of negative emotion (e.g. fear) and preattentive function on startle response. Currently, there is no single paradigm that incorporates both FPS and PPI, making it impossible to examine the potential interactions between fear and attention in the regulation of startle response. In this study, we developed an integrated FPS and PPI test protocol and validated it with psychoactive drugs. In Experiment 1, male Sprague-Dawley rats were randomly assigned to one of five groups, receiving either Light -Shock conditioning trials, non-overlapping Lights and Shocks, Light alone, Shock alone, or no Light and Shock. They were then tested for startle response and PPI concurrently, under the Light or No Light. FPS was observed only in rats subjected to fear conditioning, whereas all rats showed PPI and startle habituation. Experiment 2 used this paradigm and demonstrated a dissociative effect between diazepam (an anxiolytic drug) and phencyclidine (a nonselective NMDA receptor antagonist) on FPS and PPI. Diazepam suppressed both FPS and PPI, while PCP selectively disrupted PPI but not FPS. The diazepam's anxiolytic effect on FPS was further confirmed in the elevated plus maze test. Together, our findings indicate that our paradigm combines FPS and PPI into a single paradigm, and that is useful to examine potential interactions between multiple psychological processes, to identify the common neural substrates and to screen new drugs with multiple psychoactive effects.

1. Introduction

The startle response is an innate motoric response to a sudden and intense stimulus. It protects the integrity of the body and facilitates escape from potential danger. Startle can be elicited by acoustic, tactile and visual stimuli in a variety of animal species and in humans, although the most commonly investigated phenomenon is acoustic startle response. Because the startle response magnitude can be modulated by a variety of psychological variables, such as fear, anxiety, learning, memory and attention, it becomes a valuable behavioral tool to assess neurobiological mechanisms of psychological functions. Fear-potentiated startle (FPS) and prepulse inhibition (PPI) are two exemplary paradigms that serve this purpose [1].

Fear-potentiated startle refers to a phenomenon that the magnitude of the acoustic startle reflex is augmented in the presence of a cue (e.g., a light) that has previously been paired with a shock [2]. In the test, a neutral stimulus (termed conditioned stimulus, CS) is first paired with a shock, and then the animal's startle reflex is compared in either the presence or the absence of the CS. FPS is operationally defined by elevated startle amplitude in the presence of the CS and is supposed to measure a central state of fear. This paradigm is thus often used to study the neurobiology of conditioned fear and for the identification of potential anxiolytic drugs [3]. For example, using FPS, Davis and his colleagues suggested that the central and

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basolateral amygdala play a critical role in the mediation of fear learning and memory, as lesions of the central, or the basolateral nucleus of the amygdala block the impacts of conditioned fear on the startle response [4]. Psychopharmacological studies document that anxiolytic drugs that reduce overall excitability of the CNS, such as ethanol or diazepam, but not other classes of psychoactive drugs, have a common effect of attenuating the CS-elicited enhancement of startle response and having no effect on the baseline startle magnitude [4–7]. This feature has been used to identify potential anxiolytic drugs.

PPI is developed to study the sensorimotor gating ability, a preattentive process that is involved in early stages of information filtering [8]. PPI is observed when the amplitude of startle reflex elicited by an intense startling stimulus (e.g., 120 dB white noise) is reduced when the startle stimulus is immediately preceded by a weak prepulse (e.g. 80 dB white noise). PPI has often been used to study attention deficits associated with severe mental disorders such as schizophrenia and to screen potential antipsychotic drugs. For example, it has been widely used as a translational model of schizophrenia [9,10], as animals treated with psychotomimetic drugs, such as amphetamine (a potent psychostimulant targeting monoamine transporters), quinpirole (a $D_{2/3}$ agonist), PCP (a nonselective NMDA antagonist) or MK-801 (a nonselective NMDA antagonist) also exhibit PPI deficits [11–15]. Further, it has also been successfully used to screen chemical compounds with potential antipsychotic activity [16,17], to rank order clinical potency of approved antipsychotics [9], and to differentiate atypical from typical antipsychotics [18].

Because each paradigm serves relatively independent purposes, FPS and PPI are often used separately. To the best of our knowledge, there is no paradigm that incorporates the fear-potentiated component with the PPI component. However, such a combined paradigm would have some advantages. For example, it would allow an examination of interactive effects of fear and attention on the regulation of startle response, while a single paradigm is unable to do. Furthermore, the integrated paradigm is useful for identifying the shared neural substrates underlying conditioned fear (as measured in FPS) and attentional filtering ability (as measured in PPI). For instance, lesions of the basolateral amygdala are found to disrupt FPS and PPI in separate tests [4,19]. The new paradigm would be able to corroborate this finding in a single test. Finally, such an integrated paradigm would be efficiently used to identify novel compounds possessing dual anxiolytic and antipsychotic property in a single test. It also better serves as a behavioral model of severe mental disorders because most disorders have abnormal functions in multiple domains. In the present study, we report development of an integrated FPS and PPI test protocol by demonstrating the effectiveness of this paradigm in recording conditioned fear and sensorimotor gating ability simultaneously. In addition, we provide pharmacological validation showing that two psychoactive drugs (PCP and diazepam) maintain their selective and dissociative effects on FPS and PPI.

2. Materials and methods

2.1. Subjects

Male adult Sprague-Dawley rats (~2–3 months old, Charles River, Portage, MI) were housed two per cage (30.48 cm × 29.21 cm × 17.78 cm), with food and water available ad libitum. The colony was under a 12/12 h light/k cycle (lights on from 7:00 am to 7:00 pm), with temperature maintained at 22 ± 1° and humidity around 32%. Experiments were conducted during the light portion of the cycle. All procedures were approved by the animal care committees at the University of Nebraska-Lincoln.

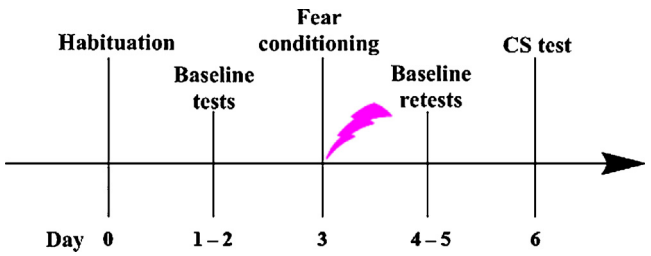

Fig. 1. A schematic depiction of the experimental procedures used in Experiments 1 and 2.

2.2. Startle and elevated plus maze apparatus

Six startle monitor systems (Kinder Scientific, Julian, CA) controlled by a PC were used. They were housed in compact sound attenuation cabinets (35.56 cm wide × 27.62 cm deep × 49.53 cm high). A speaker (diameter: 11 cm) mounted on the cabinet's ceiling was used to generate acoustic stimuli. During tests, rats were placed in a restrainer (17.2 cm long × 9.0 cm wide) with an adjustable ceiling positioned atop the box, providing only limited restraint while prohibiting ambulation. The startle response was measured by a piezoelectric sensing platform on the floor in a time window of 100 ms, beginning at the onset of the startle-eliciting stimulus (pulse). The peak value within the record window indexes the magnitude of the startle response. The CS (light) was delivered by an E light Bulb (18 V, 6 W, Eiko 40717) mounted on the cabinet's ceiling.

The EPM consisted of two open arms (50 cm × 10 cm), two enclosed arms (50 cm × 10 cm) and a central platform (10 cm × 10 cm) made of black Plexiglas. Each arm was supported by a sturdy plastic leg and was elevated 70 cm above the floor. The two enclosed arms had high walls (40 cm in height), while the two open arms had raised edges (1.0 cm in height) along each side and end to decrease the possibility of falling during drug testing.

2.3. Drugs

Diazepam (Sigma-Aldrich, St. Louis, MO) was dissolved in 30% N,N-Dimethylformamide (DMF, Sigma-Aldrich). Phencyclidine hydrochloride (PCP, a gift from NIDA Chemical Synthesis and Drug Supply Program [RTI, Research Triangle Park, NC]) was dissolved in 0.9% saline. Diazepam was injected subcutaneously (s.c.) and PCP intraperitoneally (i.p.).

2.4. Experiment 1: behavioral validation of an integrated fear-potentiated startle and prepulse inhibition paradigm

Experiment 1 systematically evaluated the effectiveness of the integrated FPS and PPI paradigm to simultaneously record conditioned fear and sensorimotor gating ability. Forty adult male rats were randomly assigned to five groups (n = 8/group): the CS+, the CS−, the Light-only, the Shock-only, or no-Light-Shock group. They only differed on the fear conditioning day. The overall experimental procedure consisted of the following four phases: Baseline tests of startle and PPI, Fear conditioning, Post-conditioning retests of startle and PPI, and Fear-potentiated startle and PPI test (Fig. 1).

2.4.1. Baseline tests of startle and PPI (Day 1 and Day 2)

Rats were first habituated to the startle chambers for 20 min under 70 dB background noise (Day 0), then they were tested for their baseline startle response and PPI daily for 2 days. Each daily session started with a 5-min period acclimatization with