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Experience Increases the Prepulse Inhibition of the Acoustic Startle Response in Mice

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The authors have previously shown that inhibition of the acoustic startle response by a prepulse increases when it is repetitively elicited over days. The present experiments show in C3H and C57 mice that this change is caused by an increase in prepulse inhibition (PPI) and not by a decrease in prepulse facilitation. This PPI increase is only evoked if prepulses and startle stimuli are repeatedly given in a temporally paired ("contingent") order, proposing an associative learning process. (Only in C57 mice, PPI was additionally increased by adaptation in the same, but not in a different, context). As an underlying mechanism for this PPI increase by experience, the authors hypothesize Hebbian plasticity of an inhibitory synapse.

Keywords: startle, prepulse inhibition, prepulse facilitation, learning, context

Brief and relatively weak stimuli, called *prepulses*, presented before a startle-eliciting stimulus can modulate the magnitude of the elicited response (reviewed in Koch, 1999). Inhibition of the acoustic startle response (ASR), referred to as *prepulse inhibition* (PPI), is observed when the startle-eliciting stimulus is preceded by a weak, nonstartling sound prepulse for about 30–500 ms (e.g., Hoffman, & Ison, 1980; Plappert, Pilz, & Schnitzler, 2004). PPI reflects a filter mechanism that allows an individual to ignore irrelevant stimuli and ensures undisturbed processing of relevant stimuli (for review, see Koch, 1999). The neuronal circuit underlying acoustic PPI is well understood: It is thought that the prepulse activates an inhibitory loop, including the ascending auditory pathway, which, in turn, activates a cholinergic pathway from the pedunculopontine tegmental nucleus (PPTg) to the pontine reticular nucleus (PNC), which constitutes the sensorimotor interface in the startle pathway. This supposedly causes an inhibition of excitability in the PNC, leading to decreased processing of the succeeding startle stimulus (for review, see Fendt, Li, & Yeomans, 2001; Koch, 1999).

In contrast, prepulses with short interpulse intervals (IPIs) of 0–30 ms can facilitate the ASR. This is referred to as *prepulse facilitation* (PPF; e.g., Ison, Taylor, Bowen, & Schwarzkopf, 1997; Plappert et al., 2004). In contrast to PPI, there is only speculation about the neural mechanisms involved in PPF. It has been hypothesized that PPF is produced by an arousal effect of the prepulse, thus intensifying the reaction to the startle stimulus that follows this prepulse (Reijmers & Peeters, 1994). Our studies have led us to believe that the prepulse elicits both PPI and PPF and that both are cumulative in effect, thus determining the final prepulse effect that may be either inhibitory or facilitating (discussed in detail in Plappert et al., 2004).

There is ongoing debate on whether the amount of the inhibitory prepulse effect is influenced by experience, that is, whether it increases over several test days. In rats, several studies have indicated such an influence (Crofton, Dean, & Sheets, 1990; Dean, Sheets, Crofton, & Reiter, 1990; Mansbach & Geyer, 1991; Martin-Iversen, 1999; Reijmers & Peeters, 1994; Wu, Krueger, Ison, & Gerrard, 1984). However, researchers have found in other studies that the prepulse effect is already present with full amount at the first trial (Hoffman, Marsh, & Stein, 1969; Hoffman, & Wible, 1970; Ison, Hammond, & Krauter, 1973; Krauter, Leonard, & Ison, 1973; Russo, Reiter, & Ison, 1975; Schulz, Fendt, Pedersen, & Koch, 2001). Gewirtz and Davis (1995) found in rats and Quednow et al. (2005) found in humans even a decrease of the prepulse effect during repetitive elicitation. These contradictory findings may have been because of species differences and different experimental procedures and/or prepulse parameters used (i.e., stimulus modality, length, and intensity; discussed in Reijmers & Peeters, 1994).

In contrast to results with rats and humans, we found in several inbred strains of mice a strong and reliable increase of the inhibitory prepulse effect over several test days (Plappert et al., 2004). Reijmers and Peeters (1994) have speculated that the arousal effect of the prepulses (i.e., the PPF component) may decay as the mouse gains familiarity with the test sessions, resulting in an increase in the measured inhibitory prepulse effect. This would mean that experience does not increase PPI but rather decreases PPF. The purpose of Experiment 1 was to test this hypothesis.

Concerning the underlying mechanism of the increase in inhibition, Crofton et al. (1990) postulated an associative learning process as a major factor. They showed that repeated daily testing caused an increase in inhibition only when the preliminary stimulus and the startle stimuli were presented in a fixed temporal

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relationship (i.e., "contingently"). However, these results were shown for the gap inhibition paradigm, that is, using gaps in background noise as prepulses. Gap prepulses are regarded as weaker than the acoustic bursts used in the PPI paradigm (Crofton et al., 1990). In addition, the IPI at which the gaps were given was longer (170 ms) than that normally used in the prepulse inhibition paradigm (50–100 ms), possibly weakening the inhibitory impact of the gaps even further. It is also conceivable that the inhibitory effect of these weak gap prepulses develops more slowly than that of strong acoustic burst prepulses (discussed in Crofton et al., 1990) and that a different mechanism underlies this process. Therefore, in Experiment 2, we investigated whether an associative learning mechanism also occurs during repetitive presentation of "normal," nongap and relatively intense, prepulses. If so, then only repetitive associated presentation of prepulse and startle stimulus would produce an increase in the inhibitory prepulse effect. However, should presentation of either the prepulse or the startle stimulus alone suffice to evoke this effect, this would indicate a nonassociative mechanism. The results of Crofton et al. (1990) are unclear in this respect: In addition to an increasing effect of "contingent" stimulus presentation on gap inhibition, results showed a small increase after presentation of startle stimuli alone. This was not observed after presentation of startle stimuli and prepulses in a variable temporal relationship.

A potential effect of repetitive context exposition on the prepulse effect was examined in Experiment 3.

All three experiments were carried out with two different inbred strains of mice: C3H and C57BL/6J (C57). The C3H strain was used because it exhibits both strong PPF and PPI (Plappert, Rodenbücher, & Pilz, 2005), whereas PPI is strong and PPF is very weak in the C57 strain (Plappert et al., 2004, 2005).

Experiment 1: Effect of Repetitive Elicitation of PPI or PPF Over Consecutive Test Days

In Experiment 1, we investigated whether an increase in the inhibitory prepulse effect is in reality not an increase in PPI but a decrease in PPF. We elicited either strong PPF or PPI in C3H or in C57 mice by using in each case a specific prepulse condition in which the respective modulation has been shown to be pronounced (in past experiments): Strong PPF was evoked by presenting tone prepulses at a short IPI of 12.5 ms and strong PPI by presenting noise prepulses at a long IPI of 50 ms. PPI or PPF were elicited repeatedly over 8 test days, and shifts in the two during this period were recorded.

Method

Subjects. Fifty naive female C3H/HeN (C3H) mice and 39 naive female C57/BL6J (C57) mice were obtained from Harlan Winkelmann, Borchen, Germany. All mice were 7–9 weeks old at the beginning of the experiments. Two weeks before testing, the mice were kept in groups of five and housed in cages containing nesting material under a 12:12-hr light–dark schedule (lights on at 6 a.m.), at 24 ± 1°C and humidity 60 ± 5% throughout the study. They received food and water ad lib. Testing took place during the light period.

Materials. The startle response was measured in a "ballistic chamber" situated inside a sound-attenuated chamber lit by a 9-W cold light bulb. The apparatus consisted of a platform on which the test cage was fastened. The test cage was a 5 × 9 × 5 cm wire mesh cage with a metal floor plate. The platform was mounted on piezo accelerometers. The output of the transducer was amplified and filtered from 2 to 500 Hz (University of Tübingen, Piezo-Amp-System, Tübingen, Germany). The resulting voltage was sampled by an analog-to-digital converter located within a computer (Microstar data acquisition processor DAP 1200, Washington, DC). Startle amplitude was calculated as the difference between peak-to-peak voltage during a time window of 50 ms after stimulus onset and peak-to-peak voltage in the 50-ms time window before stimulus onset.

Acoustic stimuli and steady white background noise were generated by a computer using a digital signal processor board (Medav, SigGen, Uttenreuth, Germany) and delivered through a loudspeaker (Visaton HTM 5.6, Haan, Germany) placed at a distance of 35 cm from the test cage inside the sound-attenuated chamber. The SPL within the cage was measured with a 0.5-in. (1.3-cm) condenser microphone (Brüel and Kjaer, audio equipment; Model 4113, Naerum, Denmark) with a measuring amplifier (Brüel and Kjaer, Model 2606). The level of the acoustic stimuli in dB was SPL PEAK, the level of the background noise in dB was SPL root mean square (RMS) relative to 0.02 mPa.

The experiment was performed in one of four identical chambers, each with identical setups. Each mouse was tested daily at about the same time in the same chamber. The tests were started simultaneously in all four chambers. During the experiment, the mice were observed via a video camera positioned inside the startle chamber.

Procedure. To avoid stressing the mice by touching them, the test cage was placed within the home cage and left there until the mice entered the test cage voluntarily. Test cages were closed when all 4 mice had entered then brought to the test chamber and attached to the measuring platform. There the mice were given 5 min to adapt to the experimental environment inside the sound-attenuated chamber. During this time, the background noise was kept constant. The mice were then put back into the home cage without touching them. This adaptation procedure was performed on 3 consecutive days prior to the first experiment and once at the start of each day of the experiment. The experiment was begun at least 30 min after this adaptation procedure, with an additional adaptation time of 5 min at a steady level of background noise (50 dB SPL RMS).

PPI and PPF were tested over 8 consecutive days: PPI was tested in 26 C3H and in 29 C57 mice, and PPF was tested in 24 C3H and in 10 C57 mice. The startle stimuli were noise pulses (20-ms duration, 0-ms rise-decay times, 105 dB SPL). The prepulses used to elicit PPI were noise pulses (65 dB SPL, 10-ms duration, including 0.4-ms rise-decay times) presented at an IPI of 50 ms between prepulse onset and startle stimulus onset. PPF was elicited with 14 kHz prepulses (65 dB SPL, 10-ms duration, including 0.4-ms rise-decay times) presented at an IPI of 12.5 ms. To measure PPI or PPF, 20 startle stimuli were presented during a habituation phase, and 40 startle stimuli alone or 40 startle stimuli preceded by a prepulse in a test phase in a balanced order with a constant intertrial interval (ITI) of 15 s. Possible responses to prepulses were calculated as the difference between peak-to-peak voltage during the 50-ms time window before and 50 ms after prepulse onset. Prepulses elicited no ASR, and the means measured were always within one standard deviation of 0 mV.

Statistics. The mean startle amplitude without prepulses (S) was calculated for each mouse; ASRs to the 20 initial habituating startle stimuli were not included in this value. Moreover, the mean ASR amplitude with prepulses (PS) was calculated for each prepulse condition and each mouse. The prepulse effect was measured for each mouse as the percentage of ASR change [100 × (PS − S)/S] so that negative values were recorded for the PPI and positive values for the PPF.

The influences of time (in terms of test days), strain (C3H or C57), prepulse condition (short or long IPI), and interactions on PPI or on ASR amplitude, respectively, were calculated with a general linear model (GLM) using individual mice as repeated measures, time as a continuous factor, and strain and prepulse conditions as factors. The differences between the ASR on Days 1 and 5 and the mean ASR difference between Days 1–4 and Days 5–8 were estimated by a dependent *t* test. To show the trend of PPI over the test days, an exponential model function, $f(x) = y_0 + a \times (1 - e^{(-t \times x)})$, was fitted to the data, and r^2 was determined.

The ASR to the first startle stimulus with prepulse was taken as a value PS1 in order to estimate the effect of the first prepulse for each mouse. The mean ASR to two startle stimuli without prepulses preceding and following PS1 was taken as S1. The percentage of ASR change was calculated for each mouse as above [100 × (PS1 − S1)/S1]. Because of the low (S1) or nonexistent (PS1) averaging of this method, there was a high variance of the resultant values. For this reason, we report medians, interquartile ranges, and one-sample Wilcoxon's tests for the effects of the first prepulse.

Results

In the C3H strain, 14 kHz prepulses presented at a short IPI of 12.5 ms produced PPF (mean ASR change over days: 90%; see Figure 1a1), whereas noise prepulses at a long IPI of 50 ms produced PPI (mean ASR change over days: −47%; see Figure 1a2, $t(48) = 14.8$, $p < .0001$). The amount of PPF did not change over the test days ($F(1, 237) < 1$). Consequently, there was no difference in PPF between Days 1 and 5 (dependent $t(23) = 1.66$, $p = .11$). In contrast to PPF, PPI increased over days, ($F(1, 202) = 68.15$, $p < .0001$), and reached a nearly constant level on Day 5. There was no further change from Day 5 to Day 8 ($F(1, 86) = 1.85$, $p = .18$). PPI differed highly significantly between Days 1 and 5 (dependent $t(28) = 6.47$, $p < .0001$).

In the C57 strain, both prepulse conditions produced PPI (see Figure 1b). Although the amount of the PPI increase over the test days was higher in the long-IPI condition (mean ASR change over days: −73%) than in the short-IPI condition (mean ASR change over days: −41%, $t(37) = 4.06$, $p = .0002$), PPI increased significantly over days in both prepulse conditions ($F(1, 69) \geq 5.81$, $p \leq .019$) and reached a nearly constant level on Day 5 in both conditions. There was no further change from Day 5 to Day 8 ($F(1, 29) \leq 1.85$, $p \geq .18$). PPI differed significantly between Days 1 and 5 in both conditions ($t(9) \geq 5.40$, $p \leq .0004$).

In both strains, the absolute ASR amplitudes for startle alone (C3H, Day 1: 107.3 ± 11.1 mV, Day 8: 54.2 ± 4.2 mV; C57, Day 1: 55.8 ± 6.8 mV, Day 8: 43.6 ± 4.8mV) and startle preceded by prepulses (C3H, Day 1: 80.7 ± 6.3 mV, Day 8: 22.9 ± 3.8 mV;

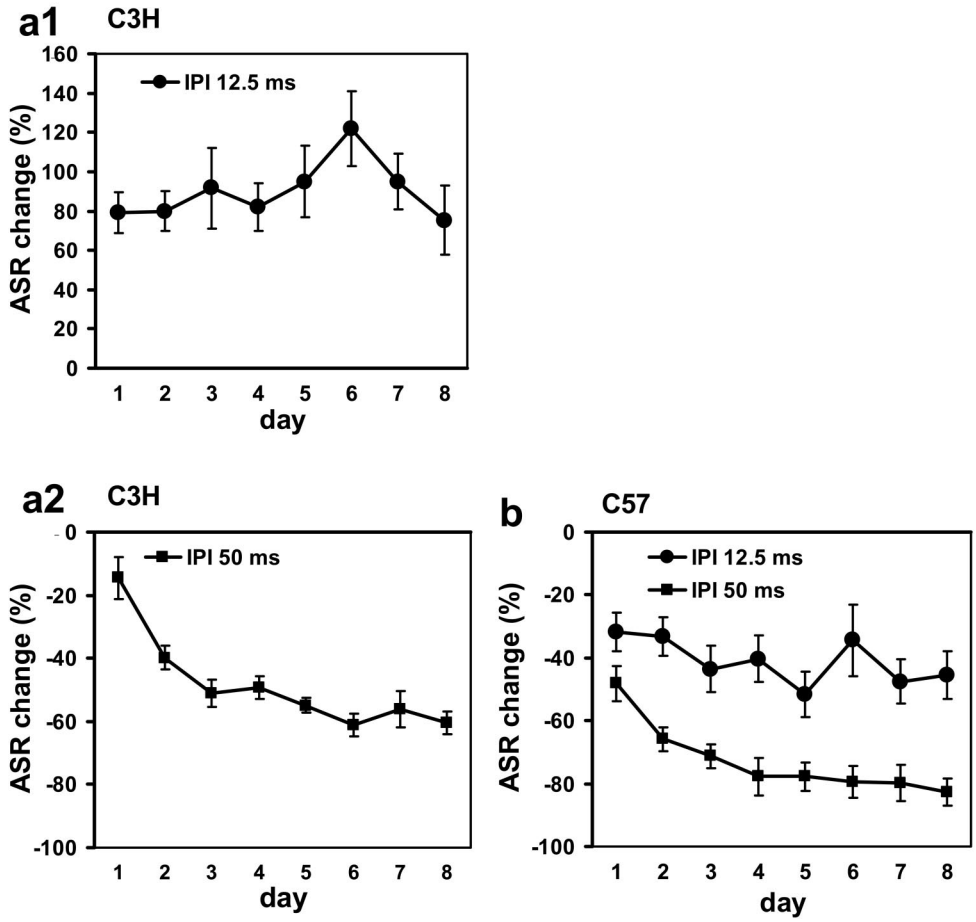

Figure 1. Percentage of acoustic startle response (ASR) change evoked by prepulses during 8 days of testing. a1: C3H strain: 14 kHz prepulses were presented at an interpulse interval (IPI) of 12.5 ms between prepulse and startle stimulus. Prepulses facilitated (i.e., increased) ASR; the facilitation did not change significantly over the period of days. Error bars represent the standard error of the mean ($n = 32$). a2: C3H strain: Noise prepulses presented at an IPI of 50 ms ($n = 26$). These prepulses inhibited ASR; the inhibition increased over days ($p < .0001$). b: C57 strain: 14 kHz (IPI = 12.5 ms) or noise (IPI = 50 ms) prepulses presented ($n = 29$ or 10). In both cases, prepulses inhibited ASR; inhibition increased over days ($p < .0001$).

C57, Day 1: 29.3 ± 4.3 mV, Day 8: 9.6 ± 1.9 mV) decreased over the test days (GLM: significant change over days, $F(1, 181) \geq 13.74$, $p \leq .0003$). This decrease was in both cases stronger in the prepulse-startle condition than in the startle-alone condition, leading to an increase in the amplitude difference over the test days (difference Day 8 − Day 1, C3H: 4.7 ± 9.3 mV, C57: 7.4 ± 4.5 mV). This increase over the days was only significant in the C57 strain (GLM: $F(1, 202) = 4.35$, $p = .038$) but not in the C3H strain (GLM: $F(1, 181) < 1$).

Comparing the strains, the mean PPI produced by noise prepulses at an IPI of 50 ms was lower in the C3H strain (−47%) than in the C57 strain (−73%; GLM: highly significant effect of strain), $F(1, 383) = 165.7$, $p < .001$. PPI increased similarly over the test days in both mouse strains (GLM: highly significant change over days), $F(1, 383) = 165.7$, $p < .0001$; there was no interaction between strain and day, indicating that the trends of PPI over the test days did not differ between the two strains, $F(1, 383) = 2.22$, $p = .14$. An exponential model function described the PPI trends very well in both strains (C3H, time constant: 1.28 days, $r^2 = .98$; C57, time constant: 1.49 days, $r^2 = .99$).

In the C3H strain, the effect of the very first prepulse on Day 1 with long IPI was not significantly different from zero (*Mdn*: 7.7%, interquartile range: −172.1%–+62.6%; one-sample Wilcoxon's test: $p = .38$). In the C57 strain, the very first prepulse with long IPI already elicited a significant PPI (*Mdn*: 47.6%, interquartile range: 4.6%–92.7%; one-sample Wilcoxon's test: $p = .006$).

Discussion

Prepulses with short (12.5 ms) IPI elicited PPF in the C3H strain but not in the C57 strain. Prepulses with long (50 ms) IPI elicited PPI in both strains but more weakly in C3H mice than in C57 mice. These results indicate that the PPF component of the prepulse is much stronger in C3H mice than in C57 mice. There are strong indications in the literature that a prepulse elicits both PPF and PPI independently of one another and that both together determine the extent of the resulting ASR change (discussed in Plappert et al., 2004). The strong PPF component in the C3H strain was apparent when prepulses with short IPI were used (compared with no PPF in the C57 strain) and in the comparatively weaker PPI when prepulses with long IPI were used. A strong PPF component in the C3H strain would also explain why the effect of the very first prepulse with long IPI was not significantly different from zero in this strain. In contrast, in the C57 strain, in which the PPF component is weak or nonexistent, the first prepulse with long IPI evoked a significant PPI. This shows that PPI can already occur at the first trial in mice, as also shown, for example, by Ison et al. (1973) for rats and by Graham and Hackley (1991) for humans.

We observed a strong increase in PPI in both the C3H and C57 strains over the period of test days. This supports the conclusion reached by several authors (Crofton et al., 1990; Dean et al., 1990; Mansbach & Geyer, 1991; Martin-Iversen, 1999; Plappert et al., 2004; Reijmers & Peeters, 1994; Wu et al., 1984) that PPI increases during repetitive elicitation.

Our results clearly indicate that the increase in the inhibitory prepulse effect over the period of test days is caused by an increase in PPI and not by a decrease in PPF. In the C3H strain, PPI elicited by prepulses during a long IPI of 50 ms before the startle stimulus increased during the period of test days. In contrast, PPF elicited by prepulses during a short IPI of 12.5 ms remained nearly constant over the period of days in this strain. (The increase, if any, was small.) Because PPF remained constant over the period of days, it is very unlikely that a PPF change contributed to the observed PPI change. No PPF was elicited in the C57 strain by the short-IPI condition, indicating that PPF is low in this strain (see also Plappert et al., 2004). The finding that PPI strongly increased in this strain despite the absence of PPF is additional corroboration for the thesis that the PPI increase does not depend on a PPF change.

Our data, therefore, tend to refute the hypothesis of Reijmers and Peeters (1994) that the increase of the measured inhibitory prepulse effect is caused by a decrease in the arousal effect of the prepulse, that is, a decrease in the PPF component as the mouse becomes accustomed to the test sessions.

It has already been shown that absolute startle amplitudes are not very important concerning the PPI effect (Ison, Bowen, Pak, & Guiterrez, 1997; Plappert et al., 2004). This was also the case in the present study. There was no indication that the course of absolute startle amplitudes over days artificially induced the percentage of PPI increase observed because even the absolute difference between the amplitude to startle stimuli alone and to startle stimuli preceded by prepulses increased in both strains over days.

Crofton et al. (1990) offered the hypothesis that an increase in PPI is evoked only by associated presentation of prepulse and startle stimuli. The purpose of Experiment 2 was to further explore this possibility.

Experiment 2: Effect of Different Pretreatments on PPI and PPF

In Experiment 2, we tested whether the presentation of prepulse and startle stimulus in a fixed time interval is a necessary condition for the increase in PPI during repetitive elicitation. This would indicate an associative learning process. Alternatively, the presentation of each stimulus alone or the two stimuli in a variable time interval could be sufficient to produce a PPI increase. C3H or C57 mice were pretreated during Days 1–4 in one of four different ways: (a) presentation of the test context without stimulation, (b) startle stimulus alone, (c) prepulse alone, and (d) prepulse and startle stimulus together in a variable temporal relationship, that is, noncontingently. In the noncontingent case, we chose IPIs between prepulse and startle stimulus, which were at least 3 s, thus probably preventing any activation of the prepulse circuit during startle stimulation. On Days 5–8, the prepulse effect was tested in all groups as in Experiment 1 (contingent presentation of prepulse and startle stimulus; see Experiment 1).

Method

Subjects. One hundred nineteen naïve female C3H mice and 61 naïve female C57 mice were obtained from Harlan Winkelmann, Borchen, Germany. The mice were of the same age and were housed as described in Experiment 1. Mice from one consignment of the supplier were statistically assigned to Experiment 1 or to Experiment 2.

Materials. The apparatus was the same as in Experiment 1.

Procedure. The adaptation procedure and the stimulus parameters were the same as in Experiment 1.

PPI and PPF were tested as in Experiment 1 on Days 5–8 in both strains following 4 days in which the mice underwent one of four different pretreatments:

1. The procedure used for the noncontingent (NC) test group (C3H PPI: $n = 12$; C3H PPF, $n = 15$; C57 PPI: $n = 14$) was the same as for PPI or PPF measurement in Experiment 1 (see the *Method* section in Experiment 1) except that the startle stimuli and the prepulses were presented in an unpaired manner. The IPIs were 3, 6, 9, or 12 s. Each IPI condition was presented 10 times (i.e., a total of 40 trials) in a pseudorandomized order.

2. In the startle stimulus-alone (SS) group (C3H PPI: $n = 12$, C3H PPF: $n = 15$, C57 PPI: $n = 17$), 100 startle stimuli alone were given at an ITI of 15 s (as in normal PPI or PPF measurement), without prepulses.

3. In the prepulse-alone (PP) group (C3H PPI: $n = 15$, C3H PPF, $n = 14$, C57 PPI: $n = 16$), 40 prepulses alone were given after an additional adaptation time of 5 min at an ITI of 30 s to ensure the same number of prepulses and the same total duration of the experiment like in the normal PPI and PPF measurement.

4. In the test-context adaptation (TC) group (C3H PPI: $n = 23$, C3H PPF: $n = 13$, C57 PPI: $n = 14$), the mice were exposed for only 30 min to the test context without stimulation (as in normal PPI and PPF measurement).

The measurements were performed alternatively with those of the control group in Experiment 1.

Statistics. The effects of time in terms of test days, of group (test group or control group), and of interactions were calculated with GLM, using time as continuous factor, group as nominal factor, and individual mice as the repeated measures factor. In one case, a post hoc Tukey's honestly significant difference (HSD) test was calculated to compare means of different groups.

Results

Figure 2 shows the PPI trends over Days 1–8 for the control group without pretreatment (data from Experiment 1) and Days 5–8 for the four test groups with different pretreatments on Days 1–4 for the C3H and the C57 strains, respectively.

C3H strain, PPI. PPI in the C3H strain (see Figure 2a) increased during the testing days (GLM: highly significant change of PPI over days, $F(1, 182) = 34.4$, $p < .0001$), with no difference among the test groups (GLM: $F(3, 182) < 1$). There was no significant interaction between the test group and day, indicating that PPI trends over the days did not differ among the test groups, ($F(3, 182) = 2.20$, $p = .090$). The PPI curves of the test groups were therefore averaged and compared with those of the control group (i.e., the group without pretreatment). Pretreatments in the test groups had no significant effect on PPI: The mean trend of PPI of the test groups did not differ from the trend of PPI of the control group on Days 1–4 (GLM: no effect of group, $F(1, 282) = 2.65$, $p = .10$; significant change over days, $F(1, 282) = 61.0$, $p < .0001$; but no significant Day × Group interaction, $F(1, 282) = 2.18$, $p = .14$). Thus, the increase in PPI during Days 1–4 was produced only by contingent stimuli presentation in the control group and not by the pretreatments in the test groups. On Days 5–8, PPI was significantly higher in the control group than in the test groups (GLM: $F(1, 262) = 82.9$, $p < .0001$), and the trends of PPI over the test days differed between control group and test groups (GLM: highly significant change over days, $F(1, 262) = 14.84$, $p < .0001$); there was a highly significant Day × Group interaction ($F(1, 262) = 8.22$, $p = .0001$).

C3H strain, PPF. In contrast to PPI, PPF in the C3H strain (data not shown) did not change over the period of days in all test groups (GLM: no difference between the test groups, $F(3, 167) = 2.22$, $p = .088$); there was no change of PPF over days, ($F(1, 167) < 1$), and no Test Group × Day interaction, indicating that the trends of PPI over the period of days did not differ among the test groups ($F(3, 167) < 1$). Because there was also no change in PPF over the period of days in the control group (see Experiment 1), the trends of PPF did not differ between the test groups and the control group on Days 1–4: in spite of a group effect (GLM: $F(1, 241) = 10.97$, $p = .001$); there was no effect of day ($F(1, 241) < 1$) and no Day × Group interaction ($F(1, 241) < 1$). There was also no difference in the trends of PPF when Days 5–8 were compared between both groups (GLM: no group effect, $F(1, 241) = 2.52$, $p = .11$); there was no change over days ($F(1, 241) < 1$) and no Day × Group interaction ($F(1, 241) < 1$). These data indicate that PPF is a stable and invariable phenomenon that remained unchanged by experience with any stimulus constellation tested in this study.

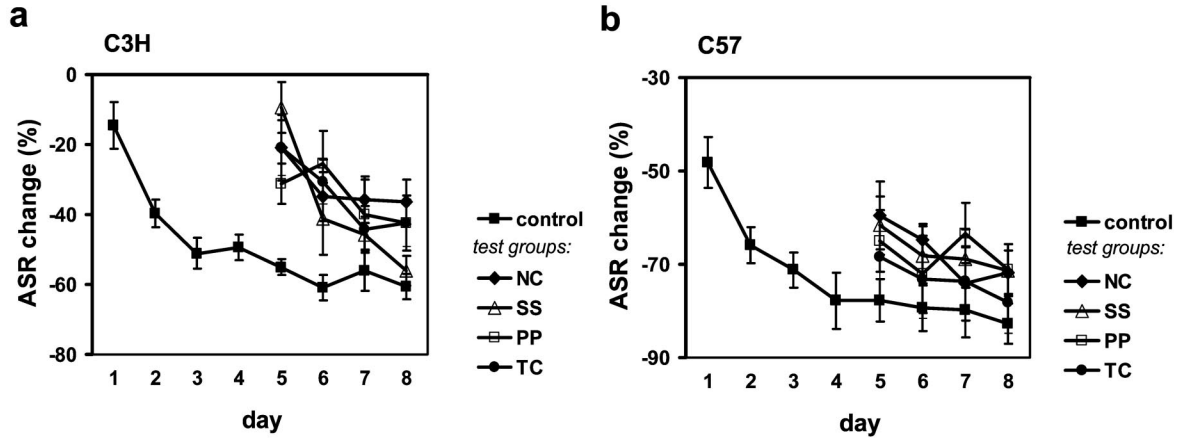

Figure 2. Percentage of acoustic startle response (ASR) change evoked by prepulses presented 50 ms before the startle stimulus. a: C3H strain: Control— 8 days of testing (data from Experiment 1); test groups—(n = 12–23) testing during Days 5–8 after 4 days of one of four different pretreatments. b: C57 strain: Control (data from Experiment 1) and test groups (n = 14–17) treated as in Figure 2a. Errors bars represent the standard error of the mean. NC = noncontingent presentation of prepulse and startle stimulus; SS = startle stimulus-alone presentation; PP = prepulse-alone presentation; TC = adaptation to the test context.

C57 strain, PPI. In the C57 strain, there was also no significant difference in PPI between the four test groups (see Figure 2b; GLM: no significant difference between the test groups ($F(3, 179) = 2.49$, $p = .061$). PPI tended to be higher in the TC group (in which the mice were presented only with the test context without stimulation) than in the other test groups, but this was not significant (Tukey's HSD: $p > .05$, for all comparisons). As in the C3H strain, PPI also increased over days (see Figure 2b; highly significant change of PPI over days ($F(1, 179) = 13.21$, $p = .0004$), and there was no significant Test Group × Day interaction ($F(3, 179) < 1$). The PPI curves of the test groups were therefore averaged. In contrast to the C3H strain, however, the pretreatments in the test groups caused a borderline increase in PPI: PPI was different between the test groups on Days 5–8 and the control group on Days 1–4 (GLM: borderline significant effect of group, $F(1, 268) = 3.69$, $p = .056$). Furthermore, the mean PPI trend of the test groups showed a lower slope than that of the control group on Days 1–4 (GLM: highly significant PPI change over days ($F(1, 268) = 67.9$, $p < .0001$) and a highly significant interaction between groups and days, indicating that the PPI trends over the days differed among the groups ($F(1, 268) = 19.85$, $p < .0001$). This flattened course of PPI of the test groups on Days 5–8 resembled the course of the control group on Days 5–8. Indeed, there was no difference in the mean trend of PPI of the test groups compared with that of the control group on Days 5–8 (GLM: highly significant change over days, $F(1, 268) = 9.92$, $p = .0018$), but there was no significant interaction between groups and day ($F(1, 268) < 1$). However, the PPI increase caused by pretreatments in the test groups was smaller than the increase caused by contingent stimulus presentation in the control group during Days 5–8: There was still a highly significant difference between the test groups and the control group when compared on Days 5–8 (GLM: $F(1, 268) = 50.1$, $p < .0001$).

Discussion

Numerous studies in the literature in rats and humans have shown that previous experience with the prepulse alone has no effect on the PPI (Crofton et al., 1990; Hoffman et al., 1969; Ison et al., 1973; Russo et al., 1975; Schell, Wynn, Dawson, Sinaii, & Niebala, 2000; Wu et al., 1984). This is in accordance with our findings. Likewise, noncontingent presentation of the prepulse and the startle stimulus (Crofton et al., 1990) and adaptation (Hoffman et al., 1969) had no influence on PPI.

However, all kinds of effects have been reported concerning previous experience with the startle stimulus per se: no effect with brief electric shocks (Russo et al., 1975), small effect when using gaps in background noise as prepulses (Crofton et al., 1990), and a strong effect when weak visual prepulses were used (Ison et al., 1973). In our experiment with relatively strong auditory prepulses, none of these factors had an effect on PPI in C3H mice. Only the contingent presentation of prepulse and startle stimulus evoked an increase in PPI.

In C57 mice, contingent stimulus presentation was the main cause for the PPI increase. In contrast to the C3H strain, however, PPI was additionally increased by the pretreatments of the test groups. In other words, a further factor contributed to the increase in PPI in the C57 strain during repetitive testing. This can only have been a factor occurring in all different pretreatments because the amount of PPI increase induced by pretreatment was similar in all four test groups. One factor common to all four test groups was the exposition of the mice to the test context during the pretreatments. It is conceivable that attention directed toward the context decreases during repetitive context presentation, leading to an increasing salience of the prepulse relative to the background (discussed in Restivo, Passino, Middei, & Ammassari-Teule, 2002). (The tendency of the additional PPI increase to be highest in the adaptation group [TC], which experienced only the test context without any stimulation, may be an additional indication for this.) This increased salience of the prepulse would lead to an increased intensity of the prepulse, thereby evoking stronger PPI (Plappert et al., 2004). If this is true, then a contextual change between pretreatments and PPI testing would eliminate the increased effect of the pretreatments on PPI. This was tested in Experiment 3.

Experiment 3: Effect of Exposition to a Different Context on PPI in the C57 Strain

If repeated exposition to the same test context was responsible for the observed increase in PPI in the C57 strain test groups, then this increase would not appear after adaptation to a different context. A further test group (different-context adaptation; DC) was therefore adapted on Days 1–4 to a context different from the "normal" test context, and PPI was then tested in this different test context on Days 5–8 as in the other test groups.

Method

Subjects. Twelve naïve female C57 mice were obtained from Harlan Winkelmann, Borchen, Germany. All mice were of the same age and were housed as in Experiment 1. Mice from one consignment of the supplier were statistically assigned to Experiment 1 or to Experiment 3.

Apparatus. The apparatus for PPI testing was the same as in Experiment 1. For adaptation to a different context, an apparatus (Context 2) was used that differed from the normal test chamber (Context 1) in several aspects: The apparatus was located in a different test room. The adaptation cage was placed inside a sound-attenuated chamber of different geometry and with vertically striped walls. The cage was triangular in shape and consisted of 11 × 11 × 11 cm Plexiglas walls and a floor grid with steel bars placed 1 cm apart. The cage was 30 cm high and was open toward the top (where the loudspeaker was located). The apparatus was illuminated by a red 5-W cold light bulb and was scented with eucalyptus oil. The background noise was 35 dB SPL RMS low frequency noise in contrast to the 50 dB of white noise in Context 1.

Procedure. The adaptation procedure and the stimulus parameters were the same as in Experiment 1.

In the DC test group, the mice were adapted for 30 min like the TC test group in Experiment 2 but in a different context (see above) than that of the previous PPI tests. PPI was measured only on Days 5–8. The measurements were performed in alternation with the measurement of the control group in Experiment 1 and the TC group of Experiment 2.

Statistics. The same statistics were used as those in Experiment 2.

Results

Figure 3 shows the results of the DC test group. PPI increased over the period of days (GLM: $F(1, 35) = 20.0$, $p < .0001$). However, in contrast to all other test groups of the C57 strain (see Experiment 2), the PPI trend of the DC test group increased as steeply as in the control group on Days 1–4: The mean PPI trend for DC was the same as that of the control group on Days 1–4

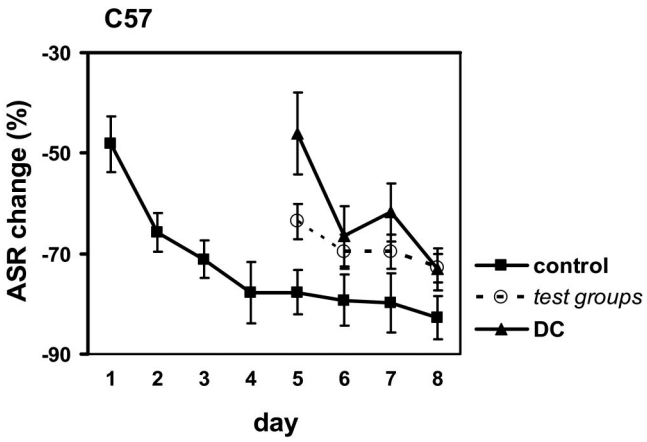

Figure 3. Percentage of acoustic startle response (ASR) change evoked by prepulses presented 50 ms before the startle stimulus in the C57 strain. Control (data from Experiment 1), mean of the test groups (data from Experiment 2), and a further test group (i.e., different context [DC] adaptation; $n = 12$), which was adapted to a different context on Days 1–4 and tested on Days 5–8. Error bars represent the standard error of the mean.

(GLM: no effect of group, $F(1, 121) = 2.07$, $p = .15$); there was a highly significant PPI change over days ($F(1, 121) = 50$, $p < .0001$) but no interaction between groups and day ($F(1, 121) < 1$). In addition, it differed from that of the control group on Days 5–8 (GLM: highly significant effect of group, $F(1, 121) = 59.4$, $p < .0001$, and change over days, $F(1, 121) = 19.4$, $p < .0001$), and there was a significant interaction between groups and day ($F(1, 121) = 8.52$, $p = .0042$). There was also a significant difference from the TC group of Experiment 2 in that the experimental procedure was identical, but the mice were adapted on Days 1–4 to the same context instead of to a different context ($F(1, 78) = 16.7$, $p = .0001$).

Discussion

In Experiment 2, PPI increased in all C57 groups after the mice were adapted to the test context on Days 1–4 regardless of whether the mice received stimuli in this context or not. This PPI increase was no longer observable if the context was changed after adaptation on Days 1–4. This clearly suggests that repetitive context presentation causes an increase in PPI in the C57 strain. This PPI increase is cumulative with the effect of contingent presentation of prepulse and startle stimuli (see Experiment 2). One possible reason why this was observed only in C57 is that this strain relies extensively on contextual information and is specifically able to form complex relationships between various environmental stimuli (Paylor, Tracy, Wehner, & Rudy, 1994; Thinus-Blanc, Save, Rossi-Arnaud, Tozzi, & Ammassari-Teule, 1996, cited in Restivo et al., 2002). Perhaps the C57 strain learns over a period of days to recognize the context as familiar, thus permitting the prepulse to "light up" against the contextual stimuli. This may lead to increased effectiveness of the prepulse in evoking PPI. However, despite this increased effect of context presentation on PPI in the C57 strain, the main effect of the PPI increase over a period of test days was produced in both strains by the repetitive contingent presentation of the prepulse and the startle stimulus.

General Discussion

In this study, we found a strong PPI increase in two strains of mice, C3H and C57, over a period of days. The increase was exponential with time constants of 1.3–1.5 days and occurred mainly in the first 4 days. This finding confirms similar outcomes of other authors (Crofton et al., 1990; Dean et al., 1990; Mansbach & Geyer, 1991; Martin-Iversen, 1999; Plappert et al., 2004; Reijmers & Peeters, 1994; Wu et al., 1984). PPI increases in C3H despite a constant PPF during the whole testing period and in C57 despite the absence of PPF in this strain. This strongly suggests that the PPI increase is not because of a PPF decrease. In addition, the discrepancy of the courses of facilitation and inhibition over days is a further hint that PPF and PPI are completely independent processes (Mansbach & Geyer, 1991; Plappert et al., 2004; Swerdlow, Shoemaker, Pitcher, Goins, & Platten, 2002).

The neuronal circuit underlying acoustic PPI includes the ascending auditory pathway, which, in turn, activates a cholinergic pathway from the PPTg to the PNC (which constitutes the sensorimotor interface in the startle pathway). This activation supposedly causes an inhibition of excitability in the PNC, leading to decreased processing of the succeeding startle stimulus (for review, see Fendt et al., 2001; Koch, 1999). One of our working hypotheses was that repetitive activation of this inhibitory loop may increase the inhibitory impact of prepulses by changing the efficacy of this circuit. However, mere stimulation of the PPI pathway does not explain all present results. If prepulses are given alone, or if they are given in a noncontingent manner with a leading interval probably long enough that inhibition disappeared when startle is elicited, then no PPI increase can be observed (in C3H mice, and in C57 mice when the context effect is neglected). Obviously, it is necessary for the full PPI increase that startle is elicited in the time window when PPI is (still) active. Because pairing of the two stimuli is a necessary prerequisite of the observed PPI increase by experience, an associative learning process seems to be necessary to explain why the PPI increase is only (in its full extent) observed after contingent presentation of prepulse and startle stimulus.

This means that the site mediating the PPI change needs information about both, prepulse and startle stimulus, as well as their temporal relationship. All this information is present in the PNC neurons (mediating startle) and their inhibitory synapses from the PPTg (mediating PPI). The simplest way to explain our results is a Hebbian mechanism at this site: The number or impact of cholinergic receptors on the PNC neurons are increased when prepulses activate the receptors, and, simultaneously, the neuron is activated by startle stimuli. Upregulation (or another genetic mechanism) would be in concert with our finding that the process has an observed time constant of greater than 1 day. Alternatives are much more complex mechanisms involving alterations in the PPI pathway prior to the PNC synapse or in the systems modulating PPI (which are reviewed in Koch, 1999) or alterations changing the impact of acoustic stimuli (discussed also in Crofton et al., 1990).

The finding that PPI changes by experience has implications for future research (accordingly, also discussed in Crofton et al., 1990). First, the above PPI procedure can be used not only to study sensorimotor processing, as is extensively done now (see, e.g., Swerdlow, Geyer, & Braff, 2001, for a review), but also to study the observed behavioral plasticity. Because the neural pathway of

the ASR and the PPI are well understood (see Fendt et al., 2001; Koch, 1999), the PPI increase may be a useful new model for studying the neural basis underlying this learning process. Second, a deficit in PPI is widely used in animal models to study the deficit in sensorimotor processing in schizophrenia patients (see, e.g., Swerdlow & Geyer, 1998, for a review). In future use of this animal model (at least in mice), care must be taken to separate the unlearned PPI component from learning effects.

In summary, the present study showed that the increase in the prepulse effect during a period of test trials is caused by an increase in PPI and not by a decrease in PPF. This increase in PPI is only observed if prepulses and startle stimuli are given in an associative manner. Future work will be required to investigate more clearly the nature of this assumed learning process. In C57 mice (but not in C3H mice), the repetitive presentation of the test context additionally produces a comparably small PPI increase. We conclude that repetitive context presentation leads to a reduction of attention to the contextual stimuli and thus to an increased salience of the prepulse (and therefore to an increased perceived intensity). The reason for the strain specificity of this context effect remains to be explored in further studies.

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