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Research report

Prepulse inhibition predicts working memory performance whilst startle habituation predicts spatial reference memory retention in C57BL/6 mice

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HIGHLIGHTS

- Two forms of startle plasticity independently predict cognitive performance in mice.
- Prepulse inhibition at low prepulse positively correlates with working memory scores.
- Strong overall startle habituation is associated with superior memory retention.
- The predictive value of prepulse inhibition warrants further investigation.

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ABSTRACT

Prepulse inhibition (PPI) of the acoustic startle reflex refers to the attenuation of the startle response to an intense pulse stimulus when it is shortly preceded by a weak non-startling prepulse stimulus. It is a well-established high-throughput translational measure of pre-attentive sensory gating, and its impairment is detected in several neuropsychiatric diseases including schizophrenia. It has been hypothesized that PPI might be associated with, or predictive of, cognitive deficiency in such diseases, and therefore provide an efficient assay for screening drugs with potential pro-cognitive efficacy. Free from any predetermined disease model, the present study evaluated in a homogeneous cohort of inbred C57BL/6 mice the presence of a statistical link between PPI expression and cognitive performance. Performance indices in a spatial reference memory test and a working memory test conducted in the Morris water maze, and contextual fear conditioning were correlated against pre-existing baseline PPI expression. A specific correlative link between working memory and PPI induced by weak (but not strong) prepulse was revealed. In addition, a correlation between habituation of the startle reflex and reference memory was identified for the first time: a stronger overt habituation effect was associated with superior spatial search accuracy. The PPI paradigm thus provides two independent predictors of dissociable cognitive traits in normal C57BL/6 mice; and they might serve as potential markers for high-throughput evaluation of potential cognitive enhancers, especially in the context of schizophrenia where deficits in startle habituation and PPI co-exist.

1. Introduction

Prepulse inhibition (PPI) of the acoustic startle reflex refers to the reduction in the startle response to an intense auditory 'pulse' stimulus when it is shortly preceded by a weak non-startling 'prepulse' stimulus [14,35]. PPI represents an automatic pre-attentive gating mechanism protecting the processing of the initial prepulse from distraction by the subsequent pulse stimulus, and its expression is modulated by higher cognitive processes [37]. A potential link between PPI and higher cognitive function has been proposed such that a stronger magnitude of PPI might be associated with, or predictive of, superior cognitive performance [19,42]. Such a

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relationship is also suggested by clinical populations including patients with schizophrenia in which PPI deficits and cognitive impairments frequently co-exist [39,80]. Thus far, evidence for a potential link between PPI and cognition in the general population is weak and inconsistent [80]. Nevertheless, recent studies in healthy volunteers found that PPI was positively correlated with strategy formation, planning efficiency, and execution speed in cognitive tasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB) [9,10,16,31], and working memory performance as measured by the Letter-Number Span Task [47].

Because PPI is readily translatable across species and can be tested in similar fashions in humans and rodents, the PPI paradigm has increasingly been applied as a test of face validity for animal models of neuropsychiatric diseases characterized by abnormal sensorimotor gating, notably schizophrenia amongst other diseases [28,69]. Whilst a number of manipulations are known to similarly affect PPI and cognitive function in animals [34,79], the relationship between PPI expression and cognitive performance in non-perturbed healthy animals remains poorly understood. We have previously reported that in placebo-treated healthy volunteers, weak PPI expression correlated with poor strategy score on the spatial working memory test from the CANTAB [16]. As a translational parallel, the present study aims to evaluate in a homogeneous cohort of adult C57BL/6 mice whether PPI expression might statistically predict performance in typical tests of learning and memory in rodents including spatial reference and working memory in the Morris water maze and contextual fear conditioning in the conditioned freezing paradigm. Cognitive assays were performed after evaluation of PPI so that PPI was measured free from any possible transfer effects [e.g., 43].

Beside PPI, another robust form of startle plasticity is habituation [18,24,71], referring to the cross-species phenomenon that repeated presentations of the startling stimulus lead to a decrease in the startle magnitude [38]. According to the dual-process theory by Groves and Thompson [38], changes in the observed behavioural response to repeated presentations of a sensory stimulus is governed by two independent and antagonistic neural mechanisms: a decremental process termed "habituation process" leading to decreased responding, and an incremental process termed "sensitization process" potentiating the response magnitude. It is presently uncertain whether a link exists between startle habituation as a simple form of non-associative learning and more complex memory processes. However, such an association might be anticipated in hippocampus-dependent tasks such as spatial reference memory or contextual conditioning given the critical involvement of the hippocampus in habituation processes [48,54,74]. Furthermore, spatial learning and habituation – amongst other behaviours – have been found to be similarly sensitive to manipulations of the dopaminergic and glutamatergic systems [3,5–7,22,40,48,51], suggesting the possibility of at least a partial overlap in the underlying neural mechanism. Dopaminergic dysfunction in particular has been central to theories on the neuropsychology of schizophrenia [37,44,67], and habituation deficits have been repeatedly reported in schizophrenia patients [12,13,21,26,55] including first episode schizophrenics [49,50]. Notwithstanding, deficiency in glutamatergic neurotransmission–also implicated in schizophrenia – including signalling via NMDA, AMPA and metabotropic glutamate (mGlu) receptors, have been linked to PPI disruption [11,26,57,73] and habituation deficits [6,7,45,62]. The present study therefore included an overall measure of within-session startle habituation as a variable to be correlated with cognitive performance. This has enabled us to identify a hitherto unreported correlative link between startle habituation and reference memory performance in mice. Following this new lead, we further employed a between-group approach to directly contrast animals showing overt habituation against those showing overt sensitization to better define the relationship between reference memory performance and startle habituation/sensitization [38].

Table 1
Sequence and timing of behavioural tests performed in the same cohort of adult male C57BL/6 mice.

Days	Tests	Manipulations / procedures
1 - 14	*Acclimatization to new animal vivarium*	
15	Prepulse inhibition (PPI)	Acoustic startle reflex
16 - 20	*Free period*	
21	Reference Memory Test Watermaze (in Room 1)	Pre-training
22		Acquisition
23		Acquisition
24		Acquisition
25		Acquisition
26		Probe Test
27 - 34	*Free period*	
35	Working Memory Test Watermaze (in Room 2)	Delay = 15s
36		Delay = 15s
37		Delay = 15s
38		Delay = 15s
39		Delay = 10 min
40		Delay = 10 min
41		Delay = 10 min
42		Delay = 10 min
43 - 48	*Free period*	
49	Context Conditioned Freezing	Conditioning
50		Retention Test (context A)
51		Test (in neutral context B)
52		Retention Test (context A)
53		Test (in neutral context B)

2. Materials and methods

2.1. Subjects

A cohort of 23 naïve male C57BL/6 mice was obtained from our in-house specific pathogen free colony derived from C57BL/6J breeding pairs originating from Charles River (Germany). At the start of the experiments, the animals were 12 weeks old. They were housed in groups of 4–5 in Macrolon Type III cages (Techniplast, Milan, Italy) with ad lib. food and water throughout the entire experimental period. They were held in a temperature controlled room (21 °C), with relative humidity set at 55%, and kept under a reversed 12:12 h light/dark cycle (lights off: 07:00–19:00 h). All tests were conducted in the dark phase of the cycle. Sufficient time (indicated as 'free period') was allowed between tests to minimize transfer effects as depicted in Table 1. All procedures described in the present study had been previously approved by the Zurich Cantonal Veterinary Office, in adherence to the "Principles of Laboratory Animal Care" (NIH publication No. 86-23, revised 1985). All efforts were made to minimize the number of animals used and their potential suffering.

2.2. Prepulse inhibition of acoustic startle reflex

2.2.1. Apparatus

The apparatus consisted of four acoustic startle chambers for mice (SR-LAB, San Diego Instruments, San Diego, CA, USA) as fully described elsewhere [76].

2.2.2. Procedure

During a PPI session, the subjects were presented with a series of discrete trials comprising a mixture of four types of trials. These included pulse-alone trials, prepulse-plus-pulse trials, prepulse-alone trials, and no-stimulus trials in which no discrete stimulus other than the constant background noise (65 dB_A) was presented. The pulse stimulus was 120 dB_A in intensity and 40 ms in duration. Five different prepulse intensities were used: 69, 73, 77, 81, and 85 dB_A, corresponding to 4, 8, 12, 16,