Behavioural Brain Research 242 (2013) 166–177

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

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

Philipp Singer[a,1], Jonas Hauser[a,2], Luis LLano Lopez[b], Daria Peleg-Raibstein[a,3], Joram Feldon[a], Pascual A. Gargiulo[b], Benjamin K. Yee[a,*,1]

[a] Laboratory of Behavioural Neurobiology, Swiss Federal Institute of Technology Zurich, CH-8063 Schwerzenbach, Switzerland
[b] Laboratorio de Neurociencias y Psicología Experimental, Instituto de Medicina y Biología Experimental de Cuyo (IMBECU), Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, 5500, Argentina

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.

ARTICLE INFO

Article history:
Received 5 July 2012
Received in revised form 1 December 2012
Accepted 6 December 2012
Available online xxx

Keywords:
Cognition
Correlation
Individual difference
Learning
Prepulse inhibition
Schizophrenia
Sensorimotor gating

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

* Corresponding author. Present address: The Robert S. Dow Neurobiology Laboratories, Legacy Research Institute, 1225 NE Second Avenue, Portland, OR 97232, United States. Tel.: +1 503 413 2581; fax: +1 503 413 5465.
E-mail address: byee@downneurobiology.org (B.K. Yee).
[1] Present address: The Robert S. Dow Neurobiology Laboratories, Legacy Research Institute, 1225 NE Second Avenue, Portland, OR 97232, United States.
[2] Present address: Cognitive, Perceptual and Brain Sciences, Division of Psychology & Language Sciences, University College London, 26 Bedford Way, London WC1H 0AP, United Kingdom.
[3] Present address: Laboratory of Translational Nutrition Biology, Swiss Federal Institute of Technology Zurich, CH-8063 Schwerzenbach, Switzerland.

0166-4328/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2012.12.011

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,

and 20 dB units above background, respectively. The duration of prepulse stimuli was 20 ms. The stimulus onset asynchrony (SOA) between the prepulse and pulse stimuli on prepulse-plus-pulse trials was 100 ms, which was equivalent to an inter-stimulus interval (ISI) of 80 ms. A session began with the animals being placed into the Plexiglas enclosure. They were acclimatized to the apparatus for 2 min before the first trial began. The first six trials consisted of startle-alone trials, which served to habituate and stabilize the animals' startle response. Subsequently, the animals were presented with twelve blocks of discrete test trials. Each block consisted of one trial of each of the following trial types: pulse-alone, prepulse-plus-pulse of each of the five levels of prepulse, prepulse-alone of each of the five levels of prepulse, and no-stimulus. The session was concluded with a final block of six consecutive pulse-alone trials. The interval between successive trials (i.e., inter-trial interval, ITI) was variable with a mean of 15 s (ranging from 10 to 20 s).

Vibrations of the Plexiglas enclosure caused by the whole-body startle response of the animal were converted into analogue signals by a piezoelectric unit attached to the platform. These signals were digitized and stored by a computer. A total of 130 readings were taken at 0.5-ms intervals (i.e., spanning across 65 ms), starting at the onset of the startle stimulus in pulse-alone and prepulse-plus-pulse trials, and at the onset of the prepulse stimulus in prepulse-alone trials. The average amplitude (in arbitrary units) over the 65 ms was used to index reactivity. PPI obtained at each prepulse intensity was indexed by percent PPI (%PPI) calculated as %PPI = (1 − (prepulse-*plus*-pulse/pulse-*alone*)) × 100%.

2.3. Spatial reference and working memory in the Morris water maze

2.3.1. Apparatus

The water maze consisted of a circular fibreglass tank, 102 cm in diameter and 36 cm deep [see 65 for a full description of its construction]. A transparent Plexiglas cylinder (diameter = 7 cm) was used as the escape platform, with its surface submerged 0.5 cm below the water surface and therefore invisible to the mice. The location of the platform was marked by a local cue in the form of a white circular disk (measuring 12 cm in diameter) positioned horizontally at a height of 12 cm above the centre of the platform. A digital camera was installed above the water maze to record the animals' swim path in every trial. The Ethovision tracking system (Noldus, Wageningen, The Netherlands) was used to calculate the two dependent measures, escape latency and path-length, on each and every trial. The two water maze experiments were conducted in two different rooms (Room 1 and Room 2), each enriched with a unique set of distal spatial cues. The reference memory test was conducted first in Room 1. The working memory test took place in Room 2 after a test-free recovery period of 1 week.

2.3.2. Pre-training

To familiarize the animals to the apparatus and to swimming in the maze they were pre-trained using a visible platform. The platform was positioned in the centre of the maze and each animal underwent two consecutive trials, with the starting positions randomly selected from four possible release points (N, E, S, and W). In the first trial, the subject was gently released from the starting point with its head facing the platform location. In the second trial, it was released from the starting point facing the wall of the maze. The animals were allowed a maximum of 60 s to locate the escape platform. Upon reaching the platform they spent an inter-trial interval (ITI) of 15 s on it before the second trial commenced. If an animal failed to locate the platform within the 60 s time limit, it was guided to it by the experimenter and allowed to stay on it for 15 s. In that case, a maximal escape latency of 60 s was recorded.

2.3.3. Reference memory

On days 1–4 the animals were given two trials per day with the hidden platform located in a constant position in the centre of one of the four possible quadrants (NE, NW, SE, and SW). To start a trial, the animal was always released from one of four possible starting points (N, E, S, and W) with its head facing the wall of the maze. The starting positions were randomized across trials and days for each mouse. As previously, when an animal failed to locate the platform within the time limit of 60 s, it was guided to it. The ITI was 15 s during which the animals stayed on the platform. On day 5, a probe test was conducted to assess memory retention. During the probe test, the platform was removed, and the mice were allowed to swim freely in the maze for 60 s. Each animal's search pattern was analysed by calculating the percentage of time and percentage of path length spent in each of the four quadrants.

2.3.4. Working memory

This lasted 8 days with two trials per day. The hidden platform was now placed in a new position each day and remained in that position for both daily trials. Working memory was indexed by the improvement on Trial 2 compared with Trial 1 when the platform location was unknown to the animals. After 4 days of testing with an ITI of 15 s, the ITI was extended to 10 min for the next 4 training days. Over the extended ITI, the mice were kept in an opaque waiting box in the testing room. Eight platform positions were defined: 4 located at a distance of 35 cm (in the N, E, S and W directions), and another 4 at a distance of 15 cm (in the NE, NW, SE, and SW directions) from the centre of the maze. The start position was randomized across trials and days from eight possible release points (N, E, S, W, NE, NW, SE, and SW) for each mouse.

2.4. Contextual conditioning in the conditioned freezing paradigm

2.4.1. Apparatus

The apparatus has been fully described before [58]. In brief, two sets of four conditioning chambers installed in separate testing rooms were used to provide two distinct contexts. The first set of chambers (context 'A') comprised four Coulbourn Instruments (Allentown, PA, USA) operant chambers (Model E10-10). The second set of chambers (context 'B') comprised four cylindrical (19 cm in diameter) enclosures made of clear Plexiglas resting on a metal mesh floor. Illumination inside the chamber was provided by either a visible light (context 'A') or an invisible infrared light source (context 'B'). A digital camera was mounted in each chamber and captured images at a rate of 1 Hz. The algorithm of the freezing response detection procedure has been validated and fully described before [60]. In brief, successive digitized images obtained at a rate of 1 Hz were compared. The number of pixels difference between adjacent frames was then computed. If this was less than 0.05% of the total number of pixels in a frame, the animal was considered to be freezing in that 1-s interval.

2.4.2. Procedure

The test comprised three stages: (i) conditioning to context 'A', (ii) assessment of conditioned response to the shocked context 'A' and (iii) evaluation of freezing behaviour in the neutral (non-shocked) context 'B'. On day 1, the animals received three un-signalled electrical foot shocks (1 s, set at 0.3 mA) in context 'A'. The first shock-delivery was administered 180 s after placing the animals into the chambers. Successive shocks deliveries were administered every 180 s. The conditioning session was concluded with a final shock-free 180-s interval. On day 2, all mice were returned to context 'A'. They were placed in the chambers for 240 s and freezing levels were recorded in the absence of any further stimulus. On day 3, mice were given a similar 240 s test session in the neutral context 'B'. A similar 'A'–'B' test block was conducted on days 4 and 5, respectively.

2.5. Statistical analysis

All data were analysed by parametric analysis of variance (ANOVA). To assist interpretation of statistically significant outcomes, supplementary restricted analyses applied to subsets of the data or Fisher's least significance difference (LSD) post hoc comparisons were conducted. All statistical analyses were carried out using IBM SPSS Statistics (version 19). A Type-I error rate (p-value) of 0.05 was consistently taken as the yardstick for statistical significance. To better conform to the homogeneity and normality assumptions of parametric ANOVA a natural logarithmic data transformation was performed on the reactivity scores obtained in the PPI experiment [16].

Pearson's product moment correlations were conducted to evaluate possible associations between specific behavioural indexes obtained in the PPI experiment and performance indexes from the three cognitive tests (as defined below and graphically summarized in Fig. 1). All correlations were scrutinized by case-wise diagnostics to identify data points with possible undue statistical influence that might bias interpretation and limit generalization of our conclusion [17]. To this end, inspection of scatter plot was assisted with computation of individual Cook's distance (D_i) as a measure of influence. Cases with D_i > the median of the $F_{(p,N-p)}$ distribution (where p = number of parameters = 2 for simple linear regression, and N = total number of data points) was recommended as a yardstick for exclusion and re-analysis [52]. Additional diagnostic statistics reported by IBM SPSS Statistics: standardized DFBeta's and standardized DFFit, were also consulted. The process was repeated if necessary. When applicable and instructive, the correlative outcomes and regression lines with and without exclusions are graphically illustrated to allow clear visualization of the impact of the identified data points with excessive influence.

2.5.1. PPI-related predictor variables

- *PPI*: The five %PPI scores (generated by different levels of prepulse intensity: 69, 73, 77, 81 and 85 dB) were evaluated separately as predictor of subsequent cognitive performance. Examination of inter-correlation between %PPI scores obtained across different prepulse intensities justified this approach instead of a single summary average score to index PPI. As illustrated in Fig. 1C, PPI generated by the weakest prepulse did not correlate well with that obtained with stronger prepulse stimuli.
- *Startle reaction*: mean startle reactivity on pulse-alone trials presented in the middle block of trials.
- *Startle habituation*: difference score from the first to the last block of pulse-alone trials, i.e., (first–last). Thus, a positive value indicates habituation and a negative value sensitization.
- *Prepulse-elicited reactivity*: the linear derivative (slope) of the prepulse reactivity curve shown in Fig. 1A over the range from background to 85-dB prepulse. The indexation of prepulse reactivity by the slope was justified by the fact that the main effect of prepulse intensity was associated with a highly significant linear trend (p < 0.001) accounting for >90% of the variance.

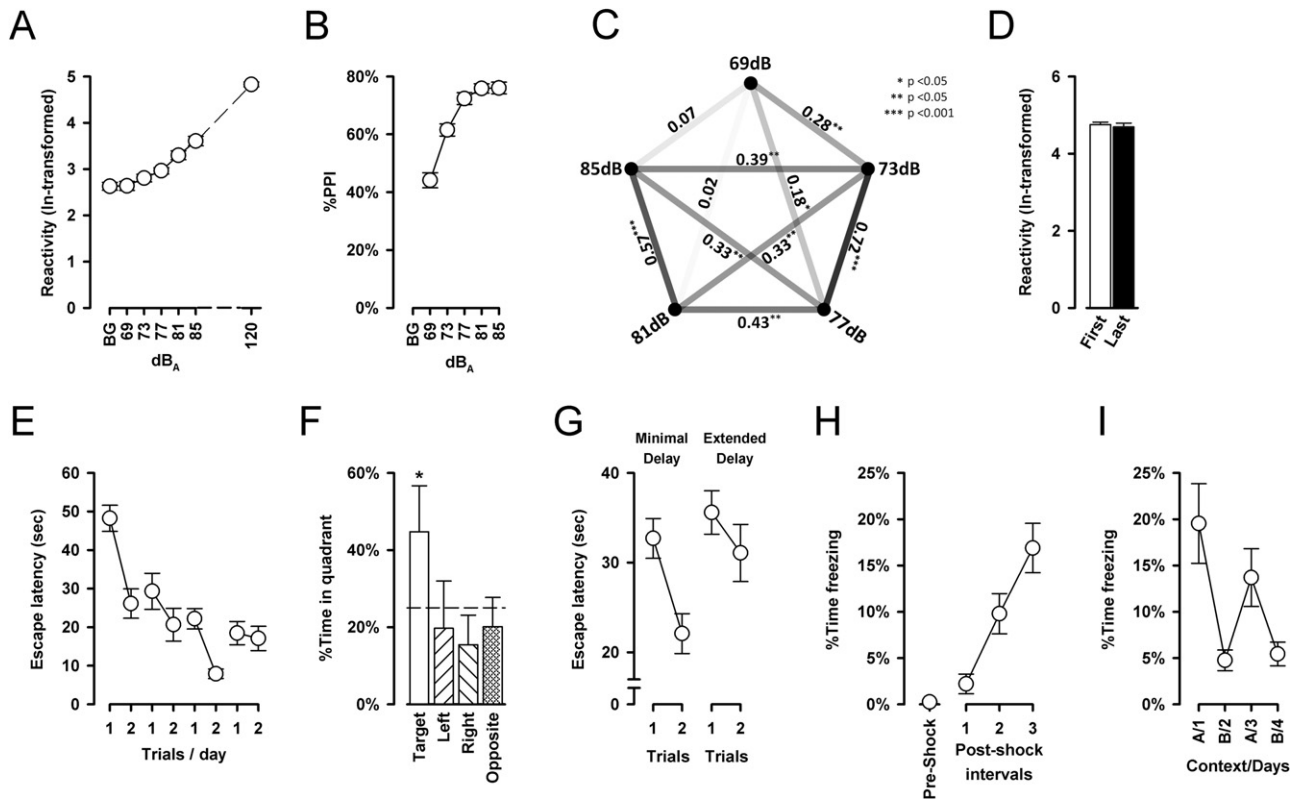

Fig. 1. Summary of all behavioural data. (A) Reactivity on background ('BG'), prepulse-alone (69, 73, 77, 82, 85 dB_A) and pulse-alone (120 dB_A) trials. (B) %PPI as a function of increasing prepulse intensity. (C) Shared variance between %PPI obtained with different prepulse intensities is indicated here by R^2 and represented also by the intensity of shading connecting every pair-wise correlation. (D) Startle reaction on the first and last blocks of pulse-alone trials. (E) Acquisition of reference memory as indicated by a decrease in escape latency across trials [$F_{(1,22)} = 23.66$, $p < 0.001$] and days [$F_{(3,66)} = 15.87$, $p < 0.001$]. (F) %Time spent in each of the four quadrants during the probe test. The animals exhibited a clear search preference for the target quadrant [$F_{(3,66)} = 29.96$, $p < 0.001$]. * denotes that performance was significantly above chance level (25%) based on one-sample *t*-tests ($p < 0.005$). (G) Working memory performance was indexed by an improvement from Trial 1 to Trial 2 [$F_{(1,22)} = 23.77$, $p < 0.001$] which was seen at both delay conditions but appeared weaker at the extended delay. (H) Development of the conditioned freezing response was reflected by a highly significant increase in percent time freeing across the three post-shock intervals [$F_{(2,42)} = 26.26$, $p < 0.001$], that was primarily linear in shape [$F_{(1,21)} = 37.44$, $p < 0.001$] accounting for 99.96% of the variance of the post-shock intervals effect. (I) Expression of contextual fear conditioning was demonstrated by significantly higher freezing levels in the shocked context 'A' (days 1 and 3) relative to the neutral (non-shocked) context 'B' (days 2 and 4) [$F_{(1,21)} = 19.97$, $p < 0.001$]. All values refer to mean ± standard error (SE). $N = 23$ except for data shown in (H) and (I) where $N = 22$.

2.5.2. Cognitive dependent variables

2.5.2.1. Reference memory.

- *Escape performance in acquisition* = average escape latency (or path-length) across trials 2–8. The first trial was excluded here because the location of the platform was not yet known to the animals on that trial).
- *Search accuracy in retention probe test* = percentage of time (or path-length) in the target quadrant (tQ).

2.5.2.2. Working memory.

- A saving score was calculated as the reduction in escape latency (or path-length) from Trial 1 to Trial 2 averaged across delays and days (Fig. 1G).

2.5.2.3. Conditioned freezing.

- *Acquisition:* indexation of acquisition was based on the increase of % time freezing over the three successive post-shock intervals (Fig. 1H). This was represented statistically by the within-subject effect of post-shock intervals [$F_{(2,42)} = 26.26$, $p < 0.001$]. Since this statistical effect was primarily expressed as a linear increment over successive post-shock intervals, as evidenced by the fact that the linear component of the post-shock intervals [$F_{(1,21)} = 37.44$, $p < 0.001$] explained over 99.96% of the variance (i.e., sum of squares) partitioned to the post-shock intervals effect, we elected to calculate the linear component of individual animals' acquisition curve across the three post-shock intervals to index acquisition. This is equivalent to the slope of the least-square line fitted across the three post-shock intervals, which would be in the units of % time freezing per interval.
- *Retention*: expression of conditioned freezing to the training context 24 h after acquisition was indexed by the percent time freezing recorded in the first re-exposure test when the animals were returned to context 'A' (indicated as 'A/1' in Fig. 1I) without any shock US.

3. Results

3.1. Summary of behavioural results

The group average performance indices derived from the PPI experiment are summarized in Fig. 1A–D. The relevant cognitive measures obtained in the subsequent cognitive assays performed in the same animals are illustrated in Fig. 1E–I. The data depicted are averaged over the 23 subjects, except for the conditioned freezing experiment (Fig. 1H and I), in which the sample size was reduced to 22 due to the loss of one animal's data because of a technical failure on the critical test day. The overall patterns of results obtained in individual tests are highly consistent with expectation and our past experience.

3.2. PPI as a predictor of working memory performance

PPI emerged only as a potential significant predictor of working memory performance (Fig. 2A). This is in sharp contrast to the absence of any statistical link with performance in the reference

A

%PPI at prepulse:	Reference Memory Performance: Acquisition: Latency	Reference Memory Performance: Acquisition: Path-length	Reference Memory Performance: Retention: %Time in tQ	Reference Memory Performance: Retention: %Path in tQ	Working Memory Performance: Latency	Working Memory Performance: Path-length	Contextual Conditioned Freezing: Acquisition	Contextual Conditioned Freezing: Retention
69 dB	+0.34	+0.34	-0.01	-0.02	**+0.53**	**+0.50**	-0.24	-0.41
73 dB	+0.06	+0.00	+0.11	+0.09	+0.29	+0.22	+0.26	+0.21
77 dB	-0.01	-0.05	-0.17	-0.20	+0.34	+0.29	+0.17	+0.15
81 dB	+0.10	+0.08	+0.06	+0.04	-0.03	-0.10	-0.04	+0.01
85 dB	+0.11	+0.10	+0.06	+0.03	-0.11	-0.16	+0.16	+0.10
***N*=**	23	23	23	23	23	23	22	22

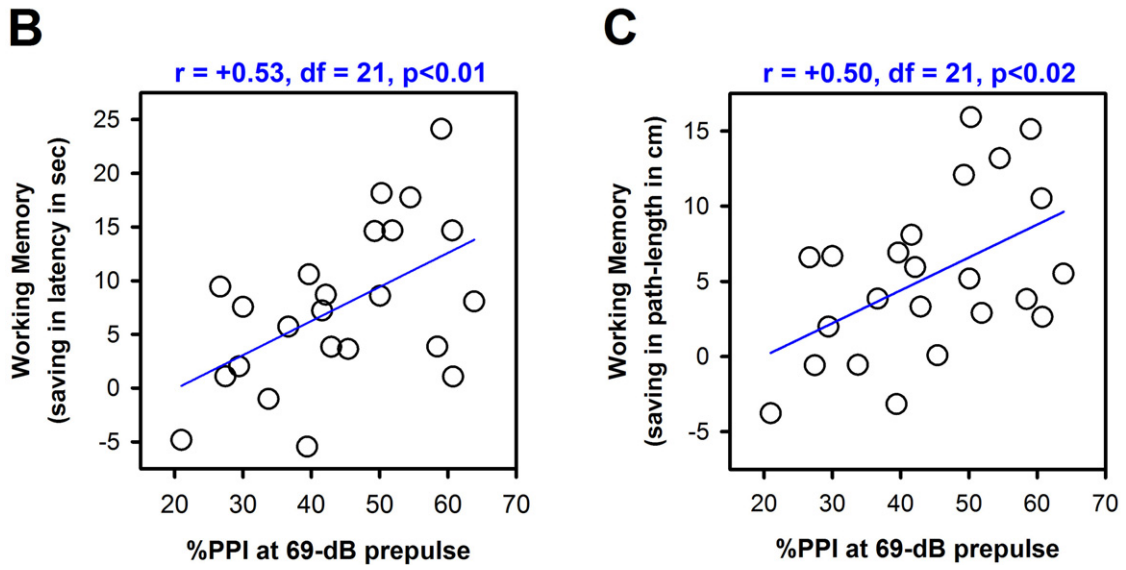

Fig. 2. PPI predicts working memory performance. (A) Correlation matrix between individual %PPI scores obtained at each of the five prepulse intensities (69–85 dB) against distinct performance scores derived from the three cognitive tests. Amongst the correlation coefficients illustrated, only that between %PPI obtained with 69-dB prepulse stimulus and working memory performance achieved statistical significance. Below, the levels of significance and linear regression lines between %PPI obtained with 69-dB prepulse stimulus (abscissa) and saving scores (represented by the ordinate) in escape latency (B) or path-length (C) from Trial 1 to Trial 2 are illustrated. The depicted correlations in (B) and (C) are relatively free from potential outliers with maximum Cook's distance of the illustrated correlations were 0.27 and 0.14, respectively.

memory test or contextual conditioned freezing. Specifically, saving scores, defined as reduction in escape latency or path-length from the first trial (when platform location was not known) to the second trial, correlated specifically with %PPI obtained with the weakest prepulse here at 69 dB (Fig. 2B and C). The effect sizes of the two correlations were similar, accounting for about 25–28% of the variance. Stepwise multiple regressions with all five %PPI scores entered as predictors at the same time similarly indicated that the %PPI score obtained in the 69-dB prepulse condition was the only significant predictor.

When the working memory performances at the two delay conditions were separately investigated, we found that the correlative link with PPI was more pronounced at the shorter delay. The correlation remained significant at $p \leq 0.05$ [$r = 0.41$–0.42, df = 21] at the shorter delay, but not so at the long delay. The latter might be due to weaker learning observed with the extended delay condition (see Fig. 1G), thus the working memory scores might be somewhat more noisy and thereby limited the statistical power to detect the link between the two behavioural traits.

3.3. Startle habituation as a predictor of reference memory performance

Initial analyses revealed seemingly contradictory correlative relationships between startle habituation and reference memory performance (Fig. 3A). Whilst stronger habituation was related to slower escape latency or longer path-length during acquisition (Fig. 3B and C), an opposite relationship was observed in terms of spatial search accuracy in the retention probe test, in which stronger habituation predicted more focal search in the target quadrant (Fig. 3D and E). However, there were strong indications that the apparent association between habituation and acquisition performance was heavily influenced by two subjects (highlighted in red in Fig. 3B and C), and their exclusions would be deemed appropriate. Exclusion of the first subject (#30) was justified by the large Cook's distance of this data point depicted in the scatter plots of Fig. 3B and C (1.24 and 0.88, respectively), which exceeded the recommended cut-off at 0.717 (= the median of the $F_{2,21}$ distribution). Following this, exclusion of the second subject (#17) was justified by recalculation of the Cook's distances: with values (0.73 and 1.49, from the scatter plots in Fig. 3B and C, respectively) exceeding the recommended cut-off at 0.718 (= the median of $F_{2,20}$ distribution). The final corrected analyses suggested that habituation accounted for a very small proportion of variation in acquisition performance (1.4% and 0.04% variance in escape latency and path-length, respectively).

On the other hand, no such concerns were raised in the correlative analyses between startle habituation and probe test performance with the maximum Cook's distance being 0.132 (Fig. 3D) and 0.136 (Fig. 3E). Indeed, removal of the two highly

A

Startle Habituation	Reference Memory Performance: Acquisition: Latency	Reference Memory Performance: Acquisition: Path-length	Reference Memory Performance: Retention: %Time in tQ	Reference Memory Performance: Retention: %Path in tQ	Working Memory Performance: Latency	Working Memory Performance: Path-length	Contextual Conditioned Freezing: Acquisition	Contextual Conditioned Freezing: Retention
Initial correlation *	+0.52[a]	+0.44[b]	**+0.42**	**+0.47**	+0.18	+0.16	- 0.35	+0.13
Final correlation	+0.02[a]	+0.12[b]						
Final *N* =	21	21	23	23	23	23	23	23

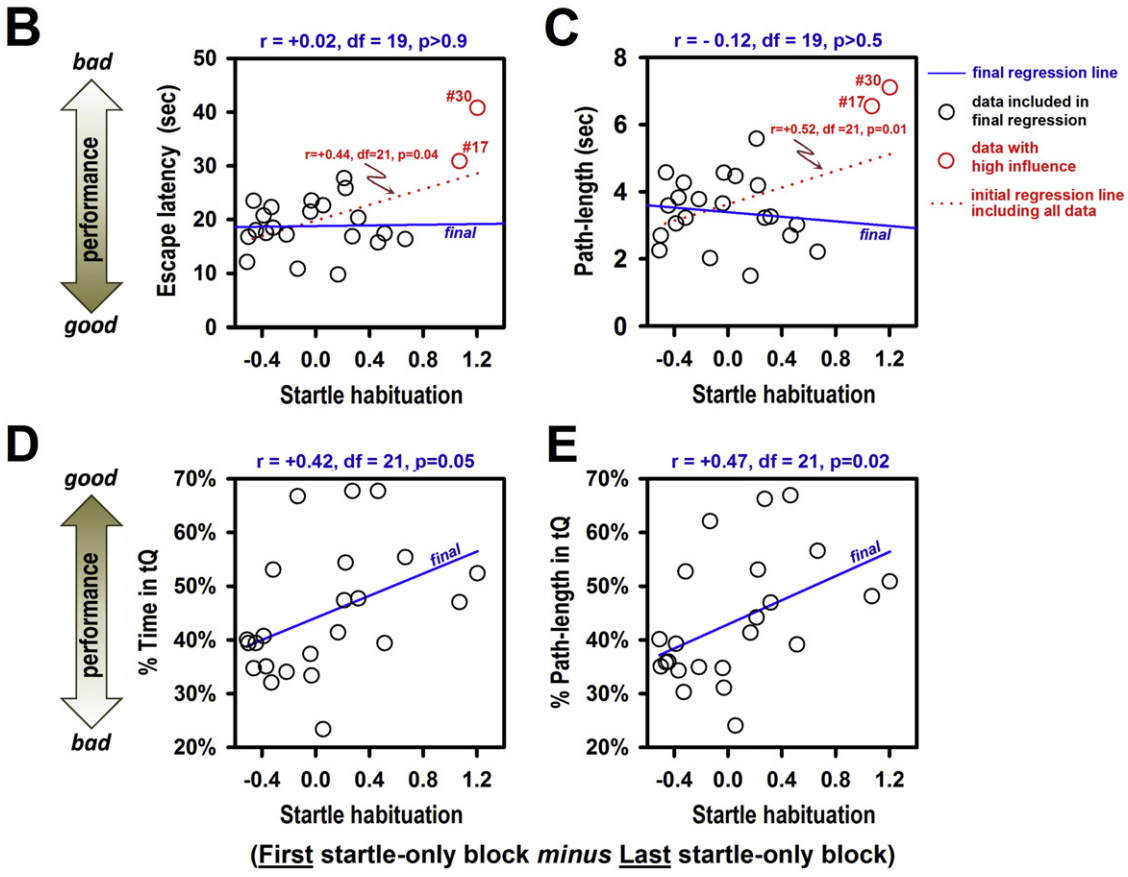

Fig. 3. Startle habituation predicts retention but not acquisition performance in the water maze reference memory test. (A) The table summarizes the outcomes of the correlative analysis of the habituation index against different behavioural scores derived from the three cognitive tests. Final significant correlations are indicated in bold type. When the initial and final correlations differed, both statistics are illustrated. *Footnotes*: [a] the correction involved exclusions of two subjects with large Cook's distances (1.24 and 0.73); [b] the correction involved exclusions of the same two subjects that were also associated with large Cook's distances (0.88 and 1.49). (B) and (C) depict the scatter plots of startle habituation against the escape latency and path-length measures of acquisition, respectively, as defined in Section 2.5.2. The final regression lines are depicted in blue. For the purpose of comparison, the initial uncorrected regression lines are depicted in dotted red lines. (D) and (E) depict the scatter plots of startle habituation against the retention probe test performance expressed as proportion time spent, and path-length recorded, respectively, in the target quadrant (tQ). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

influential subjects identified in the previous analyses would not have altered the statistical outcomes of the two correlations against probe test performance (Fig. 2C and D). If anything, the correlation coefficients would increase in value. Thus, we concluded that only the correlations between habituation and retention test performance were robust.

3.3.1. Subdivision of subjects based on startle habituation

To further explore the observed association between startle habituation and reference memory, we next adopted a between-subject approach whereby two groups of animals showing either habituation or sensitization of the startle reaction were segregated. A subject was considered a '*habituator*' or '*sensitizer*' when its startle habituation index was significantly (i.e., $p < 0.05$) above or below a habituation value of zero. Briefly, the index of zero startle habituation ("Δ of First – Last blocks", represented by the *x*-axis of Fig. 4A and B) indicates no change of average startle reaction from the first to the last block of pulse-only trials – marked by the vertical reference line inside the yellow box in Fig. 4A and B. The width of the yellow boxes corresponds to the upper and lower boundaries of deviation = $0 \pm 2.074 \times$ SDs = {−0.210 to +0.210}, beyond which would indicate significant deviation from zero at $p < 0.05$, based on one-sample *t*-test with 22 degrees of freedom (because $N = 23$). Animals to the right of the yellow area were classified as '*habituators*' ($n = 9$, with positive Δ values significantly larger than zero), and to the left of the yellow area '*sensitizers*' ($n = 9$, with negative Δ values significantly below zero). Animals inside the yellow boundaries were classified as '*neither*' ($n = 5$). The mean startle habituation indices of the latter two groups are illustrated in the horizontal bar plots above the scatter plots in Fig. 4A and B.

Fig. 4C directly contrasts the startle average from the first against the last blocks of pulse-only trials, and illustrates the magnitude

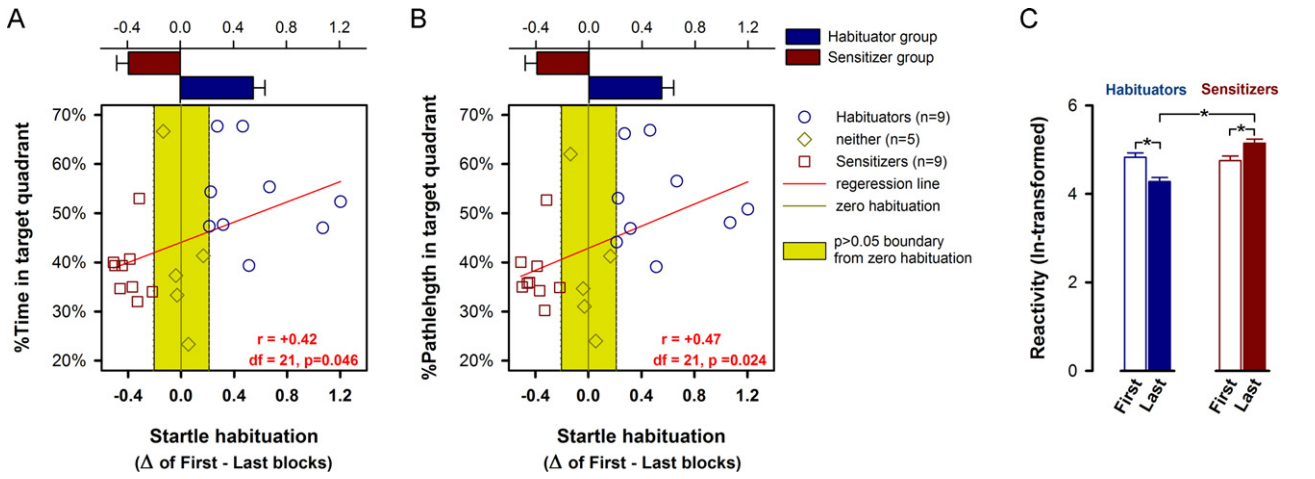

Fig. 4. Subdivision of the animals according to habituation index. (A) and (B) Scatter plots showing individual probe test performance and startle habituation as well as the allocation of each subject ($N = 23$) to one of three possible groups: habituators ($n = 9$), sensitizers ($n = 9$), and neither ($n = 5$). For derivation of the boundaries within which (marked by yellow) animals did not exhibit a significant change from the first to the last block of pulse-alone startle reaction is explained in the text, such that animals to the right of the yellow box were classified as "*habituators*" and to the left "*sensitizers*", showing significant decrease and increase of startle reaction from the first to the last block, respectively. The mean (±SE) startle habituation of the two habituated and sensitized groups are illustrated in the bar plots on the top. (C) Startle reaction (ln-transformed) of the habituator and sensitizer groups on the first and last blocks of pulse-alone trials (values refer to mean ± SE). * indicates significant pair-wise comparison based on the mean-square error terms associated with the significant Group by Blocks interaction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of habituation and sensitization in the identified "*habituators*" and "*sensitizers*". As expected, a 2 × 2 (Group × Blocks) ANOVA of the ln-transformed reactivity data yielded a highly significant group × blocks interaction [$F_{(1,16)} = 55.80$, $p < 0.001$] (Fig. 4C). The presence of significant habituated and sensitized response by the last block [p's < 0.0005] was confirmed by supplementary post hoc pairwise comparisons, which also indicated that *habituators* and *sensitizers* differed significantly only in their startle response in the last block ($p < 0.0001$) but not their startle reactivity in the first block ($p = 0.27$).

3.3.2. Habituators vs. sensitizers in the reference memory test

Acquisition of spatial reference memory across the 8 trials was assessed by two separate 2 × 8 (Group × Trials) ANOVAs of escape latency and path-length. As illustrated in Fig. 5A–D, escape performance clearly improved across trials yielding a significant main effect of trials in terms of both latency [$F_{(7,112)} = 10.32$, $p < 0.001$] and path-length [$F_{(7,112)} = 8.48$, $p < 0.001$]. Neither the main effect of group nor its interaction with trials attained statistical significance indicating that acquisition of the reference memory task did not markedly differ between the two groups of mice. Similar analysis of swim speed also revealed no significant group differences. The average speed of the two groups was: habituator = 0.18 ms^{-1}, and sensitizer = 0.19 ms^{-1}, SE = 0.01 ms^{-1}.

Retention of reference memory was assessed by the animals' search preference for the target quadrant (Fig. 5C–F). To this end, the percentage of time and path-length in each of the four quadrants were submitted to two separate 2 × 4 (Group × Quadrants) ANOVAs. The overall presence of reference memory was indicated by a significant main effect of quadrants [%time: $F_{(3,48)} = 41.22$, $p < 0.001$, %path-length: $F_{(3,48)} = 39.85$, $p < 0.001$]. However, the preference for the target quadrant was clearly stronger in the *habituators* than the *sensitizers* yielding a highly significant group × quadrants interaction [%time: $F_{(3,48)} = 8.32$, $p < 0.001$, %path-length: $F_{(3,48)} = 8.53$, $p < 0.001$]. Subsequent restricted analysis to the target quadrant revealed a significant main effect of group [%time: $F_{(1,16)} = 14.75$, $p = 0.001$, %path-length: $F_{(1,16)} = 15.29$, $p = 0.001$] confirming the impression that the *habituators* exhibited a superior search preference than the *sensitizers*. Nevertheless, preference for the target quadrant was significantly above chance level (25%) in both groups based on one-sample t-tests of percent time [*habituators*: $t = 8.91$, *sensitizers*: $t = 6.61$, df = 8, $p < 0.001$] or percent path-length [*habituators*: $t = 8.70$, *sensitizers*: $t = 5.94$, df = 8, $p < 0.001$] in the target quadrant. Swim speed was highly comparable between the two groups (*habituators* = 0.19 ms^{-1}, and *sensitizers* = 0.20 ms^{-1}, SE = 0.01 ms^{-1}).

3.3.3. Habituators vs. sensitizers in other tests

As expected from the correlative outcomes summarized in Fig. 3A, the two groups (*habituators* vs. *sensitizers*) never significantly differed from each other across all measures taken from the PPI, working memory and conditioned freezing experiments (data not shown).

3.4. Startle reaction to the pulse stimulus and reactivity to the prepulse stimuli as predictors of cognitive performance

As summarized in Table 2, neither the startle reaction to the pulse stimulus nor the reactivity to the prepulse stimuli is a significant predictor of cognitive performance. The maximum correlative coefficient [+0.41] revealed was between mean startle reactivity (on pulse-alone trials) against the path-length measure of acquisition performance in the reference memory test, which just exceeded the criterion of statistical significance [$p = 0.052$]. However, this correlative link was not substantiated by the alternative performance measure based on the latency measure [$r = +0.33$, $p = 0.12$]. All other reported coefficients remained far from statistical significance.

4. Discussion

In a random cohort of homogenous wild type adult male mice of the inbred C57BL/6 strain, the present study has provided evidence in support of a positive statistical association between individual variations in PPI expression and working memory function. At the same time, we demonstrated a novel and specific association between startle habituation and spatial search accuracy in the retention test of spatial reference memory such that animals exhibiting strong overall startle habituation outperformed

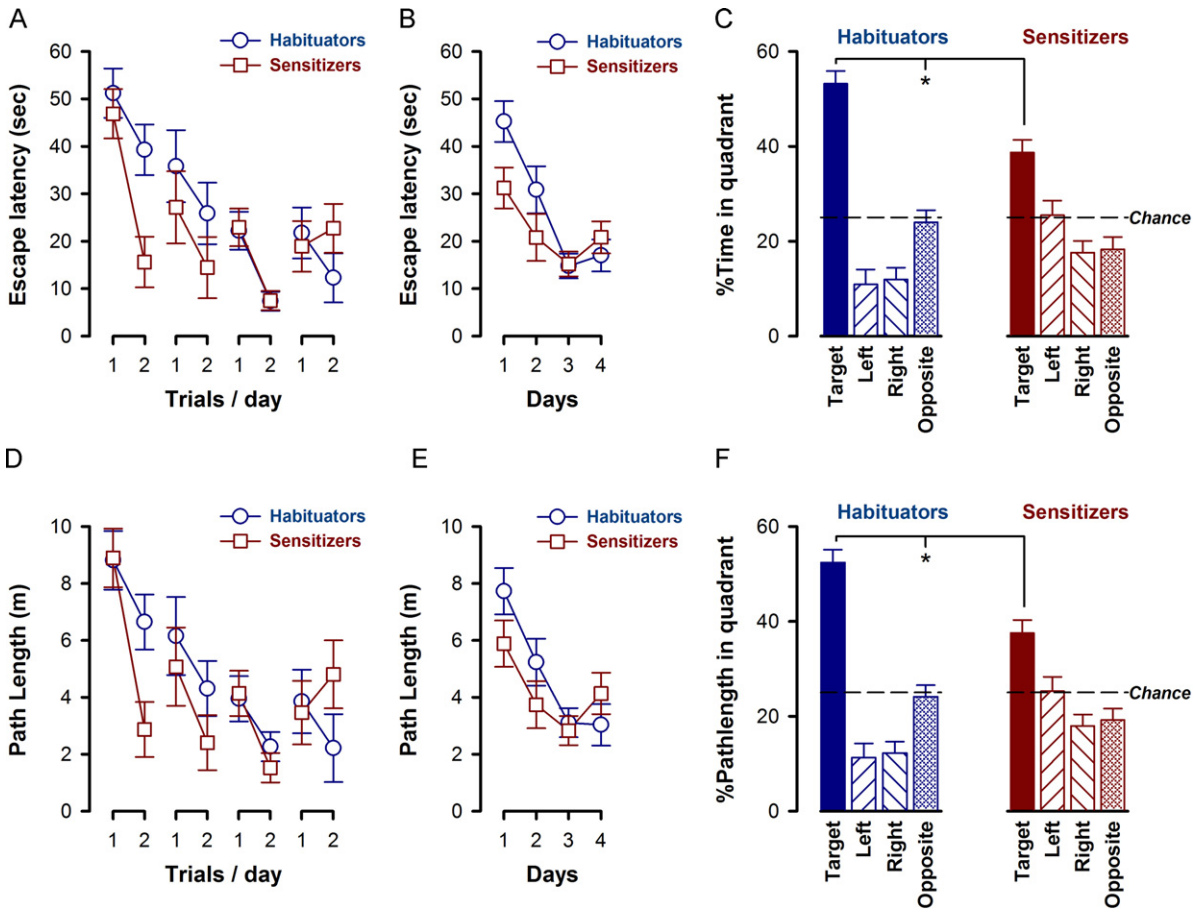

Fig. 5. Habituators vs. sensitizers in reference memory performance. Escape performance in terms of latency (A and B) and path-length (D and E) across the 8 trials did not differ between the habituated and sensitized groups. However, the habituator group showed improved search accuracy in the probe test relative to the sensitizer group in terms of both %time (C) and %path-length (F) in the target quadrant (*, $p < 0.05$ based on restricted analysis of the target quadrant following the emergence of a significant 2×4 (Group × Quadrants) interaction. All values refer to mean ± SE.

those that did not. The identified correlations between distinct behavioural traits provide further qualifications on the use of the common PPI paradigm as a translational paradigm, even though they were demonstrated here on the basis of individual differences presumably attributed to environmental and or developmental causes rather than genetic factors since an inbred mouse strain was used.

4.1. The link between sensorimotor gating and working memory

In considering PPI as a potential high-throughput assay to probe cognitive functions or to predict pro-cognitive drug action, it is imperative to distinguish the cognitive domains that are orthogonal to PPI from those that parallel PPI. However, as pointed out by Young et al. [80] only few studies have investigated this in humans and the limited existing data do not substantiate the claim [e.g., 29 and 30] that PPI is a reliable correlate of general cognitive function in humans. This is in spite of the coexistence of PPI and cognitive deficiency in several diseases including schizophrenia [25]. From one perspective, the present parallel investigation in laboratory mice might be taken as support for Young et al.'s [80] interpretation, because baseline PPI failed to predict subsequent cognitive performance in the majority of tests subsequently conducted in the same animals. Yet, a clear and unique link with working memory performance was successfully identified. Importantly, this link is not without precedence, Csomor et al. [16] have previously reported a correlation between the formation of an appropriate search strategy in the CANTAB spatial working memory task and the magnitude of PPI amongst placebo-treated healthy volunteers. This emphasis on strategy rather than memory retention capacity is supported by the inverse correlation between PPI magnitude and thinking time in the CANTAB stocking test (a version of the Tower

Table 2
Summary of the correlative outcomes of startle reaction and prepulse reactivity with cognitive performance. None of the correlation coefficients achieved statistical significance.

	Reference memory performance				Working memory performance		Contextual conditioned freezing	
	Acquisition		Retention					
	Latency	Path-length	%Time in tQ	%Path in tQ	Latency	Path-length	Acquisition	Retention
Startle reaction	+0.33	+0.41	− 0.25	− 0.26	− 0.22	− 0.16	− 0.24	−0.28
Prepulse reactivity	− 0.02	+0.02	− 0.04	− 0.03	− 0.22	− 0.15	− 0.06	+0.18
N=	23	23	23	23	23	23	22	22

of Hanoi puzzle), identified in the same study [16]. The equivalent correlation revealed in the mice here was seemingly more clearly seen in the short rather than the extended delay condition. If strong PPI is predictive of retention capacity, then the correlation would have been at least as clear (if not more so) in the longer delay condition. Instead, weak PPI may reflect executive deficiency, as concluded by Bitsios et al. [9], leading to premature action before a strategy is fully formed, or the formation of less efficient strategies. Thus, the animals could not effectively translate their knowledge of the escape platform's whereabouts revealed to them on the first trial into faster escape on the second trial.

Notably, the correlations between PPI and working memory performance were not invariably observed across all PPI test conditions. Csomor et al. [16] reported a significant PPI – working memory correlation only with a SOA of 60 but not 120 ms. Here, we only detected it with the lowest prepulse intensity at 4 dB units above background noise. Indeed, the correlation here would have been masked if the PPI magnitude were averaged across the five prepulse intensities. Such specification cannot be readily predicted or explained. Although, we cannot exclude a possible ceiling effect at prepulses of higher intensity that might limit individual variability here. By contrast, such an argument cannot be applied to Csomor et al.'s [16] data, since PPI was stronger at the 60 ms than 120 ms SOA condition. Yet, the possibility that PPI generated by prepulse of low intensity might be qualitatively different from that generated by more intense prepulse is highlighted by the lack of correlation between them (Fig. 1C). It is unfortunate that Csomor et al. [16] did not vary prepulse intensity but only SOA, and vice versa here. More comprehensive parametric analyses would be highly instructive in defining the best PPI test conditions that might maximize the prediction of working memory function by PPI.

Although PPI is a pre-attentive process, sustained focal attention can play a critical role in its expression [19,20,63,64]. We have recently shown that PPI was positively correlated with sustained attention or vigilance in a visual two-choice discrimination task in C57BL/6 mice [8]. There is also preliminary evidence suggesting that negative priming, a test of selective attention and inhibition, might be correlated with PPI performance in healthy humans [24, but see 68]. This may imply that PPI is probably more closely related to general attention-related processes [63,64] rather than neuroplastic processes underlying memory formation and/or retention in typical learning paradigms for rodents. Nevertheless, PPI deficiency remains a common observation in schizophrenia patients as well as their healthy relatives and is therefore widely considered as an endophenotype of the disease [70]. And, deterioration in attentional functioning is an early sign of schizophrenia [81] as well as a good predictor of global functional recovery in first-episode patients [33]. Hence, the prognostic value of PPI as an early predictor and correlate of schizophrenia psychopathology certainly deserves further investigation in both clinical and preclinical settings.

4.2. The link between startle habituation and reference memory retention

Here, the presence of a unique correlation between PPI and working memory performance was contrasted by the specific association between startle habituation and reference memory retention indexed by the spatial search accuracy in the probe test. The correlative analyses initially also yielded a significant correlation between habituation and escape performance across the four days of reference memory acquisition training, which would instead suggest that stronger habituation predicted poorer acquisition performance in the water maze reference memory task. However, closer examination revealed that this correlative relationship was heavily biased by two influential outliers with excessive statistical leverage; and once these animals were excluded, the correlation was far from statistical significance (Fig. 3B and C). Removal of the two influential outliers did not affect any other correlative outcomes. Importantly, the correlation between habituation and probe test performance remained statistically significant, suggesting that the link between habituation and reference memory retention is specific and robust (see Fig. 3D and E). To further explore this hypothesis we subsequently singled out those animals that exhibited significant habituation or sensitization of the startle response for a direct comparison. In agreement with the correlative analysis, the habituated group specifically outperformed the sensitized group on the probe test. However, the search preference for the target quadrant was clearly above chance level in both groups indicating that reference memory was not severely disrupted in the 'sensitized group'. The question arises as to whether the demonstrated group difference stemmed from an improvement in the 'habituated group' and/or deterioration in the 'sensitized group'. We addressed this by comparison with the remaining animals that exhibited neither habituation nor sensitization on statistical grounds as a pseudo-control group. As illustrated in Fig. 6, the sensitized and control groups are closely matched indicating that the presence of a sensitized startle response by the end of the PPI test session did not worsen reference memory retention. By contrast, it appears that the overt presence of strong overall startle habituation might predict enhanced reference memory in C57BL/6 mice.

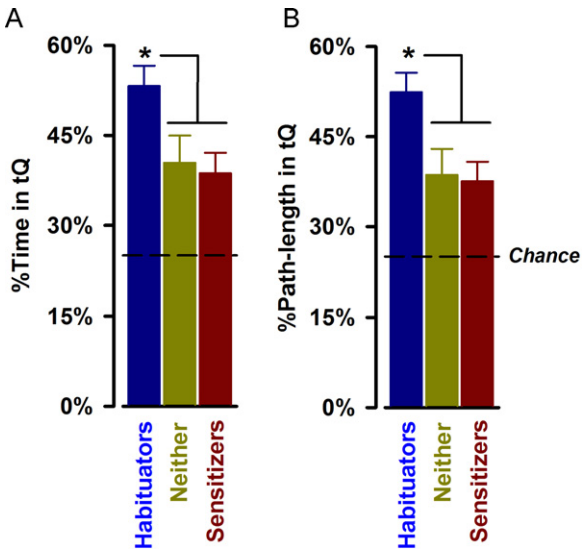

Fig. 6. Probe test performance. The habituated group ($n = 9$) exhibited increased preference for the target quadrant than both the sensitized group ($n = 9$) and the 'neither' group ($n = 5$) showing neither habituation nor sensitization. This was similarly seen in terms of (A) percent time and (B) percent path-length in the target quadrant (tQ). $^*p < 0.05$ based on post hoc comparisons following the emergence of a significant group effect in the respective one-way ANOVA. All values refer to mean ± SE.

This prediction, however, does not conform to a known strain difference in spatial cognition between C57BL/6 and DBA/2 mice. Whilst the DBA/2 strain is associated with weaker spatial memory and related contextual processing compared with the C57BL/6 strain [e.g., 4, 56, 59, 78], DBA/2 mice typically exhibited stronger and more robust startle habituation than C57BL/6 mice [66]. However, this does not preclude the possibility that the within-strain relationship identified here might be similarly observed in other strains of mice or rats. This can only be satisfactorily addressed empirically, and the answer would help to define the importance and scope of the present novel finding.

Our choice of C57BL/6 mice in the present study has inadvertently provided us a unique opportunity for a qualitative contrast

between individuals with a propensity for startle habituation and non-*habituators*, but generalization of the present finding to other strains of mice and other species in which overall habituation is more robustly seen may not be straightforward. Regarding the measurement of habituation here, it is important to qualify that habituation was indexed by comparing the startle response at the beginning with the end of the (PPI) test session. In-between, many startle stimuli with or without prepulses were presented. C57BL/6 mice typically exhibit varying degrees of sensitization and instances of dishabituation during this extended period of exposures to the pulse stimulus, in spite of rapid initial habituation. As reported elsewhere [77], such sensitized responses are mostly observed with more intense pulse stimuli (like here, at ≈120 dB) and in animals with strong baseline startle reactivity. Hence, the C57BL/6 strain typically does not show a robust startle habituation effect in this measure in comparison with other mouse or rat strains [27,66,71,77]. This had enabled us to obtain a near symmetrical segregation of C57BL/6 mice that exhibited overall response habituation against those showing overall response sensitization. From this perspective, our habituation index may also be interpreted as a measure of sensitization. This is perfectly in line with Groves and Thompson's dual process theory [38], which explicitly states that a stimulus has both response-decreasing (habituating) as well as response-increasing (sensitizing) influence on the subsequent response. This does not undermine the validity of our observed correlation, but it must be recognized that one cannot decide whether the cognitive test results correlate with the underlying habituation or sensitization processes (or both). Indeed, whilst short-term habituation is likely dependent on synaptic mechanisms within the brainstem startle pathway [46,72], sensitization involves extrinsic modulation of the startle pathway [e.g., 23, 53]. One may therefore suspect that mechanisms involved in sensitization are more likely impacting on other cognitive functions.

The novel link between startle habituation and spatial reference memory retention revealed here, likely involves multiple neural mechanisms. Like the link between PPI and working memory, both dopaminergic and glutamatergic mechanisms are obvious candidates (see Section 1) and it would be premature to single out any particular one. Halberstadt and Geyer [40] suggest that the D_1 receptor might exert inhibitory control over sensitization and at the same time plays a key role in the habituation process. However, whilst genetic disruption of the D_1 receptor induces both habituation [40] and reference memory [22] deficits, working memory [75] as well as associative learning [36,41] are also severely affected. This pattern only partially matches our correlative observations. Augmentation of D_1 receptor activity has been proposed as a potential therapeutic strategy to restore cognitive dysfunction in schizophrenia [1,2,15,32,61], but its impact on startle habituation has not been carefully evaluated. A facilitation effect in the latter would further strengthen the impact and significance of our finding here.

5. Conclusion

The present study shows that individual differences in two independent forms of startle plasticity – PPI and habituation – are statistically associated with performance in two distinct spatial memory tests taxing working and reference memory, respectively. These identified links appear highly specific and are relevant to the diagnostic significance of PPI in health and disease. Whilst the positive correlation between PPI magnitude and working memory performance represents an important finding seemingly translatable to humans, the novel suggestion of the potential link between startle habituation and other higher cognitive functions in C57BL/6 mice certainly deserves further probing, especially with a causal perspective that is lacking here.

Acknowledgements

The study was supported by the Swiss Federal Institute of Technology, and formed parts of the practical education in behavioural neuroscience. We are very grateful to the excellent technical support by Peter Schmid and the animal husbandry staffs at the Laboratory of Behavioural Neurobiology.

References

[1] Abi-Dargham A, Moore H. Prefrontal DA transmission at D1 receptors and the pathology of schizophrenia. The Neuroscientist 2003;9:404–16.
[2] Abi-Dargham A. Do we still believe in the dopamine hypothesis? New data bring new evidence. International Journal of Neuropsychopharmacology 2004;7(Suppl. 1):1–5.
[3] Altinbilek B, Manahan-Vaughan D. A specific role for group II metabotropic glutamate receptors in hippocampal long-term depression and spatial memory. Neuroscience 2009;158:149–58.
[4] Ammassari-Teule M, Passino E, Restivo L, de Marsanich B. Fear conditioning in C57/BL/6 and DBA/2 mice: variability in nucleus accumbens function according to the strain predisposition to show contextual- or cue-based responding. European Journal of Neuroscience 2000;12:4467–74.
[5] Bannerman DM, Rawlins JN, Good MA. The drugs don't work-or do they? Pharmacological and transgenic studies of the contribution of NMDA and GluR-A-containing AMPA receptors to hippocampal-dependent memory. Psychopharmacology 2006;188:552–66.
[6] Bespalov A, Jongen-Rêlo AL, van Gaalen M, Harich S, Schoemaker H, Gross G. Habituation deficits induced by metabotropic glutamate receptors 2/3 receptor blockade in mice: reversal by antipsychotic drugs. Journal of Pharmacology and Experimental Therapeutics 2007;320:944–50.
[7] Bickel S, Lipp HP, Umbricht D. Early auditory sensory processing deficits in mouse mutants with reduced NMDA receptor function. Neuropsychopharmacology 2008;33:1680–9.
[8] Bitanihirwe BK, Dubroqua S, Singer P, Feldon J, Yee BK. Sensorimotor gating and vigilance-dependent choice accuracy: a within-subject correlative analysis in wild-type C57BL/6 mice. Behavioural Brain Research 2011;217:178–87.
[9] Bitsios P, Giakoumaki SG, Theou K, Frangou S. Increased prepulse inhibition of the acoustic startle response is associated with better strategy formation and execution times in healthy males. Neuropsychologia 2006;44:2494–9.
[10] Bitsios P, Giakoumaki SG. Relationship of prepulse inhibition of the startle reflex to attentional and executive mechanisms in man. International Journal of Psychophysiology 2005;55:229–41.
[11] Brody SA, Dulawa SC, Conquet F, Geyer MA. Assessment of a prepulse inhibition deficit in a mutant mouse lacking mGlu5 receptors. Molecular Psychiatry 2004;9:35–41.
[12] Bolino F, Di Michele V, Di Cicco L, Manna V, Daneluzzo E, Casacchia M. Sensorimotor gating and habituation evoked by electro-cutaneous stimulation in schizophrenia. Biological Psychiatry 1994;36:670–9.
[13] Bolino F, Manna V, Di Cicco L, Di Michele V, Daneluzzo E, Rossi A, et al. Startle reflex habituation in functional psychoses: a controlled study. Neuroscience Letters 1992;145:126–8.
[14] Braff DL, Geyer MA. Sensorimotor gating and schizophrenia. Human and animal model studies. Archives of General Psychiatry 1990;47:181–8.
[15] Castner SA, Williams GV, Goldman-Rakic PS. Reversal of antipsychotic-induced working memory deficits by short-term dopamine D1 receptor stimulation. Science 2000;287:2020–2.
[16] Csomor PA, Stadler RR, Feldon J, Yee BK, Geyer MA, Vollenweider FX. Haloperidol differentially modulates prepulse inhibition and p50 suppression in healthy humans stratified for low and high gating levels. Neuropsychopharmacology 2008;33:497–512.
[17] Cook RD. Detection of influential observation in linear regression. Technometrics 1977;19:15–8.
[18] Davis M. Differential retention of sensitization and habituation of the startle response in the rat. Journal of Comparative and Physiological Psychology 1972;78:260–7.
[19] Dawson ME, Hazlett EA, Filion DL, Nuechterlein KH, Schell AM. Attention and schizophrenia: impaired modulation of the startle reflex. Journal of Abnormal Psychology 1993;102:633–41.
[20] Dawson ME, Schell AM, Hazlett EA, Nuechterlein KH, Filion DL. On the clinical and cognitive meaning of impaired sensorimotor gating in schizophrenia. Psychiatry Research 2000;96:187–97.
[21] Duncan EJ, Szilagyi S, Efferen TR, Schwartz MP, Parwani A, Chakravorty S, et al. Effect of treatment status on prepulse inhibition of acoustic startle in schizophrenia. Psychopharmacology 2003;167:63–71.
[22] El-Ghundi M, Fletcher PJ, Drago J, Sibley DR, O'Dowd BF, George SR. Spatial learning deficit in dopamine D(1) receptor knockout mice. European Journal of Pharmacology 1999;383:95–106.

[23] Fendt M, Koch M, Schnitzler HU. Amygdaloid noradrenaline is involved in the sensitization of the acoustic startle response in rats. Pharmacology Biochemistry and Behavior 1994;48:307–14.
[24] Filion D, Kelly KA, Hazlett EA. Behavioral analogies of short lead interval startle habituation. In: Dawson ME, Schell AM, Bohmelt A, editors. Startle modification: implications for neuroscience, cognitive science, and clinical science. Cambridge, UK: Cambridge University Press; 1999. p. 270–83.
[25] Geyer MA. The family of sensorimotor gating disorders: comorbidities or diagnostic overlaps? Neurotoxicity Research 2006;10:211–20.
[26] Geyer MA, Braff DL. Startle habituation and sensorimotor gating in schizophrenia and related animal models. Schizophrenia Bulletin 1987;13: 643–68.
[27] Geyer MA, Dulawa SC. Assessment of murine startle reactivity, prepulse inhibition, and habituation. Current Protocols in Neuroscience 2003;(Suppl. 24):8.17.1–15.
[28] Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology 2001;156:117–54.
[29] Geyer MA. Are cross-species measures of sensorimotor gating useful for the discovery of procognitive cotreatments for schizophrenia? Dialogues in Clinical Neuroscience 2006;8:9–16.
[30] Geyer MA. Developing translational animal models for symptoms of schizophrenia or bipolar mania. Neurotoxicity Research 2008;14:71–8.
[31] Giakoumaki SG, Bitsios P, Frangou S. The level of prepulse inhibition in healthy individuals may index cortical modulation of early information processing. Brain Research 2006;1078:168–70.
[32] Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams GV. Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. Psychopharmacology 2004;174:3–16.
[33] González-Blanch C, Perez-Iglesias R, Pardo-García G, Rodríguez-Sánchez JM, Martínez-García O, Vázquez-Barquero JL, et al. Prognostic value of cognitive functioning for global functional recovery in first-episode schizophrenia. Psychological Medicine 2010;40:935–44.
[34] Gould TJ, Rukstalis M, Lewis MC. Atomoxetine and nicotine enhance prepulse inhibition of acoustic startle in C57BL/6 mice. Neuroscience Letters 2005;377:85–90.
[35] Graham FK. Presidential Address, 1974. The more or less startling effects of weak prestimulation. Psychophysiology 1975;12:238–48.
[36] Granado N, Ortiz O, Suárez LM, Martín ED, Ceña V, Solís JM, et al. D1 but not D5 dopamine receptors are critical for LTP, spatial learning, and LTP-Induced arc and zif268 expression in the hippocampus. Cerebral Cortex 2008;18:1–12.
[37] Gray JA, Feldon J, Rawlins JNP, Hemsley DR, Smith AD. The neuropsychology of schizophrenia. Behavioral and Brain Sciences 1991;14:1–81.
[38] Groves PM, Thompson RF. Habituation: a dual-process theory. Psychological Review 1970;77:419–50.
[39] Hagan JJ, Jones DN. Predicting drug efficacy for cognitive deficits in schizophrenia. Schizophrenia Bulletin 2005;31:830–53.
[40] Halberstadt AL, Geyer MA. Habituation and sensitization of acoustic startle: opposite influences of dopamine D1 and D2-family receptors. Neurobiology of Learning and Memory 2009;92:243–8.
[41] Hitchcock JM, Sananes CB, Davis M. Sensitization of the startle reflex by footshock: blockade by lesions of the central nucleus of the amygdala or its efferent pathway to the brainstem. Behavioral Neuroscience 1989;103: 509–18.
[42] Holstein DH, Csomor PA, Geyer MA, Huber T, Brugger N, Studerus E, et al. The effects of sertindole on sensory gating, sensorimotor gating, and cognition in healthy volunteers. Journal of Psychopharmacology 2011;25: 1600–13.
[43] Ishii D, Matsuzawa D, Fujita Y, Sutoh C, Ohtsuka H, Matsuda S, et al. Enhancement of acoustic prepulse inhibition by contextual fear conditioning in mice is maintained even after contextual fear extinction. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2010;34:183–8.
[44] Kapur S. Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. American Journal of Psychiatry 2003;160:13–23.
[45] Klamer D, Pålsson E, Revesz A, Engel JA, Svensson L. Habituation of acoustic startle is disrupted by psychotomimetic drugs: differential dependence on dopaminergic and nitric oxide modulatory mechanisms. Psychopharmacology 2004;176:440–50.
[46] Koch M. The neurobiology of startle. Progress in Neurobiology 1999;59: 107–28.
[47] Light GA, Braff DL, Sprock J, Cadenhead K, Swerdlow NR. Prepulse inhibition of startle is related to higher order cognition in women. Society for Neuroscience – Abstracts 2007, 806.17.
[48] Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM, et al. Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. Journal of Neuroscience 1997;17:5196–205.
[49] Ludewig K, Geyer MA, Vollenweider FX. Deficits in prepulse inhibition and habituation in never-medicated, first-episode schizophrenia. Biological Psychiatry 2003;54:121–8.
[50] Ludewig K, Ludewig S, Seitz A, Obrist M, Geyer MA, Vollenweider FX. The acoustic startle reflex and its modulation: effects of age and gender in humans. Biological Psychology 2003;63:311–23.
[51] Lyon L, Burnet PW, Kew JN, Corti C, Rawlins JN, Lane T, et al. Fractionation of spatial memory in GRM2/3 (mGlu2/mGlu3) double knockout mice reveals a role for group II metabotropic glutamate receptors at the interface between arousal and cognition. Neuropsychopharmacology 2011;36: 2616–28.
[52] McDonald B. A teaching note on Cook's distance – a guideline. Research Letters in the Information and Mathematical Sciences 2002;3:127–8.
[53] Ortiz O, Delgado-García JM, Espadas I, Bahí A, Trullas R, Dreyer JL, et al. Associative learning and CA3-CA1 synaptic plasticity are impaired in D1R null, Drd1a−/− mice and in hippocampal siRNA silenced Drd1a mice. Journal of Neuroscience 2010;30:12288–300.
[54] Oswald CJ, Yee BK, Rawlins JN, Bannerman DB, Good M, Honey RC. The influence of selective lesions to components of the hippocampal system on the orienting response, habituation and latent inhibition. European Journal of Neuroscience 2002;15:1983–90.
[55] Parwani A, Duncan EJ, Bartlett E, Madonick SH, Efferen TR, Rajan R, et al. Impaired prepulse inhibition of acoustic startle in schizophrenia. Biological Psychiatry 2000;47:662–9.
[56] Passino E, Middei S, Restivo L, Bertaina-Anglade V, Ammassari-Teule M. Genetic approach to variability of memory systems: analysis of place vs. response learning and fos-related expression in hippocampal and striatal areas of C57BL/6 and DBA/2 mice. Hippocampus 2002;12:63–75.
[57] Pietraszek M, Nagel J, Gravius A, Schäfer D, Danysz W. The role of group I metabotropic glutamate receptors in schizophrenia. Amino Acids 2007;32(February (2)):173–8.
[58] Pietropaolo S, Mintz M, Feldon J, Yee BK. The behavioral sequela following the prevention of home-cage grid-climbing activity in C57BL/6 mice. Behavioral Neuroscience 2007;121:345–55.
[59] Restivo L, Passino E, Middei S, Ammassari-Teule M. The strain-specific involvement of nucleus accumbens in latent inhibition might depend on differences in processing configural- and cue-based information between C57BL/6 and DBA mice. Brain Research Bulletin 2002;57:35–9.
[60] Richmond MA, Murphy CA, Pouzet B, Schmid P, Rawlins JN, Feldon J. A computer controlled analysis of freezing behaviour. Journal of Neuroscience Methods 1998;86:91–9.
[61] Roberts BM, Seymour PA, Schmidt CJ, Williams GV, Castner SA. Amelioration of ketamine-induced working memory deficits by dopamine D1 receptor agonists. Psychopharmacology 2010;210:407–18.
[62] Sanderson DJ, Bannerman DM. The role of habituation in hippocampus-dependent spatial working memory tasks: evidence from GluA1 AMPA receptor subunit knockout mice. Hippocampus 2012;22:981–94.
[63] Scholes KE, Martin-Iverson MT. Disturbed prepulse inhibition in patients with schizophrenia is consequential to dysfunction of selective attention. Psychophysiology 2010;47:223–35.
[64] Scholes KE, Martin-Iverson MT. Relationships between prepulse inhibition and cognition are mediated by attentional processes. Behavioural Brain Research 2009;205:456–67.
[65] Singer P, Boison D, Mohler H, Feldon J, Yee BK. Deletion of glycine transporter 1 (GlyT1) in forebrain neurons facilitates reversal learning: enhanced cognitive adaptability? Behavioral Neuroscience 2009;123:1012–27.
[66] Singer P, Feldon J, Yee BK. Are DBA/2 mice associated with schizophrenia-like endophenotypes? A behavioural contrast with C57BL/6 mice. Psychopharmacology 2009;206:677–98.
[67] Swerdlow NR, Koob GF. Dopamine, schizophrenia, mania, and depression: toward a unified hypothesis of cortico-striatopallido-thalamic function. Behavioral and Brain Sciences 1987;10:197–208.
[68] Swerdlow NR, Filion D, Geyer MA, Braff DL. Normal personality correlates of sensorimotor, cognitive, and visuospatial gating. Biological Psychiatry 1995;37:286–99.
[69] Swerdlow NR, Platten A, Shoemaker J, Pitcher L, Auerbach P. Effects of pergolide on sensorimotor gating of the startle reflex in rats. Psychopharmacology 2001;158:230–40.
[70] Turetsky BI, Calkins ME, Light GA, Olincy A, Radant AD, Swerdlow NR. Neurophysiological endophenotypes of schizophrenia: the viability of selected candidate measures. Schizophrenia Bulletin 2007;33:69–94.
[71] Valsamis B, Schmid S. Habituation and prepulse inhibition of acoustic startle in rodents. Journal of Visualized Experiments 2011;55:3446.
[72] Weber M, Schnitzler HU, Schmid S. Synaptic plasticity in the acoustic startle pathway: the neuronal basis for short-term habituation? European Journal of Neuroscience 2002;16:1325–32.
[73] Wiedholz LM, Owens WA, Horton RE, Feyder M, Karlsson RM, Hefner K, et al. Mice lacking the AMPA GluR1 receptor exhibit striatal hyperdopaminergia and 'schizophrenia-related' behaviors. Molecular Psychiatry 2008;13: 631–40.
[74] Wright JW, Murphy ES, Elijah IE, Holtfreter KL, Davis CJ, Olson ML, et al. Influence of hippocampectomy on habituation, exploratory behavior, and spatial memory in rats. Brain Research 2004;1023:1–14.
[75] Xing B, Guo J, Meng X, Wei SG, Li SB. The dopamine D1 but not D3 receptor plays a fundamental role in spatial working memory and BDNF expression in prefrontal cortex of mice. Behavioural Brain Research 2012;235: 36–41.
[76] Yee BK, Chang DL, Feldon J. The effects of dizocilpine and phencyclidine on prepulse inhibition of the acoustic startle reflex and on prepulse-elicited reactivity in C57BL6 mice. Neuropsychopharmacology 2004;29:1865–77.
[77] Yee BK, Chang T, Pietropaolo S, Feldon J. The expression of prepulse inhibition of the acoustic startle reflex as a function of three pulse stimulus intensities, three prepulse stimulus intensities, and three levels of startle responsiveness in C57BL6/J mice. Behavioural Brain Research 2005;163:265–76.

[78] Youn J, Ellenbroek BA, van Eck I, Roubos S, Verhage M, Stiedl O. Finding the right motivation: genotype-dependent differences in effective reinforcements for spatial learning. Behavioural Brain Research 2012;226:397–403.
[79] Young JW, Finlayson K, Spratt C, Marston HM, Crawford N, Kelly JS, et al. Nicotine improves sustained attention in mice: evidence for involvement of the alpha7 nicotinic acetylcholine receptor. Neuropsychopharmacology 2004;29:891–900.
[80] Young JW, Powell SB, Risbrough V, Marston HM, Geyer MA. Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. Pharmacology & Therapeutics 2009;122:150–202.
[81] Yung AR, McGorry PD. The prodromal phase of first-episode psychosis: past and current conceptualizations. Schizophrenia Bulletin 1996;22: 353–70.