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Early Auditory Sensory Processing Deficits in Mouse Mutants with Reduced NMDA Receptor Function

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Cognitive deficits in schizophrenia include impairments at automatic, preattentive stages of sensory information processing. These deficits are evident in the prepulse inhibition- (PPI) and habituation of the auditory startle response paradigm, the paired tone paradigm in the EEG, and the peak recovery function of auditory evoked potentials (AEP). Administration of NMDA receptor antagonists reliably disrupts PPI and habituation of the startle, but not gating of AEPs in rodents. In the peak recovery paradigm, patients with schizophrenia and primates treated with NMDA receptor antagonists show reduced maximal response at long interstimulus intervals (ISI), but normal responses at short ISIs. Thus reduced NMDA receptor signalling may underlie alterations in these paradigms observed in schizophrenia. We tested the paradigms mentioned in mouse mutants with reduced expression of the NR1 subunit of the NMDA receptor ($N = 15$) and their wild-type littermates ($N = 16$). The NR1 mutant mice showed impaired habituation and PPI of the auditory startle response, as well as impaired gating in the paired tone paradigm. Deficits between the two gating measures did not correlate, corroborating previous evidence that these paradigms measure distinct processes. In the peak recovery paradigm, the NR1 mutants showed increased responses of the AEPs P1 and N1 at short ISIs but no difference between groups were observed at long ISIs. In conclusion, the NR1 hypomorphic mice modelled sensory and sensorimotor gating and startle habituation deficits observed in schizophrenia, but failed to model alterations in the peak recovery function.

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INTRODUCTION

Patients with schizophrenia show deficits in early sensory information processing in the visual as well as the auditory modality (Butler *et al,* 2005; Javitt *et al,* 2000b). These impairments can be measured in behavioral paradigms (eg tone matching tasks, habituation, and prepulse inhibition of the startle response (PPI)), or EEG paradigms (mismatch negativity, P300, paired tone paradigm, peak recovery paradigm). Much effort in recent translational and genetic psychiatric research has concentrated on using these measurements to characterize endophenotypes of schizophrenia, that is neurobiological markers of fundamental biological processes underlying the disease. Endophenotypes are more closely linked to neurobiological underpinnings of disease processes than clinical phenotypes that are mainly based on symptoms and are, therefore, assumed to be more amenable to genetic and translational research approaches (Gottesman and Gould, 2003).

Among the most widely investigated putative endophenotypes of schizophrenia are PPI and the habituation of the startle response, gating of auditory evoked potentials (AEPs), and peak recovery functions of AEPs. PPI reflects sensorimotor gating and refers to the reduction of an auditory startle response to a startling stimulus when the stimulus is preceded by a weak prepulse by 30–500 ms. Habituation describes the progressive decrease of a response to a repeatedly presented stimulus. It represents another measure of sensorimotor gating and is thought of as the simplest form of learning (Koch, 1999). In the paired tone paradigm, the first positive peak (P1, occurring at 20 ms) and the first negative peak (N1, at 40 ms) of the AEPs to the second tone are suppressed, and the extent of this suppression (S2/S1 ratio) is thought to reflect a measure of auditory gating. Gating mechanisms are thought to represent inhibitory mechanisms that restrict processing of a flood of sensory input (Light and Braff, 1999). The peak recovery function describes the increase of amplitudes of AEPs as a function of increasing interstimulus intervals (ISI). The mechanism underlying this increase in peak amplitude is thought to be a decay of the refractoriness with increasing ISI of the brain areas involved in the generation

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of the AEPs (Budd *et al,* 1998). Thus the recovery function describes aspects of auditory sensory memory (Lu *et al,* 1992).

The advantage of the described paradigms is suitability for a direct translational research approach. Furthermore, they have been widely used in studies in humans and in rodents, in particular PPI (Geyer *et al,* 2001; Light and Braff, 1999). Deficits in PPI and auditory gating are found in a variety of psychiatric diseases (Braff *et al,* 2001). Most notably, alterations in both paradigms have repeatedly been described in patients with schizophrenia and their first-degree relatives (Adler *et al,* 1982; Cadenhead *et al,* 2000; Siegel *et al,* 1984). Furthermore, patients with schizophrenia show deficits in the habituation of the auditory startle reflex (Braff *et al,* 2001; Meincke *et al,* 2004) and specific alterations in the AEP peak refractoriness paradigm (Shelley *et al,* 1999; Erwin *et al,* 1994).

Several lines of evidence indicate reduced NMDA receptor (NMDAR) signalling in schizophrenia (Moghaddam, 2003; Javitt and Zukin, 1991) and in particular in the pathophysiology of the observed startle habituation, gating, and AEP generation alterations in schizophrenia (Javitt *et al,* 2000a; Klamer *et al,* 2004; Geyer *et al,* 2001). To provide supporting evidence for this hypothesis we tested a mouse mutant with 90% reduced expression of the NR1 subunit of the NMDA receptor (subsequently named NR1 mutants in this paper) in these paradigms (Mohn *et al,* 1999). Previous studies have already shown PPI deficits in these mutants (Duncan *et al,* 2006; Fradley *et al,* 2005). The goal of our study was to test if the NR1 mutants would model deficits of schizophrenic patients in auditory gating, AEP generation, and habituation of the startle reflex.

MATERIALS AND METHODS

Animals

The study was approved by the ethics committee for animal research of the Kanton Zurich, Switzerland. The mouse mutants with 90% reduced expression of the NMDA receptor subunit 1 (NR1) were initially obtained from the University of North Carolina (Chapel Hill, NC, USA; Mohn *et al,* 1999). The heterozygous breeding animals were maintained in a pure C57Bl/6J− or 129S6/SvEv background. F1 hybrids were produced by pairing heterozygous mice from each background, the resulting homozygous (−/−) offsprings and the wild-type littermates (+/+) were used for the experiments. At the time of the testing the mice were an approximately 5–6 months old. In the gating paradigms 16 wild-type (eight females, eight males) and 15 mutant (seven females, eight males) mice were tested. A subset of the same animals was tested in the peak recovery paradigm (mutants: two females, nine males; wild types: six females, seven males).

Surgery and EEG Recordings

The surgery and recording procedure were described previously in more detail (Umbricht *et al,* 2005; Bickel *et al,* 2006). Shortly, EEG was recorded from four gold-plated screws with miniature connectors that were implanted above the frontal- (2.6 mm anterior/$\pm$1.5 mm lateral relative to bregma) and the auditory cortex (2.7 mm posterior/$\pm$3.5 mm lateral). Electrodes over the cerebellum (6 mm posterior/midline) and one between the auditory leads served as reference and ground respectively.

During recording sessions wires were connected via miniature plugs to the connectors mounted on the animal's head, allowing the animal free range of movement in the recording box. Thus, the EEG was recorded in awake and freely moving animals. EEG recordings were acquired with a Neuroscan Synamp system (Neuroscan Labs, TX, USA) (bandpass filter 1–100 Hz, 50-Hz notch filter, sampling rate 1 kHz).

Auditory stimuli were generated with a RP2 system, amplified with PA5 amplifiers, and delivered through two electrostatic loudspeakers (all Tucker-Davis Technologies, Alachua, FL). The speakers were mounted at an approximate height of 5 cm at the short sides of the recording box.

The same mice were tested twice in the PPI paradigm and once in the paired tone paradigm and peak recovery paradigm. Starting 1 week after the first PPI session and during subsequent 7 weeks the mice underwent surgery and electrophysiological recording, followed by the second PPI session.

Auditory Gating Paradigm

Two hundred pairs of auditory stimuli were presented with ISI of 500 ms and a random inter-pair interval of 9–12 s. The stimuli consisted of 10 ms spectrally rich tones, with frequencies ranging from 2 to 16 kHz in 0.5 kHz steps, and a rise/fall-time of 2 ms. The tones were of an approximate intensity of 85 dB and the continuously delivered background white noise had an intensity of 65 dB.

After the animals were placed in the recording box, they were allowed to acclimate for 2 min. In the subsequent 2 min, the mice were exposed to the background noise alone, before the paired tone paradigm started.

Peak Recovery Paradigm

Eight trains of 100 stimuli with different ISIs (0.1, 0.25, 0.55, 0.85, 1.15, 1.5, 2, 4 s) were pseudo-randomly presented four times each. The stimuli consisted of similar frequencies as in the paired-click paradigm and had a stimulus duration of 50 ms.

Prepulse Inhibition and Startle Threshold Paradigms

The auditory startle was assessed with a SM100 Hamilton Kinder startle monitor (Poway, CA, USA). The animals were put in a non-restrictive plastic box with adjustable ceiling that was placed on a piezo sensor-transducer in a sound attenuating chamber. The piezo disk recorded whole body flinches of the mice as reactions to the auditory startle tone in a time window of 250 ms after stimulus onset. The white noise bursts were presented through speakers mounted on the ceiling of the chamber. The system was calibrated with a Newton impulse calibrator every 2 weeks.

After the mice were placed in the startling box, each trial started with an acclimation period of 5 min. The PPI paradigm consisted of trials with presentation of a startle stimulus (120 dB, 40 ms) alone or a prepulse with one out of

five different prepulse intensities (58, 66, 72, 75, 81 dB, stimulus duration 20 ms) and a startle stimulus that followed the prepulse with an interstimulus interval of 100 ms. Trials were presented in blocks with each block consisting of one startle alone trial and the five different prepulse—startle stimulus trials in a fixed pseudo randomized order. A total of six blocks were presented in a session. Peak amplitude and latency were defined as maximal flinch response in a 250 ms time window after startle stimulus onset measured against baseline activity. Corresponding responses were averaged for each mouse and trial separately. PPI was measured as percentage change of the startle response magnitude following a startle stimulus preceded by a prepulse compared to the startle response to startle stimulus alone. The first PPI session was recorded 1 week before start of the electrode implantation. The second session was recorded at least 1 week following the surgery. This enabled us to control for effects of electrode implantation and investigate the influence of experience on sensorimotor gating. Startle habituation was investigated by averaging together startle alone trials, obtained in the PPI paradigm, in two consecutive blocks. Thus from the total of six startle alone trials, responses from startle alone trials in blocks 1 and 2, 3 and 4, and 5 and 6 were averaged.

Data Processing and Analysis

Data processing was performed with the Brainvision Analyzer software (Brainproducts GmbH; München, Germany) and Matlab (Mathworks Natick, USA). For the analyses of the auditory gating paradigm, epochs were constructed that contained the first and second stimulus (500 ms prestimulus baseline and a 1500 ms poststimulus interval to stimulus one). These epochs were band-pass filtered (1–80 Hz, butterworth zero phase shift filter, 24 db/oct) and baseline corrected. Epochs in which amplitudes exceeded $\pm 450\,\mu V$ in any of the four recording electrodes were excluded. Epochs were then further segmented in epochs of 50 ms pre- and 450 ms post-stimulus intervals for the first stimulus and the second stimulus and averaged for each mouse and stimulus type separately. Similar epochs were applied for the stimuli in the N1 recovery paradigm. The average of the two auditory channels was used for further analysis. AEP peaks were measured against the prestimulus baseline. P1 amplitude was defined as peak positivity within a 0–30 ms latency window; for N1, amplitude was defined as peak negativity within a 30–50 ms latency window; for P2, amplitude was defined as peak positivity within a 50–110 ms latency window. Auditory gating in the paired-click paradigm was measured by means of the ratio of the response to the second stimulus compared to the response to the first stimulus (S2S1-ratio).

Statistical Analysis

Repeated measures ANOVAs (rmANOVA) with Greenhouse-Geisser adjustments were used with group (mutants *vs* wild type) as between-subject factor for all studies. Additional within-subject factors were chosen depending on the paradigm. Such factors were the stimulus condition (first- *vs* second stimulus) in the paired-click paradigm, the ISIs in the peak recovery paradigm, the intensity of prepulses, and blocks for the habituation analysis in the PPI paradigm. The rmANOVAs were followed by *post hoc* paired- or independent samples *t*-tests if indicated. All variables were tested on normalcy of their distribution using the Kolmogorov–Smirnov test. The assumption of normal distribution was violated by one variable (latency of peak N1 to stimulus 1 in the NR1 mutants). For group comparison of this variable the non-parametric Mann–Whitney *U*-test was used. A two-tailed α level of 0.05 was considered significant and adjusted with the Bonferroni correction if multiple tests were computed.

RESULTS

Auditory Gating Paradigm

The grand average waveforms and peak measurements obtained in this paradigm are shown in Figure 1. Peak amplitudes and latencies are shown in Table 1.

Amplitudes and gating. In both groups the P1 and N1 peak to the second stimulus were significantly smaller than the corresponding peaks to the first stimulus (stimulus effects: P1: $F(1,29)=225.91$, $p<0.01$; N1: $F(1,29)=108.05$, $p<0.01$). Auditory gating for the P1 and the N1 peak was significantly impaired in the NR1 mutants compared to their wild-type littermates, as shown by the increased S2S1 ratios in the mutants (independent *t*-test: P1: $p<0.01$, N1: $p<0.01$). The peak amplitudes to the first stimulus for the P1 and the N1 component were not statistically different between the groups (P1: $p=0.3$, N1: $p=0.4$). Thus increased ratios in the NR1 mutants were due to augmented responses to the second stimulus rather than due to reduced amplitudes to the first stimulus (P1: $p<0.01$, N1: $p<0.01$).

Latencies. A rmANOVA with stimulus condition as repeated measures computed for each peak separately showed that the mean latencies of the peaks P1 and N1 were significantly longer in the NR1 mutants (effect of genotype: P1: $F(1,29)=20.25$, $p<0.01$; N1: $F(1,29)=22.78$, $p<0.01$). The latency was prolonged in response to both, the first and the second tone (P1 to tone 1/tone 2: $p=0.01$/$p<0.01$; N1: $p<0.01$/Mann–Whitney *U*-test $p<0.01$).

Peak Recovery Paradigm

Grand averages and peak measurements are displayed in Figures 2, 3 and the Supplementary Figure 2. Peak amplitudes and latencies are shown in Table 1.

P1

Amplitudes. The mean peak amplitude showed an ISI effect across both groups ($F(2.2,47.8)=76.88$, $p<0.01$) and a ISI $\times$ genotype interaction ($F(2.2,47.8)=3.51$, $p=0.03$) (Figure 2). This interaction was due to a greater increase of the P1 amplitude at short ISIs (Figure 3). To test this we computed the sequential increases of the amplitudes from short to long ISIs. A rmANOVA of these incremental increases demonstrated a significant ISI $\times$ genotype interaction ($F(3.4,74.7)=3.79$, $p=0.01$). A significant difference

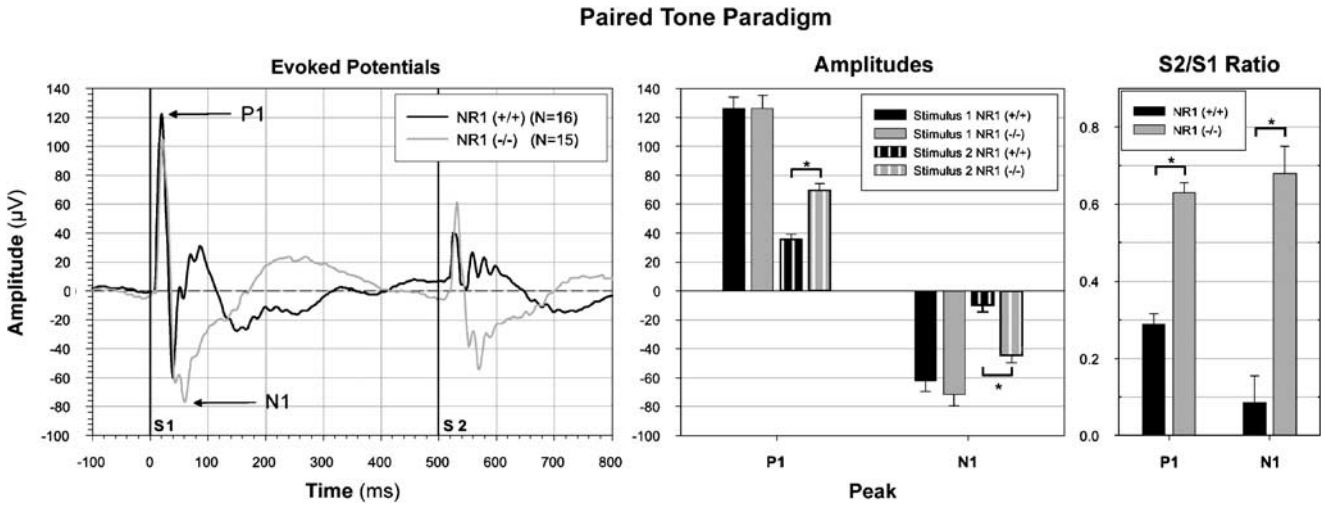

Figure 1 The grand averages of the auditory evoked potentials (left graph) show that both groups show a reduction of the peaks P1 and N1 in the response to the second tone compared to the first. However, the suppression is more pronounced in the control group as compared to the NR1 hypomorphic mice (middle graph). The graph on the right shows peak amplitude ratios (response to tone 2/response to tone 1). Also note the prolonged N1 component in the mutant group (left graph) (asterisks indicate $p<0.01$, error bars show SEM).

in the amplitude increase was found from ISI 0.25 to 0.55 s ($t=-5.2$, df $=22$, $p<0.01$).

Latencies. The NR1 hypomorphic mice showed significantly longer peak latencies across all ISIs (mutants: 20.7 ± 0.32 ms, wild types: 19 ± 0.29 ms, effect of genotype $F(1,22)=16.03$, $p<0.01$). We also observed an effect of ISI ($F(2.7,58.7)=30.05$, $p<0.01$) and a ISI $\times$ genotype interaction ($F(2.7,58.7)=2.86$, $p=0.05$). This was due to increasing latencies with lengthening of the ISI; this increase was greater in the mutant group.

N1

Amplitudes. Mean N1 peak amplitudes increased in both groups as a function of ISI ($F(3.2,69.7)=60.7$, $p<0.01$) (Figures 2 and 3). In addition, an ISI $\times$ genotype interaction was observed ($F(3.2,69.7)=53.1$, $p<0.01$) again due to a greater increase of amplitudes at short ISIs in the NR1 mutants ($F(4.1,90.1)=3.9$, $p<0.01$). The groups significantly differed the incremental amplitude increase from ISI 0.1 to 0.25 s ($t=4.6$, df $=22$, $p<0.01$).

Latencies. Similar to the P1 peak, mean peak latencies of the N1 were delayed across all ISIs in the NR1 mutant mice (mutants: 42.1 ± 0.62 ms, wild types: 38.5 ± 0.68 ms, effect of group $F(1,22)=14.88$, $p<0.01$). There was also an effect of ISI ($F(3,64.2)=4.67$, $p<0.01$) but no significant ISI $\times$ genotype interaction ($F(3,64.2)=2.4$, $p=0.08$).

P2

Amplitudes. A striking difference in the morphology of the waveforms is apparent between groups in the time window following the N1 component in both EEG paradigms (Figures 1 and 2). While wild type mice generated an ISI-dependent P2 component following N1, this component was not observed in the NR1 mutant mice (ISI $\times$ genotype $F(4,87.3)=11.7$, $p<0.01$) (Figure 2). P2 peak amplitudes in the control group increased with longer ISIs ($F(2.8,33.6)=11.1$, $p<0.01$). In the NR1 mutants an ongoing negative component that showed a similar recovery pattern with increasing ISI as the peak N1 was observed during the P2 time window, reflected by a missing ISI $\times$ peak interaction for N1 and the negative peak measured in the P2 time window in the mutant group ($F(2.7,26.6)=1.2$, $p=0.34$). We did not observe an ISI-dependent decrease of the negative gradient during this component. This would have indicated that P2 was generated, but masked by the ongoing N1 component.

Latencies. In the wild-type mice, the mean P2 peak latency was 74.6 ± 3.4 ms and there was no effect of ISI on peak latency ($F(3.9,46.9)=1.8$, $p=0.16$).

Prepulse Inhibition and Habituation of the Auditory Startle

All mice were tested twice in the PPI paradigm, once before and once after electrode implantation.

The startle magnitude in the startle alone trials did not show a session, or session $\times$ genotype effect. Furthermore, for the habituation analysis with blocks and sessions as repeated measures there was no effect of session or session $\times$ genotype interaction (for results see Supplementary Figure S1). Thus, to increase statistical power for the habituation analysis, we averaged the data of the two sessions for the startle response for each mouse. A significant effect of genotype indicated increased startle magnitudes in the NR1 mutants ($F(1,29)=8.83$, $p<0.01$). While the control group showed a habituation of the startle response, the mutant group showed no change of response amplitude during the sessions, as indicated by a significant block $\times$ genotype interaction ($F(1.6,46.2)=3.74$, $p=0.04$) (Figure 4). While in the control group responses were significantly decreased in the third compared to the first

Table 1 Peak Amplitudes and Latencies with SEM of the Auditory Evoked Potentials

		Wild type mice		NR1 mutants	
Peak	**ISI (sec)**	**Amplitude (μV)**	**Latency (ms)**	**Amplitude (μV)**	**Latency (ms)**
AEP recovery paradigm					
P1	0.1	9.4±1.6	16.0±0.2	11.6±1.7	16.3±0.2
	0.25	13.2±2.2	17.0±0.9	14.4±2.4	20.6±0.9
	0.55	19.0±3.3	19.2±0.5	36.9±3.6	21.6±0.6
	0.85	30.5±4.9	19.8±0.5	51.3±5.4	21.3±0.6
	1.15	37.4±5.3	19.8±0.4	50.5±5.8	21.3±0.4
	1.5	46.5±6.9	20.0±0.3	58.0±7.5	21.5±0.4
	2	53.5±7.4	20.2±0.3	61.5±8.1	21.2±0.3
	4	77.9±8.5	19.8±0.3	70.6±9.2	21.6±0.3
N1	0.1	−2.8±1.5	38.1±1.2	−8.5±1.6	38.3±1.3
	0.25	−1.5±2.3	37.7±1.2	−24.5±2.5	42.0±1.3
	0.55	−4.3±3.0	38.7±1.0	−32.3±3.3	42.0±1.0
	0.85	−13.0±4.2	38.4±0.9	−38.7±4.5	42.4±0.9
	1.15	−19.8±4.6	38.8±0.6	−43.0±5.0	42.6±0.6
	1.5	−28.4±5.3	38.8±0.5	−46.6±5.8	42.9±0.6
	2	−37.0±5.7	38.8±0.6	−47.6±6.2	42.9±0.6
	4	−49.7±5.3	39.1±0.5	−55.0±5.7	43.6±0.5
P2	0.1	2.0±0.9	69.4±7.3	0.8±1.0	83.0±8.0
	0.25	5.2±1.8	66.2±6.9	−4.8±2.0	85.8±7.5
	0.55	7.3±1.7	64.3±7.2	−1.6±1.9	93.4±7.9
	0.85	9.7±1.7	69.3±7.7	−1.4±1.8	87.6±8.4
	1.15	8.7±3.1	82.5±7.1	−3.8±3.4	95.5±7.7
	1.5	14.4±2.5	81.5±6.9	−10.2±2.8	94.1±7.4
	2	16.6±2.4	80.9±6.0	−5.8±2.6	93.6±6.6
	4	21.2±3.3	82.8±4.3	−7.4±3.6	100.1±4.7
	Stimulus				
Paired tone paradigm					
P1	1	126.3±8.1	19.4±0.5	114.0±8.4	21.1±0.5
	2	35.6±4.2	17.4±0.5	69.5±4.3	21.1±0.6
N1	1	−62.3±7.6	39.1±1.4	−71.5±7.8	46.7±1.4
	2	−9.8±4.8	34.4±1.5	−44.7±4.9	44.9±1.5

block ($t=5.5$, df $=15$, $p<0.01$) there was no difference in the mutant group between blocks ($t=0.85$, df $=14$, $p=0.41$).

There was no significant difference in PPI between the two sessions (effect of session $F(1,29)=3.6$, $p=0.07$) and no significant session × genotype interaction ($F(1,29)=3.6$, $p=0.88$) (Figure 5). Thus, further PPI analysis used collapsed data across the two sessions. In line with results from previous studies (Duncan *et al,* 2006; Fradley *et al,* 2005) PPI was significantly reduced in the mutants (effect of genotype $F(1,29)=5.8$, $p=0.02$). We also observed a significant intensity × genotype interaction ($F(2.7,78.3)=3.7$, $p=0.02$). Significant rmANOVAs for each group separately suggested that prepulse-dependent PPI was apparent in both groups (wild types: $F(2,29.4)=19.7$, $p<0.01$, NR1 mutants: $F(2.9,40.6)=24.1$, $p<0.01$). The significant genotype × intensity interaction was due to more pronounced prepulse dependent PPI in the mutant group compared to the wild types, in other words PPI increased more in the mutant group with increasing intensity of the prepulse (Figure 5).

There was no significant correlation of PPI, averaged across all prepulse intensities, and the S2S1 ratio of the paired-click paradigm in both groups for the peak N1 and the peak P1 in the wild type group. The NR1 mutant group showed a significant correlation between the PPI and the P1 ratio (Pearson correlation coefficient −0.576, $p=0.024$). However, this significant result was mainly driven by two

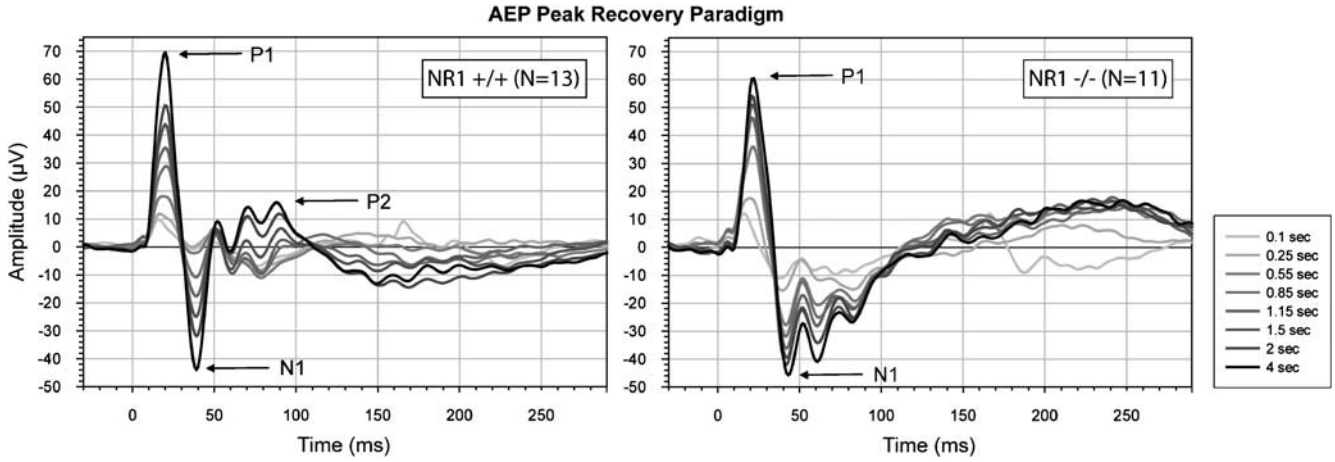

Figure 2 Grand averages of the AEP with different ISI. The amplitudes of AEP increase with ISIs in both groups. While in the wild type group (left) an ISI-dependent positive component develops following the N1 component, this component is missing in the NR1 hypomorphic mice (right).

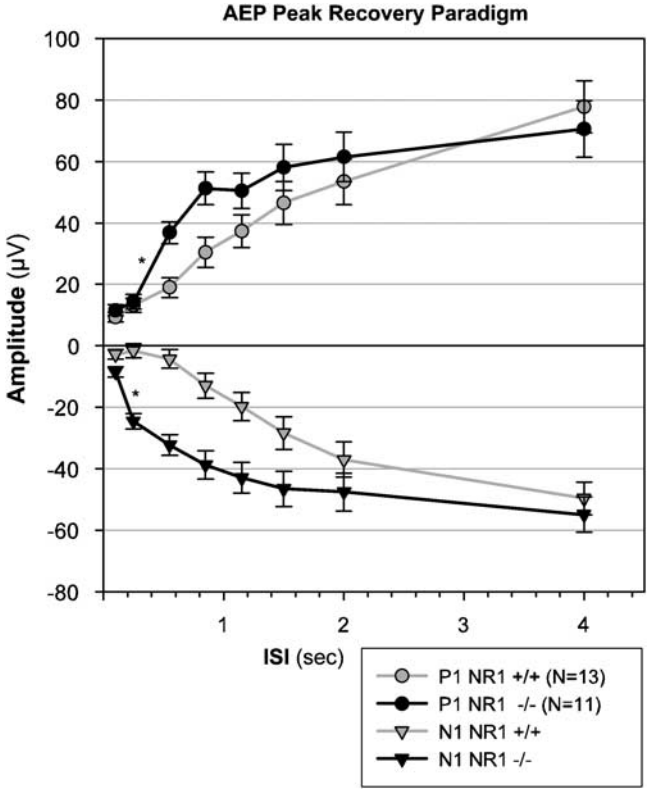

Figure 3 The NR1 mutants show larger responses at short ISI compared to the control group. However, there are no differences in the maximal responses (asterisks indicate $p<0.01$; error bars show SEM).

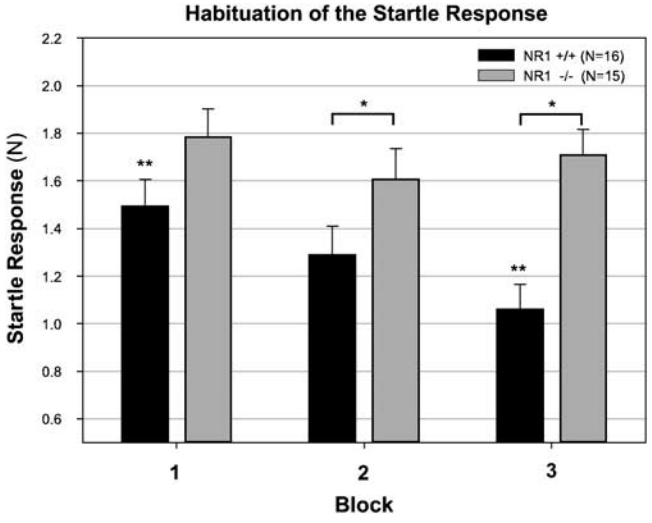

Figure 4 During the sessions the wild type mice developed habituation of the startle response. However, no habituation was observed in the NR1 mutant mice. (asterisks indicate $p<0.01$; error bars show SEM).

outliers and removal of those abolished the significant result (-0.171, $p=0.576$). Thus, these results suggest a lack of correlation between PPI and gating of peaks P1 and N1.

DISCUSSION

The present results show that mouse mutants with reduced expression of the NMDAR subunit 1 model deficits of schizophrenic patients in habituation and PPI of the auditory startle and in auditory gating in the paired tone paradigm. However, they differ in the pattern of AEP peak refractoriness.

Auditory Gating

Auditory gating was significantly disrupted for both investigated peaks (P1, N1) in the NR1 mutant mice as compared to their wild-type littermates. Decreased gating in the paired tone paradigm, that is an increased S2S1-peak ratio, can either result from a lack of suppression of the response to the second tone or a decreased response to the first tone compared to the control group. In patients with schizophrenia impaired gating is more commonly observed due to a decreased response to the first tone. However, evidence for both types of disruption can be found (Hong *et al*, 2004; Ward *et al*, 1996; Freedman *et al*, 1983). In our study decreased gating of both peaks P1 and N1 in the NR1

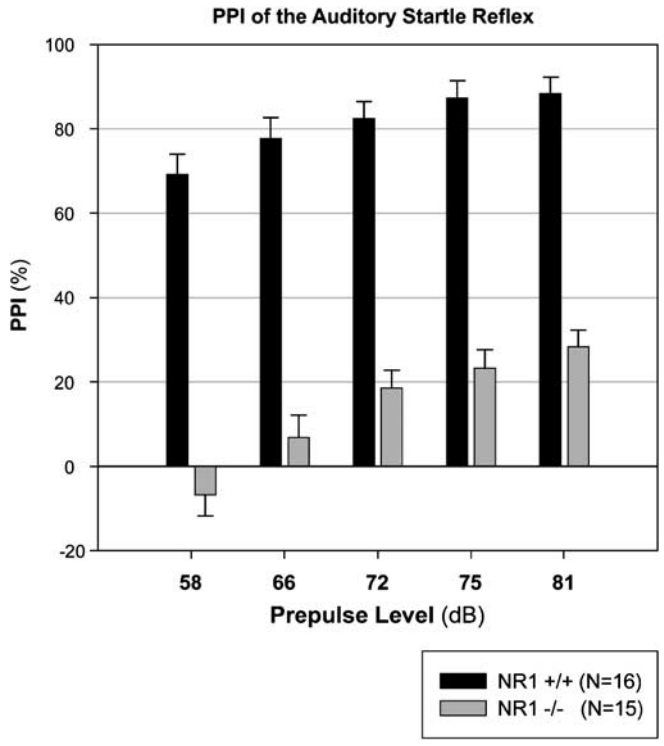

Figure 5 PPI in the NR1 mutants was decreased compared to control group at every prepulse intensity level (error bars show SEM).

mutant mice was caused by reduced suppression of the response to the second tone (Figure 1).

Based on previous studies, we reason that the mouse correlates of the P50 and N100 are the P20 and N40 respectively (named P1 and N1 in this paper) (Umbricht *et al*, 2004b; Connolly *et al,* 2004; Siegel *et al,* 2003). Most gating studies in humans have focused on the P50 component of the human auditory AEP and only few on the N100. However, gating has been shown for both peaks. In schizophrenia gating of both peaks is disrupted (Boutros *et al*, 1999; Rosburg *et al,* 2004). Thus the NR1 mutant mice show gating deficits comparable to those observed in schizophrenia.

In contrast to the reliable disruption of PPI in rodents by NMDAR antagonists, it is still controversial if reduced NMDAR signalling affects auditory gating in the paired tone paradigm. So far, most, but not all, studies in rats showed impairments of N40 -gating (the putative rat correlate of the human N100) with NMDAR blocking (Miller *et al,* 1992; de Bruin *et al,* 1999; Swerdlow *et al,* 2006). However, no effect of NMDAR antagonists on auditory gating was observed in a study in mice (Connolly *et al,* 2004). Furthermore, two studies in healthy human subjects showed no effect of ketamine on auditory gating (Oranje *et al,* 2002; van Berckel *et al,* 1998). The prominent auditory gating deficit in the NR1 mutants contrasts with the unaltered gating in several studies using acute pharmacological NMDAR blockade and possibly indicates that chronic deficient NMDAR-mediated signalling, perhaps even through development, has to be present to cause the auditory gating deficits. Hypothetically, alterations in hippocampal inhibitory interneurons may underlie the observed deficits in the NR1 mutants. CA3 interneurons have been suggested to be the crucial final neurons mediating hippocampal sensory gating (Turetsky *et al,* 2006). Repeated but not single application of NMDAR antagonists decreased the number of interneurons expressing parvalbumin (a Ca^{2+} -binding protein expressed by a subclass of interneurons) in the hippocampus of rodents (Keilhoff *et al,* 2004; Cunningham *et al,* 2006). Interestingly, alterations in hippocampal interneurons have been suggested to be involved in the pathophysiology of schizophrenia, and a specific reduction of parvalbumin expressing interneurons has been observed in the hippocampus of patients with schizophrenia (Heckers and Konradi, 2002; Zhang and Reynolds, 2002). However, further studies investigating interneurons in the NR1 mutants are needed to test this hypothesis.

The lack of a correlation between PPI and auditory gating in the paired tone paradigm further corroborates evidence from human and animal studies that these paradigms measure two distinct mechanisms (Brenner *et al,* 2004; Ellenbroek *et al,* 1999; Swerdlow *et al,* 2006).

Aep Peak Refractoriness

The NR1 mutants showed steeper rise of the P1 and N1 peak amplitudes with increasing ISI compared to the control group, suggestive of a faster decay of the auditory sensory memory trace (Lu *et al,* 1992). However, the maximal response did not differ between the groups (Figures 2 and 3). Thus our findings differ from the peak recovery profile observed in patients with schizophrenia who show a deficit in generation of the maximum P1 and N1 at long ISIs, but no difference in the slope of the peak recovery function (Roth *et al,* 1980; Shelley *et al,* 1999). Our initial hypothesis to find a similar profile in the NR1 mutant mice was based on studies in rats, non-human primates and healthy volunteers, in which such a profile was reproduced by acute application of NMDAR antagonists, pointing to a possible involvement of the NMDAR in this deficit (Javitt *et al,* 2000a; Ehlers *et al,* 1992; Umbricht *et al*, 2004a). Furthermore, previous studies have shown that the mouse AEP peaks P1, N1, and P2 display refractory curves comparable to their putative human correlates (P1, N1, P2) (Umbricht *et al*, 2004b; Maxwell *et al,* 2004). Also, in two mouse studies that investigated the effects of pharmacological NMDAR blockade P1 was increased and N1 changed dependent on mouse strain (Maxwell *et al,* 2006; Connolly *et al,* 2004). However, since only one long ISI (9 s) was used in these studies, the effect of NMDAR antagonism on responses to tones with short ISIs in mice is unknown. Taken together, most studies indicate that reduced NMDAR-mediated signalling reduces P1 and N1 in different species preferentially at long ISIs but the available evidence is currently not conclusive.

The most parsimonious explanations for the observed results in our study is that deficient NMDAR signalling throughout development, as present in the constitutive NR1 mutants, causes alterations in the underlying neural circuitry that differs from abnormalities induced by acute blockade by NMDAR antagonists and those present in schizophrenia. Other possible explanations are that (1) deficient NMDAR-mediated signalling is not the main abnormality causing alterations of the peak recovery in

schizophrenia, and (2) the P1 and N1 peaks in mice do not represent the associate human peaks although the available evidence speaks against that (Umbricht *et al*, 2004b; Maxwell *et al,* 2004).

Alterations in AEP Morphology

While the initial registration of the stimuli as represented in the first 50 ms in the AEPs showed similar morphology of the waveforms in the two groups, striking differences were apparent in the NR1 mutant mice in the time window following the N1 peak. In line with previous studies, the control group showed an ISI-dependent generation of a P2 component following N1 (Figure 2) (Umbricht *et al*, 2004b). However, in the NR1 mutants this P2 component was lacking or masked by an ongoing negative activity after the N1 component. Furthermore, the negative slow wave in the time window from 100 to 300 ms showed inverse polarity in the NR1 mutant mice (Figures 1 and 2). Thus, besides the described alterations in the early auditory information processing in the NR1 mutants, also later hierarchical stages of information processing seem to be affected by the mutation of the NMDAR. However, these findings are difficult to interpret with the current available data since no such alterations are observed in either pharmacological animal models of reduced NMDAR signalling or in patients with schizophrenia. They may indicate far-reaching consequences of reduced NMDAR signalling throughout the whole developmental period as compared to short-lasting pharmacological blockade of the NMDAR in adulthood. Single unit and AEP studies in rats suggested that the slow wave N2 and P2 represent an inhibitory process suppressing the early excitation reflected by the P1/N1 deflection observed in the cortical AEP of the rat (Metherate 1998; Sukov and Barth 1998). Thus the prolonged N1 component in the NR1 mutant mice may indicate a disruption of this inhibitory process. However as mentioned by Metherate, single unit responses and AEPs only overlap in the first 20 ms poststimulus, thus one has to be cautious when comparing findings from these two approaches (Metherate 1998). It also has to be mentioned that these alterations were not observed in earlier recordings in these mouse mutants in which a different reference electrode (above frontal cortex) than in the present study (above the cerebellum) was used. This highlights the importance of the recording setup when interpreting data and comparing different studies.

Habituation and PPI of the Startle

The very robust PPI deficits in the NR1 mutants reproduces findings from several previous studies (Duncan *et al,* 2006; Fradley *et al,* 2005) and agree with pharmacological studies that showed a reliable disruption of PPI in rodents and monkeys by NMDAR antagonists (Linn *et al,* 2003; Geyer *et al,* 2001). However, it has to be taken into account that these results contrast with most human studies, in which NMDAR antagonists either had no influence or even increased PPI (Braff *et al,* 2001; van Berckel *et al,* 1998; Abel *et al,* 2003).

Furthermore, we found group differences in the prepulse intensity dependence of the PPI. The NR1 mutants showed a bigger increase in PPI with increasing prepulse intensity compared to the wild type group. This contrasts to findings in schizophrenia, where prepulse-dependent PPI is usually unaltered (Braff *et al,* 1999; Grillon *et al,* 1992). One possible explanation for the finding in our study is a putative ceiling effect of PPI in the control group, since this group already reached relatively high PPI values in trials with the lowest prepulse intensities.

A new finding in our study is the observation of impaired habituation of the startle response in these mutants. This parallels findings in schizophrenia and pharmacological rodent models of reduced NMDAR-mediated signalling (Ludewig *et al,* 2003; Klamer *et al,* 2004). Habituation reflects a simple form of non-associative learning and its generation has been located in the afferent sensory pathway of the neural startle circuit (Pilz *et al,* 2004). It has been suggested that habituation to initially novel stimuli may be essential for processes such as orienting responses and selective attention (Geyer *et al,* 1990).

In conclusion, the present study suggests involvement of reduced NMDAR-mediated signalling in the pathophysiology of deficits in habituation and PPI of the auditory startle reflex and auditory gating in the paired tone paradigm in schizophrenia. However, the reduced P1 and N1 amplitudes at long ISI in patients with schizophrenia could not be modelled with this mouse model of NMDAR hypofunction. Thus, our results provide further supporting evidence for the hypothesis that deficient NMDAR functioning plays an important role in the pathophysiology of abnormal information processing in schizophrenia.

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DISCLOSURE/CONFLICT OF INTEREST

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