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Research report

Do transmembrane domain *neuregulin 1* mutant mice exhibit a reliable sensorimotor gating deficit?

T. Karl[a,b,c,*], T.H.J. Burne[d,e], M. Van den Buuse[f,g], R. Chesworth[a,b]

[a] *Schizophrenia Research Institute, Darlinghurst, NSW, Australia*
[b] *Neuroscience Research Australia, Randwick, NSW, Australia*
[c] *Garvan Institute of Medical Research, Darlinghurst, NSW, Australia*
[d] *Eskitis Institute for Cell and Molecular Therapies Griffith University, Brisbane, QLD, Australia*
[e] *Queensland Centre for Mental Health Research, The Park Centre for Mental Health, Richlands, QLD, Australia*
[f] *Mental Health Research Institute, Parkville, VIC, Australia*
[g] *Department of Pharmacology, University of Melbourne, VIC, Australia*

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ABSTRACT

Evidence suggests that the heterozygous transmembrane domain mutant mouse model for the schizophrenia candidate gene *neuregulin 1* (*Nrg1* HET) exhibits a deficit in prepulse inhibition (PPI). However, not all mouse models for *Nrg1* exhibit PPI deficits. Thus, our study intended to clarify the severity of the initially described PPI deficit in *Nrg1* HET mice. For this, *Nrg1* mutant mice and wild type-like littermates of one breeding colony were tested for PPI in four different phenotyping facilities in Australia employing a variety of different PPI protocols with fixed and variable interstimulus intervals (ISIs). Testing mutant and wild type-like mice in three Australian phenotyping facilities using PPI protocols with variable ISIs revealed no effect of mutant transmembrane domain *Nrg1* on sensorimotor gating. Changes to the startle response and startle response habituation were site/protocol-specific. The employment of two different PPI protocols at the same phenotyping facility revealed a protocol-dependent and site-specific facilitation of PPI in *Nrg1* mutant mice compared to wild type-like mice. In conclusion, the often-noted PPI phenotype of the transmembrane domain *Nrg1* mutant mouse model is highly PPI protocol-specific and appears sensitive to the particular conditions of the test laboratory. Our study describes wild type-like PPI under most test conditions and across three different laboratories. The research suggests that analysing one of the alleged hallmarks of animal models for schizophrenia must be done carefully: to obtain reliable PPI data it seems necessary to use more than one particular PPI protocol.

1. Introduction

Evidence suggests that the *neuregulin 1* gene (*NRG1*) is associated with schizophrenia [1,2]. *Nrg1* mRNA can be found in schizophrenia-relevant brain areas such as the prefrontal cortex and the hippocampus – in humans [3] and rodents [4]. The polypeptide influences key neurodevelopmental processes relevant to schizophrenia such as myelination and neuronal migration, and regulates the expression/activation of receptors such as N-methyl-D-aspartic acid (NMDA) and γ-aminobutyric acid receptor A ($GABA_A$) [5,6]. On a behavioural level, *NRG1* might have functional effects on prepulse inhibition (PPI), as a missense mutation in *NRG1* appears to impact on PPI of schizophrenia patients and healthy subjects [7].

PPI is a measure of sensorimotor gating, where the startle response to a startle stimulus is inhibited by a preceding prestimulus. Most schizophrenia patients demonstrate deficits in PPI [8] and pharmacological manipulations to the dopaminergic and glutamatergic systems impair PPI whereas antipsychotic treatment can facilitate PPI [9,10]. Animal models are tested in a similar manner to humans, suggesting a high level of comparability. Thus, PPI is one of the more valid paradigms for schizophrenia research and is often suggested as a key feature of schizophrenia animal models [11,12].

Alternative splicing of the schizophrenia candidate gene *NRG1* results in at least 30 distinct isoforms [13]. As a consequence, a multitude of genetic mouse models have been developed for

Abbreviations: ANOVA, analysis of variance; ASR, acoustic startle response; $GABA_A$, γ-aminobutyric acid receptor A; Garvan, Garvan Institute of Medical Research; GU, Griffith University; *Nrg1* HET, heterozygous transmembrane domain *Nrg1* mutant mouse model; ISI, interstimulus interval; MHRI, Mental Health Research Institute; NMDA, N-methyl-D-aspartic acid; Nrg1, *Neuregulin 1*; NeuRA, Neuroscience Research Australia; PPI, prepulse inhibition; RM, repeated measures; 5-HT, serotonin; SEM, standard error of the mean; WT, wild type-like.

* Corresponding author at: Neuroscience Research Australia, Barker St, Randwick, NSW 2031, Australia. Tel.: +61 2 9399 1025; fax: +61 2 9399 1005.
E-mail address: t.karl@neura.edu.au (T. Karl).

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Table 1
Overview about sensorimotor gating performance (PPI) of various genetic mouse models for neuregulin 1 isoforms/types and its main functional receptor ErbB4 [EGF = epidermal growth factor (critical for Nrg1 binding to ErbB4); PPI = prepulse inhibition; ASR = acoustic startle response; PV = parvalbumin-positive interneurons].

Target (genetic background); sex	Described in	Acoustic startle response (ASR)	PPI phenotype
Transmembrane domain Nrg1 (backcrossed to C57BL/6JArc); males	PPI Deficit [2,19] No PPI deficit [18,20]	Reduced ASR [19] Unaltered ASR [2,18,20]	Inconsistent PPI deficit; not reversible by acute clozapine treatment
EGF-like domain of *Nrg1* (C57BL/6); males	[27]	Trend ($p = .08$) for decreased ASR	No PPI deficit
EGF-like domain of *Nrg1* (C57BL/6 X 129); Females	[32]	Unaltered ASR	No PPI deficit
EGF-like domain of *Nrg1* (C57BL/6 X 129/SVEV); males	[33]	ASR not investigated	PPI not investigated
Immunoglobulin-like domain *Nrg1* (129/SV); males	[35]	ASR not investigated	PPI not investigated
Cysteine-rich domain *Nrg1* (C57BL/6); Sex not specified	[36]	ASR not investigated	PPI not investigated
Type I *Nrg1* over-expression (background not described); sex not specified	[34]	ASR not investigated	PPI not investigated
Type III *Nrg1* over-expression (background not described); sex not specified	[34]	ASR not investigated	PPI not investigated
Type III *Nrg1* (C57BL/6 X 129); males	[28]	Unaltered ASR	PPI deficit; reversible by acute nicotine treatment
Type I *Nrg1* overexpression (C57BL/6); males and females	[29]	Elevated ASR	PPI deficit and altered ASR
ErbB4 (backcrossed to C57BL/6); males	[2]	Unaltered ASR	No PPI deficit
CNS-specific ErbB4 (FVB); males	[38]	ASR not investigated	PPI not investigated
CNS-specific ErbB4 (C57BL/6); males and females	[37]	ASR not investigated	PPI not investigated
CNS-specific PV-ErbB4 (C57BL/6); sex not specified	[30]	Unaltered ASR	PPI deficit; reversible by acute diazepam treatment

the polypeptide and its main functional receptor ErbB4. Importantly, only a few of these models exhibit PPI impairments (see Table 1). The heterozygous transmembrane domain *Nrg1* mutant mouse model (*Nrg1* HET) appears to be among those [2]. *Nrg1* HET mice represent one of the few well-characterised mouse models for *Nrg1* fulfilling construct, face and partial predictive validity: (i) a missense mutation in the transmembrane region of *NRG1* is associated with schizophrenia [14], (ii) *Nrg1* HETs show hyperlocomotion [15], reduced social preference [16] and cognitive deficits [17], and (iii) hyperlocomotion is reversible by clozapine [2]. Although the initial study reported a PPI deficit for *Nrg1* HETs [2], more recent investigations have not been able to reliably replicate this finding [18–20]. Sensorimotor gating is often used as one of the two gold standards to evaluate the validity of a schizophrenia animal model; the other being hyperlocomotion [12]. Thus, the current study intends to clarify the significance of the initially described and often cited PPI deficit of transmembrane domain *neuregulin 1* mutant mice using a scenario most commonly found across different research institutes, which plan to work on a mouse model with a pre-characterised phenotype: certain aspects of the individual housing conditions and test facilities vary across sites as do the PPI protocols used. Recent research suggests that environmental standardisation across laboratories is a cause of, rather than a cure for, poor reproducibility of experimental outcomes [21]. Importantly, our research team employed the original PPI protocol [2] as well as protocols, which had been pharmacologically validated and had been used successfully for other animal models of schizophrenia in the past. We also considered the impact of different test conditions (i.e. test laboratory) by comparing the response of mice tested in two different laboratories to one particular PPI protocol (thereby using identical housing condition.

2. Material and methods

2.1. Animals

The generation of transmembrane domain *Nrg1* mutant mice (target allele: Nrg1tm2Zhou) has been described previously [2]. Test animals were adult (21–24 weeks old), male heterozygous *Nrg1*$^{+/-}$ (*Nrg1* HET) and wild type-like (WT) control *Nrg1* $^{+/+}$ littermates (backcrossed in the 15th generation on C57BL/6JArc background). Genotypes were determined after weaning (postnatal day 21) using tail tip biopsy and polymerase chain reaction amplification of selective amplicons for the knockout allele (primers for mutant *Nrg1* mice: Neo173F: 50-ATGAACTGCAGGACGAGGCA-30 and Neo6301R: 50-GCCACAGTCGATGAATCCAG-30; primers for WT control mice: 50-AACAGCCTGACTGTTAACACC-30 and 50-TGCTGTCCATCTGCACGAGACTA-30).

2.2. Housing conditions

2.2.1. Breeding colony

Mice were bred at the Garvan Institute of Medical Research (Garvan), and were housed in conventional polysulfone cages with a wire inner lid and a polysulfone transparent frame fitted with a polyester filter sheet (Tecniplast, Rydalmere, Australia). Cages were supplied with a form of minimal environmental enrichment: a red, transparent, polycarbonate igloo (certified polycarbonate mouse igloo: Bioserv, Frenchtown, USA), cellulose paper for nesting material, and a metal ring in the middle of the cage lid (3 cm in diameter). All mice were kept under a 12:12 light:dark cycle [light phase: white light (illumination: 80 lx); dark phase: red light (illumination: <2 lx)], at 21 ± 1 °C with food and water available *ad libitum*. Microbiological monitoring showed no infection of the specific pathogen free (SPF) facility holding room, with the exception of the pathogens commonly found in commercial and research facilities, *Pasteurella pneumotropica* and *Helicobacter* spp.

2.2.2. Test mouse colonies

Adult, male mice (*Nrg1* HET and WT) with no previous test experience were tested in four locations – the Garvan, Neuroscience Research Australia (NeuRA), the Mental Health Research Institute (MHRI) and Griffith University (GU). All mice were bred at the Garvan and transported to the different test facilities at least two weeks before the experiments started. Test mice at all locations were group housed (groups of 2–5) with littermates and food and water available *ad libitum*. Mice were tested in quasi-randomised order.

Garvan and NeuRA: Housing conditions of test mice were identical to the ones described for the breeding colony (see Section 2.2.1).

MHRI: Mice were housed in standard opaque plastic mouse boxes with a wire lid, standard pellet food and tap water *ad libitum*. In addition to bedding, shredded paper, sunflower seeds and a small cardboard 'hide' box was supplied as a form of minimal environmental enrichment.

GU: Mice were housed in Tecniplast cages and supplied with cellulose paper for nesting material as a form of minimal environmental enrichment.

The relevant authorities approved all procedures (Garvan: Garvan Institute/St Vincent's Hospital Animal Experimentation Ethics Committee; NeuRA: Animal Care and Ethics Committee of the University of New South Wales; MHRI: Animal Experimentation Ethics Committee of the Howard Florey Institute, University of Melbourne; GU: Griffith University Animal Ethics Committee). All experimental protocols were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.3. Sensorimotor gating (prepulse inhibition of the acoustic startle response)

Acoustic startle response (ASR) and prepulse inhibition (PPI) was tested during the light phase at all sites using identical startle chambers (SR-Lab: San Diego Instruments, San Diego, USA). All laboratories employed identical calibration techniques (as advised by San Diego Instruments). Animal enclosures were cleaned with 70% ethanol between animals.

For all protocols, startle response was measured as the average amplitude of the startle. The duration of the prepulse was 20 ms and 40 ms for the startle. All trial types for all locations were presented in a pseudorandom order. The intertrial interval was averaged over 10–25 s. ASR was calculated as the startle amplitude in arbitrary units averaged over the test trial. For ASR habituation, blocks of startle responses were averaged at the beginning, middle and end of the PPI protocol

(3 startle response blocks for 'Garvan/NeuRA' and 'GU'; 4 startle response blocks for 'MHRI'). Percentage of PPI (%PPI) was calculated as [(ASR 120 dB – prepulse response) × 100/ASR 120 dB]. For further details on the PPI protocols for each test location see below:

2.3.1. Garvan and NeuRA

Following habituation to the San Diego Instruments device (5 min for three days), and a 5 min acclimation period (70 dB background noise) upon test, the protocols were as follows:

'Stefansson': The PPI protocol used at the Garvan was identical to the one published by Stefansson and co-workers in 2002. A 16 min session was run that consisted of 56 trials. Each trial started with a 50 ms null period, followed by a 20 ms prepulse white noise of 72, 74, or 78 dB. After a 100 ms interstimulus interval (ISI), the startle stimulus was presented (a 40 ms 120 dB white noise). The total duration of the trial was 500 ms. Eight types of trials were given: prepulse (72, 74, or 78 dB) plus startle (10× per prepulse intensity), prepulse (72, 74, or 78 dB) alone (4× per prepulse intensity), startle alone (10×), and no stimulation (4×). The variable intertrial interval averaged 15 s (range 10–20 s). In the startle-alone trials, the basic auditory startle was measured, and, in the prepulse-plus-startle trials, the amount of inhibition of normal startle was measured and was expressed as a percentage of the basic startle. In the prepulse-alone trials, the response to a weak noise was measured as a control.

'Garvan': The session employed at the Garvan consisted of the following in pseudo-randomised order: ten 90 dB ASR trials, 18 × 120 dB ASR trials, two prepulse alone trials per prepulse intensity (i.e. 74/78/82/86 dB), eight PPI response trials per prepulse intensity (prepulse followed 80 ms later by a 120 dB startle pulse), and eight no pulse trials (background noise only).

'Garvan/NeuRA': This protocol was used at both the Garvan and NeuRA to allow for comparison of test site-specific effects of the PPI phenotype of *Nrg1* mutants. The session started with five 120 dB startle pulses after which four startle pulses (70/80/100/120 dB) were presented five times each in a pseudo-randomised order. After this, 75 PPI response trials (prepulse intensities of 74/82/86 dB followed by a 120 dB startle pulse) were presented five times in a quasi-randomised order employing five different ISIs (32/64/128/256/512 ms) followed by a final five 120 dB startle pulses.

2.3.2. 'MHRI'

The protocol carried out at the MHRI started with 8 115 dB pulse-alone startle stimuli (70 dB background noise). This was followed by 88 pseudo-randomised trials including 16 115 dB pulse-alone stimuli, eight no stimulus trials, and 64 prepulse trials. Prepulse trials consisted of a single 115 dB pulse preceded either 30ms or 100ms by a non-startling stimulus of 2, 4, 8 or 16 dB over the 70 dB baseline (i.e. ISIs: 10 ms and 80 ms). The session concluded with eight 115 dB pulse-alone startle stimuli.

2.3.3. 'GU'

The session run at GU started with five 110 dB startle pulses (70 dB background noise). Five blocks of 24 trials were then presented, consisting of six different trial types of pulse alone trial (70, 80, 90, 100, 110 and 120 dB) and 18 different types of prepulse and pulse trial. The prepulse had an intensity of 74, 78 or 86 dB and was presented employing six different ISIs (8/16/32/64/128/256 ms). The session finished with five 110 dB startle pulses.

2.4. Experimental procedures

Transmembrane domain *Nrg1* mutant mice and wild type-like littermates bred at the Garvan were tested for PPI in four different phenotyping facilities in Australia employing a variety of different PPI protocols with fixed and variable ISIs:

Experiment 1 was performed at the Garvan ($n = 21$ mice) and replicated the PPI protocol used in the original publication by Stefansson and co-workers describing a PPI deficit in *Nrg1* HET mice.

Experiment 2 compared the ASR, its habituation and PPI in three phenotyping facilities (MHRI, NeuRA and GU) using PPI protocols with variable ISIs ('MHRI', 'Garvan/NeuRA', 'GU'), as described above. There were $n = 62$ mice tested using the 'MHRI' protocol (38 WT, 24 *Nrg1* HET), $n = 19$ mice tested using the 'Garvan/NeuRA' protocol (9 WT, 10 *Nrg1* HET) and $n = 23$ mice tested using the 'GU' protocol (14 WT, 9 *Nrg1* HET).

Experiment 3 compared the ASR and PPI of mice (a) tested at the Garvan using two different PPI protocols ('Garvan' *versus* 'Garvan/NeuRA') and (b) tested at the Garvan or NeuRA using one identical PPI protocol (i.e. 'Garvan/NeuRA'). There were $n = 23$ mice used for the 'Garvan' protocol (13 WT, 10 *Nrg1* HET), and $n = 16$ mice used for the 'Garvan/NeuRA' protocol (8 WT, 8 *Nrg1* HET).

2.5. Statistical analysis

Data were analysed using three- and two-way repeated measures (RM) analysis of variance (ANOVA) using SPSS 17.0 software ($n \geq 8$ per genotype). The within group repeated measures factors were 'startle block', 'prepulse intensity' and 'ISI'; the between factor was 'genotype' and 'location'. Where appropriate, one-way ANOVA split by corresponding factors followed. Differences were regarded as statistically significant if $p < .05$. Data are represented as means ± SEM. Significant effects of genotype are indicated by an asterisk (*versus* WT; $^*p < .05$).

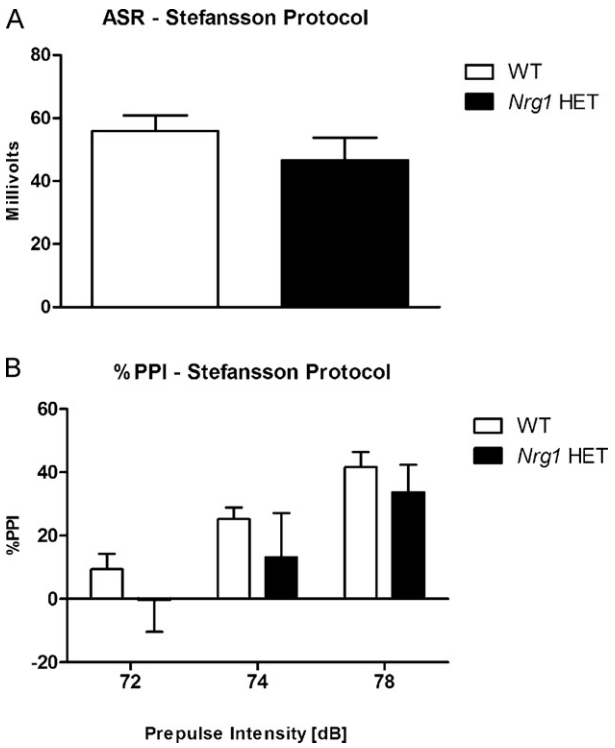

Fig. 1. (A and B) Acoustic startle response and prepulse inhibition using the 'Stefansson' protocol: (A) acoustic startle response (ASR: startle amplitude in millivolts) to a startle pulse (120 dB); (B) percentage prepulse inhibition (%PPI) for different prepulse intensities (72/74/78 dB) averaged over the test trials. Data are presented as means ± SEM.

3. Results

3.1. Experiment 1: ASR and PPI measured using the 'Stefansson' design

The acoustic startle response (ASR) to the 120 dB startle pulse of *Nrg1* mutant and wild type-like mice was similar [one-way ANOVA: $F(1,19) = 0.5$, not significant]. RM analysis of PPI over different prepulse intensities revealed no main effect of 'genotype' (Fig. 1A) but a significant effect of 'prepulse intensity' with increasing prepulse intensities elevating PPI [$F(2,38) = 14.2$, $p < .0001$; Fig. 1B].

3.2. Experiment 2: ASR and PPI measured using different PPI protocols and test locations

The ASR to a 110, 115 or 120 dB startle pulse at MHRI, NeuRA and GU revealed lower startle in *Nrg1* HETs at MHRI [one-way ANOVA: MHRI: $F(1,60) = 5.3$, $p = .03$], but no genotype effects at NeuRA and GU (not significant; Table 2).

ASR habituation occurred at MHRI in both genotypes, as shown by a two-way RM ANOVA main effect of 'startle pulse block' [$F(3,180) = 13.9$, $p < .0001$] (Fig. 2A). At NeuRA, a significant interaction between 'startle pulse block' and 'genotype' [$F(2,34) = 3.5$, $p = .04$] reflected that ASR habituation occurred in WT mice only. There was no main effect of 'genotype' [$F(1,17) = 0.1$, not significant; Fig. 2B]. At GU, ASR habituation did not occur in either genotype, as there were no main effects of 'startle pulse block' or 'genotype' (not significant; Fig. 2C).

Three-way RM ANOVA for each PPI protocol demonstrated a significant main effect of 'prepulse intensity' and of 'ISI' regardless

Table 2
Acoustic startle response in different phenotyping facilities.

PPI protocol	Startle stimulus (dB)	ASR	
		WT	*Nrg1* HET
'MHRI'	115	235.1 ± 24.3	157.1 ± 4.2*
'Garvan/NeuRA'	120	43.5 ± 4.9	41.7 ± 3.4
'GU'	110	208.5 ± 20.1	271.8 ± 32.0

Acoustic startle response (ASR: startle amplitude in millivolts) to a startle pulse (110, 115 or 120 dB) averaged over the test trial for each of the three prepulse inhibition (PPI) protocols used at the phenotyping facilities of the Mental Health Research Institute ('MHRI'), Neuroscience Research Australia ('Garvan/NeuRA') and Griffith University ('GU'). Data are presented as means ± SEM. Significant effects of genotype (one-way ANOVA) are indicated by asterisks (*$p < .05$).

of test protocol/location. %PPI increased with increasing prepulse intensities ['prepulse intensity': MHRI: $F(3,180) = 279.3$, $p < .0001$; NeuRA: $F(2,34) = 53.8$, $p < .0001$; GU: $F(2,42) = 85.5$, $p < .0001$]. At MHRI, %PPI for ISIs of 80 ms was increased compared to %PPI for ISIs of 10 ms and at NeuRA and GU, %PPI decreased with ISIs longer than

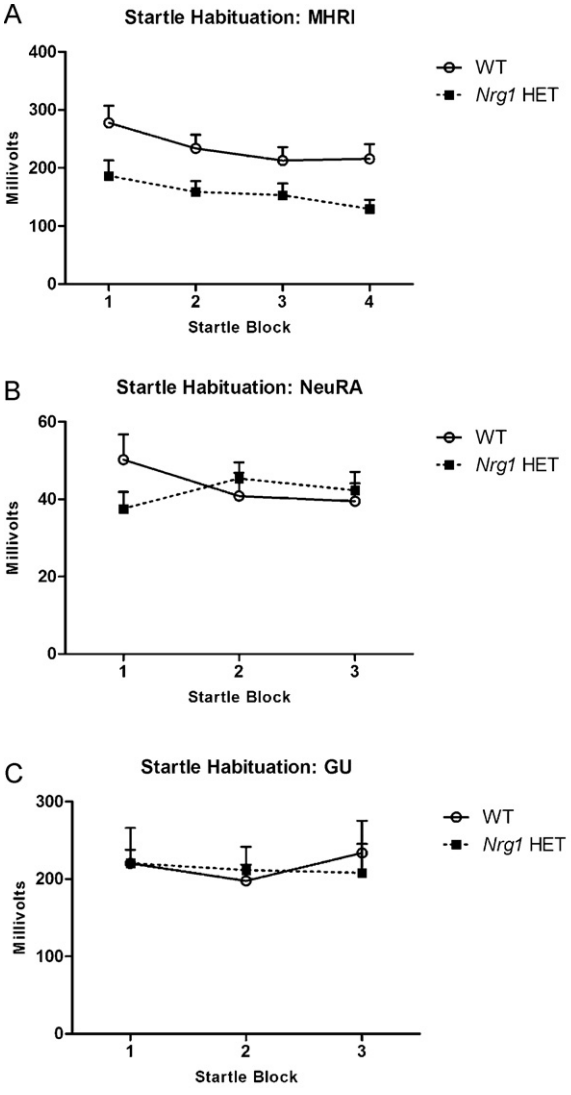

Fig. 2. (A–C) Habituation to the acoustic startle response (ASR) at three phenotyping facilities: (A) ASR habituation to an 115 dB pulse at the Mental Health Research Institute (MHRI); (B) ASR habituation to a 120 dB pulse at Neuroscience Research Australia (NeuRA) and (C) ASR habituation to a 110 dB pulse at the Griffith University (GU). Two-way RM ANOVA revealed a significant interaction of 'startle pulse block' and 'genotype' for the protocol used at the NeuRA facility ('Garvan/NeuRA'). All data are presented as means ± SEM.

Table 3
Percentage prepulse inhibition in different phenotyping facilities.

PPI protocol	Prepulse intensity (dB)	%PPI	
		WT	*Nrg1* HET
'MHRI'	72	−1.7 ± 2.9	4.5 ± 2.7
	74	17.5 ± 2.7	20.5 ± 4.2
	78	40.4 ± 3.3	49.4 ± 3.6
	86	63.8 ± 2.5	66.7 ± 2.8
	AVG	30.0 ± 2.3	35.3 ± 2.4
'Garvan/NeuRA'	74	20.6 ± 7.0	20.1 ± 5.1
	82	49.8 ± 9.3	53.2 ± 3.2
	86	52.5 ± 7.1	53.6 ± 4.0
	AVG	41.0 ± 7.4	42.3 ± 2.8
'GU'	74	11.4 ± 5.2	13.6 ± 10.5
	78	34.9 ± 5.6	49.2 ± 5.9
	86	46.8 ± 4.6	56.3 ± 4.6
	AVG	31.0 ± 4.8	39.7 ± 6.5

Percentage prepulse inhibition (%PPI – averaged over different ISIs) for different prepulse intensities (dB) and their average (AVG) is shown for each of the three PPI protocols used at the phenotyping facilities of the Mental Health Research Institute ('MHRI'), Neuroscience Research Australia ('Garvan/NeuRA') and Griffith University ('GU'). Data are presented as means ± SEM.

100 ms ['ISI': MHRI: $F(1,60) = 23.7$, $p < .0001$; NeuRA: $F(4,68) = 10.1$, $p < .0001$; GU: $F(5,105) = 8.4$, $p < .0001$]. The analysis did not detect any main effects of 'genotype' for any test protocol/location [MHRI: $F(1,60) = 2.3$, NeuRA: $F(1,17) = 0.03$, GU: $F(1,21) = 1.2$, all not significant; Table 3].

3.3. Experiment 3: ASR and PPI measured using two different PPI protocols or one PPI protocol at two test locations

ASR was assessed in two different PPI protocols ('Garvan' *versus* 'Garvan/NeuRA') at the Garvan. Using a fixed ISI protocol (i.e. 'Garvan'), startle responses to a 120 dB tone were significantly lower in mutant *Nrg1* mice compared to WT littermates [WT: 47.0 ± 4.9; *Nrg1* HET: 30.1 ± 3.4; one-way ANOVA for 'genotype': $F(1,21) = 8.5$, $p = .008$]. However, the PPI protocol with a variable ISI (i.e. 'Garvan/NeuRA') revealed no significant difference of mean ASR to the 120 dB startle tone between genotypes [WT: 31.7 ± 8.0; *Nrg1* HET: 26.6 ± 3.7, not significant]. ANOVAs detected a main effect of 'prepulse intensity' for both PPI protocols ['Garvan': $F(3,63) = 27.2$, $p < .0001$; 'Garvan/NeuRA': $F(2,28) = 20.2$, $p < .0001$; Fig. 3A] but only a main effect of 'genotype' for the latter protocol ['Garvan': $F(1,21) = 0.2$, not significant; 'Garvan/NeuRA': $F(1,14) = 4.9$, $p < .05$]. Further analysis split by 'prepulse intensity' (averaged across ISIs) revealed significantly higher %PPI in *Nrg1* HETs at the 86 dB prepulse [$F(1,14) = 5.3$, $p = .04$; Fig. 3B] but not at the 74 and 82 dB prepulses compared to control mice. Importantly, the same protocol ('Garvan/NeuRA') did not detect a genotype-specific effect when employed at NeuRA (see experiment 2).

4. Discussion

This study aimed to clarify whether a mouse model mutant for transmembrane domain *Nrg1* is characterised by a reliable impairment in prepulse inhibition – one of the hallmarks of animal models for schizophrenia – or a rather subtle alteration in this schizophrenia endophenotype. Testing mutant and wild type-like mice in three Australian phenotyping facilities (i.e. MHRI, NeuRA, GU) using pharmacologically validated PPI protocols revealed no sensorimotor gating deficit in mice mutant for the transmembrane domain *Nrg1*. In addition, the findings of the original study reporting a PPI deficit in *Nrg1* mutant mice could not be replicated. Changes to the acoustic startle response and startle response

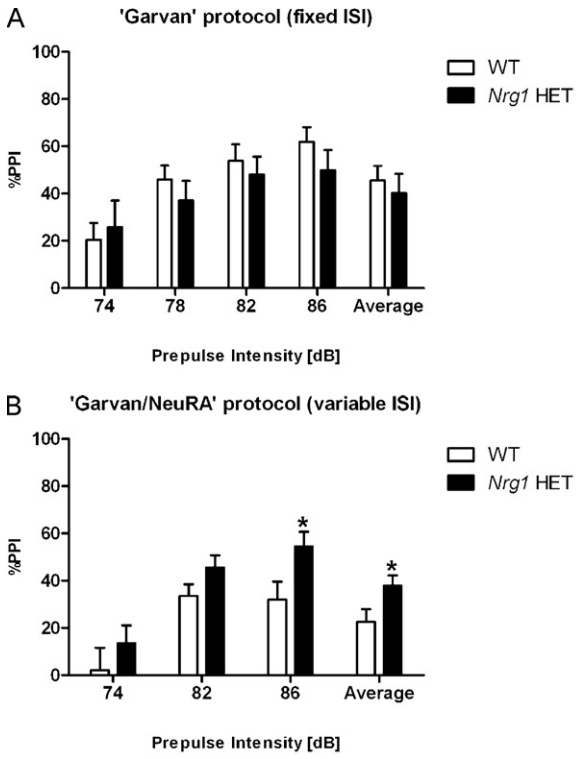

Fig. 3. (A and B) Percent prepulse inhibition (%PPI) at the Garvan using two PPI protocols (A) %PPI at four prepulses (74, 78, 82, 86 dB), and average %PPI (averaged across prepulse intensities) using a fixed interstimulus interval PPI protocol ('Garvan'); (B) %PPI at three prepulses (74, 82, 86 dB), and average %PPI (averaged across prepulse intensities) for the variable interstimulus interval protocol ('Garvan/NeuRA'). %PPI is averaged for the various interstimulus intervals. All data presented as means ± SEM. Significant effects of genotype (one-way ANOVA) are indicated by asterisks (*$p < .05$).

habituation of *Nrg1* hypomorphs were protocol/site-specific. The employment of two different PPI protocols with a fixed *versus* a variable ISI in one phenotyping facility (i.e. Garvan) revealed a protocol-dependent modification of PPI in *Nrg1* mutant mice (i.e. in response to 'Garvan/NeuRA'). Importantly, this phenomenon was site-specific as the same protocol applied in another phenotyping facility (i.e. NeuRA) failed to confirm an amplified PPI phenotype of *Nrg1* mutants.

In a first instance, we used the PPI protocol of the original study reporting PPI deficits in *Nrg1* HETs [2]. Importantly, we failed to reproduce the PPI deficit published in 2002, confirming the fragility of the reported phenotype. Differences between the original Stefansson study and our experiment can be found in the transportation of the Stefansson mice and possible housing condition differences, which can impact on PPI phenotypes [22]. It is noteworthy that no other study to date has replicated the PPI deficit reported in the Stefansson study.

Despite the fact that PPI impairments have been reported not only for schizophrenia patients but also for people suffering from other disorders, such as Tourette syndrome or Huntington's disease, it is commonly accepted as one of the hallmarks of animal models for schizophrenia. Thus, not only are PPI deficits not specific to schizophrenia, but animal research laboratories use individual and therefore unique PPI protocols, which differ in a variety of protocol characteristics (e.g. intensity and duration of prepulse and startle stimulus, ISI, protocol duration). For example, studies have shown a clear impact of different ISIs on PPI [10,23,24]. However, the level of comparability between human and animal model sensorimotor gating testing, its pharmacological sensitivity to both psychoactive as well as antipsychotic drugs and its general relevance to schizophrenia has resulted in PPI being one of the most commonly used test paradigms in schizophrenia research using animal models. Thus, PPI has been described in a multitude of genetic animal models for candidate genes for schizophrenia [12]. The transmembrane domain *Nrg1* mouse model is one of the few within the group of *Nrg1* animal models exhibiting PPI impairments, although these were not reversible by clozapine [2]. Our research suggests that this PPI deficit is highly PPI protocol-specific and sensitive to test environment differences: in the current study only one protocol (i.e. 'Garvan/Neura') detected a PPI abnormality in *Nrg1* mutant mice and this phenotype was only evident in one of two test locations. Recent research of our collaborators confirms the fragility of any detected PPI deficits, as *Nrg1* mutants exhibited impaired PPI only after acute but not chronic treatment with a vehicle solution [19].

The phenomenon that one PPI protocol reveals different *Nrg1* phenotypes in two different phenotyping facilities (i.e. Garvan and NeuRA) emphasizes the particular sensitivity of the PPI phenotype of the transmembrane domain *Nrg1* mouse model to environmental factors such as test facility characteristics. This finding is in line with observations from our laboratory showing that *Nrg1* mutant mice are more sensitive to environmental manipulations such as minimally enriched housing [15] and pharmacological challenge of the cannabinoid system [18,25]. It is important to mention that the PPI protocol in question has been pharmacologically validated at both sites using MK-801 ([26] and unpublished results) and that the other major phenotypic characteristic of the *Nrg1* mutants (i.e. hyperlocomotion) could be detected in both laboratories even when using different test equipment (Coulbourn Instruments, Whitehall, USA *versus* Med Associates Inc, St. Albans, USA; unpublished results).

The limited effect of genetic manipulations to transmembrane domain *Nrg1* on sensorimotor gating is in line with the absence of PPI deficits in other *Nrg1* models for the EGF-like domain and the ErbB4 receptor [2,27]. A few *Nrg1* mouse models exhibit PPI impairments [28–30] and even reveal predictive validity for schizophrenia as nicotine could reverse the PPI impairments of the type III *Nrg1* model [28]. However, the type I *Nrg1* mutant mouse described by Deakin and co-workers is also characterised by an increased startle response and tremor, both potentially confounding factors for sensorimotor gating measurements, and in Chen et al. [28] 30% of animals were excluded from the analysis. To establish the role of *Nrg1* in sensorimotor gating more globally other currently uncharacterised *Nrg1* animal models would have to be tested for PPI (Table 1). The lack of a pronounced prepulse inhibition deficit across mouse models for a candidate gene of schizophrenia is not limited to mouse models for *Nrg1*. For example, Gogos et al. [31] reported a lack of PPI phenotype in a genetic mouse model for the schizophrenia risk gene *catechol-O-methyltransferase*.

In conclusion, the often-noted PPI phenotype of the transmembrane domain *Nrg1* mutant mouse model is highly PPI protocol-specificity and appears sensitive to even minor environmental factors. Our study describes wild type-like PPI under almost all test conditions and across three different laboratories. Our research suggests that analysing one of the alleged hallmarks of animal models for schizophrenia must be done carefully: to obtain reliable PPI data it seems necessary to use more than one particular PPI protocol. It would be valuable to consider a variety of startle stimulus intensities for prepulse–pulse combinations as part of future research strategies into sensorimotor gating deficits of mutant mouse lines.

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