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COGNITION IN TRANSMEMBRANE DOMAIN *NEUREGULIN 1* MUTANT MICE

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Abstract—Neuregulin 1 (NRG1), which has been implicated in the development of schizophrenia, is expressed widely throughout the brain and influences key neurodevelopmental processes such as myelination and neuronal migration. The heterozygous transmembrane domain *Nrg1* mutant mouse (*Nrg1* TM HET) exhibits a neurobehavioural phenotype relevant for schizophrenia research, characterized by the development of locomotor hyperactivity, social withdrawal, increased sensitivity to environmental manipulation, and changes to the serotonergic system. As only limited data are available on the learning and memory performance of *Nrg1* TM HET mice, we conducted a comprehensive examination of these mice and their wild type-like littermates in a variety of paradigms, including fear conditioning (FC), radial arm maze (RAM), Y maze, object exploration and passive avoidance (PA). Male neuregulin 1 hypomorphic mice displayed impairments in the novel object recognition and FC tasks, including reduced interest in the novel object and reduced FC to a context, but not a discrete cue. These cognitive deficits were task-specific, as no differences were seen between mutant and control mice in spatial learning (i.e. RAM and Y maze) for both working and reference memory measures, or in the PA paradigm. These findings indicate that neuregulin 1 plays a moderate role in cognition and present further behavioural validation of this genetic mouse model for the schizophrenia candidate gene neuregulin 1.

Key words: mouse model, neuregulin 1, cognition, schizophrenia, novel object recognition, fear conditioning.

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Abbreviations: ANOVA, analysis of variance; CS/US, conditioned/unconditioned stimulus; DEX, dexamphetamine; EGF, epidermal growth factor; FC, fear conditioning; GABA, gamma-aminobutyric acid; LTP, long-term potentiation; NMDA, N-methyl-D-aspartic acid; NORT, novel object recognition task; Nrg1, neuregulin 1; *Nrg1* TM HET, heterozygous transmembrane domain *Nrg1* mutant mouse; PA, passive avoidance; RAM, radial arm maze; RM, reference memory; SEM, standard error of the mean; WM, working memory; YM, Y-maze.

The neuregulin 1 gene (*NRG1*) is localized on chromosome 8p12-p21, spans approximately 1.4 Mb (Falls, 2003) and has been reported to be associated with schizophrenia (Stefansson et al., 2002). This polypeptide is part of the growth factor family and Nrg1 mRNA can be found in schizophrenia-relevant brain areas such as the prefrontal cortex, hippocampus, cerebellum, and substantia nigra—in both humans (Law et al., 2004) and rodents (Kerber et al., 2003). Within the nervous system, Nrg1 influences key neurodevelopmental processes such as myelination, synapse formation, neuronal migration, and the regulation of expression/activation of receptors [e.g. N-methyl-D-aspartic acid (NMDA) and GABA receptor A ($GABA_A$)], all of which are thought to be involved in schizophrenia (Corfas et al., 2004; Harrison and Law, 2006). Nrg1 also appears to be involved in both short-term and long-term neural plasticity via a number of mechanisms affecting glutamatergic and GABAergic neurotransmission (Li et al., 2007; Woo et al., 2007). Its involvement in processes such as long-term potentiation (LTP)—a key mechanism underlying learning (particularly in the hippocampus and amygdala)—suggests that Nrg1 plays a role in cognitive processes (Martin et al., 2000; Malenka and Bear, 2004). Importantly, schizophrenia patients experience deficits in cognition, executive function, and memory processes (Heinrichs, 2004; Rasser et al., 2005). Further, memory impairment is a robust and stable feature of schizophrenia that includes deficits in free recall, cued recall and recognition, and does not appear affected by potential moderating factors such as illness duration or severity of psychosis (Aleman et al., 1999).

Alternative splicing of *NRG1* results in at least 30 distinct isoforms (Mei and Xiong, 2008). A missense mutation in exon 11, which codes for the transmembrane region of *NRG1*, is associated with schizophrenia (Walss-Bass et al., 2006). Importantly, the animal model for this transmembrane region, the heterozygous transmembrane domain *Nrg1* mutant mouse (i.e. *Nrg1* TM HET), shows a schizophrenia-relevant neurobehavioral phenotype, which is characterized by age-dependent hyperlocomotion (reversible by clozapine), social withdrawal, increased susceptibility to environmental risk factors such as Δ^9-tetrahydrocannabinol treatment (Stefansson et al., 2002; Boucher et al., 2007; Karl et al., 2007; O'Tuathaigh et al., 2007), and increased levels of cortical $5\text{-}HT_{2A}$ receptors (Dean et al., 2008). Furthermore, changes in short-term potentiation and impaired hippocampal LTP have previously been demonstrated in heterozygous *Nrg1* TM HET mice, and these deficits can be rescued by restoring Nrg1 signalling (Bjarnadottir et al., 2007). Despite cognitive deficits being

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one of the key features of schizophrenia in humans, there has been only one limited characterization of cognitive abilities in *Nrg1* TM HET mice to date: according to this study, *Nrg1* mutants of both sexes exhibited wild type-like spatial learning and memory in the Barnes maze (O'Tuathaigh et al., 2007). Interestingly, other *Nrg1* mouse models have demonstrated moderate learning and memory difficulties, such as the immunoglobulin-like domain *Nrg1* heterozygous mouse, which exhibited impaired latent inhibition (Rimer et al., 2005), and a mouse model for ablations of the Nrg1 receptor ErbB4 in parvalbumin-positive interneurons, which showed deficits in spatial memory (Wen et al., 2010).

To extend the understanding of the role *Nrg1* plays in cognitive processes, we examined the *Nrg1* TM HET mouse model in a battery of cognitive tasks. Using a comprehensive behavioural test battery, which included radial arm maze (RAM), passive avoidance (PA), fear conditioning (FC), novel object recognition (NORT) and Y-maze (YM) (baseline and after acute challenge with dexamphetamine (DEX)), we investigated the impact of a mutation in TM *Nrg1* on learning and memory in adult male mice. A wide range of paradigms was selected to evaluate both working (WM) and reference memory (RM) in basic, spatial and fear-related learning tests.

EXPERIMENTAL PROCEDURES

Animals

The generation of Nrg1 transmembrane domain mutant mice has been described previously (Stefansson et al., 2002). Test animals were different sets of heterozygous *Nrg1*$^{+/-}$ (*Nrg1* TM HET; Set A: $n=10$, Set B: $n=9$, Set C: $n=7$) and wild type-like control *Nrg1*$^{+/+}$ (WT; Set A: $n=12$, Set B: $n=12$, Set C: $n=7$) littermates (backcrossed in $>10^{th}$ generation on C57BL/6JArc background). Genotypes were determined after weaning (postnatal day 21) by polymerase chain reaction amplification (primers for mutant *Nrg1* TM HET mice: Neo173F: 5′-atgaactgcaggacgaggca-3′ and Neo6301R: 5′-gccacagtcgatgaatccag-3′; primers for wild type-like control mice: 5′-aacagcctgactgttaacacc-3′ and 5′-tgctgtccatctgcacgagacta-3′). Age-matched, male, adult test animals of similar genotype were pair-housed [thereby avoiding cohort removal effects; Kask et al., 2001] in polysulfone cages (1144B: Tecniplast, Rydalmere, Australia) under a 12:12-h light/dark schedule [light phase: white light (illumination: 80 lx)—dark phase: red light (illumination: <2 lx)]. Microbiological monitoring revealed no infection of the SPF facility holding room—with the exception of the pathogens commonly found in commercial and research facilities, *Pasteurella pneumotropica* and *Helicobacter spp*. All research and animal care procedures were approved by the "Garvan Institute/St Vincent's Hospital Animal Experimentation Ethics Committee" and were in accordance with the "Australian Code of Practice for the Care and Use of Animals for Scientific Purpose".

Behavioural phenotyping

For habituation purposes all test animals were transported to the testing room 1 h prior to behavioural testing. With the exception of the RAM task all experiments were performed within a time limit of 3 h per day to avoid any influence of the circadian rhythm on the animals' behavioural performance (Kopp, 2001). For this reason, animals were tested on consecutive days at similar times of the circadian rhythm for some paradigms. Environmental odours were removed from test apparatus by cleaning the test arena after each trial with 30% ethanol solution. All behavioural testing was conducted within 6 h after onset of light phase and three sets of mice were used for this investigation: set A in the PA task, baseline YM learning and the RAM testing—set B in the novel object recognition paradigm and the DEX-induced YM learning—set C in the cued and contextual FC paradigms.

Passive avoidance (PA). In this basic hippocampus-dependent learning test, the avoidance of a naturally less aversive dark compartment after it is paired with an electrical footshock indicates the retention of this memory (Bovet et al., 1969). In the training session mice were placed in a highly illuminated compartment (illumination: 70 lx; Shuttle Box System—TSE Systems, Bad Homburg, Germany). After 10 s, the door to a dark chamber was opened. Once the mouse moved into the dark chamber (illumination: <2 lx), the door was closed and a single footshock (0.4 mA for 2 s) was delivered. Mice were kept in the dark chamber for another 60 s to allow the formation of an association between the location and the footshock. In the retention session 24 h later mice were again placed in the light compartment and 10 s later the door connecting the light and dark chambers was opened. The latency to enter the dark chamber was measured each trial, with increased entry latency on the second day taken to indicate memory of the aversive stimulus.

Y-maze memory (YM). The YM apparatus was made of grey acrylic with three similar arms (width: 10 cm; length: 30 cm; height: 17 cm) placed at 120° with respect to each other. Arms were equipped with different internal visual cues placed on the side and end walls of each arm. During the training trial one arm was closed (novel arm) before mice were placed individually into one of the other two arms (start arm) facing the arm end in a quasi-randomized order. Animals were allowed to explore both arms (start and familiar arm) for 10 min. After an inter-trial interval of 1 h, test animals were returned to the YM and allowed to explore all three arms of the maze (start, familiar, and novel) for 5 min (Pang et al., 2006). The following parameters were recorded online: latency to enter and time spent in each arm as well as frequency of arm entries, grooming, and rearing. An arm entry was scored whenever animals entered an arm with more than half of its body length. Mice were tested for baseline performance or 15 min after i.p. treatment with DEX (5 mg/kg body weight; Sigma-Aldrich, Sydney, Australia) to investigate whether DEX has a more pronounced effect on the cognitive performance of *Nrg1* mutant mice.

Radial arm maze (RAM). The RAM is frequently used to study spatial learning and memory capabilities in rodents (Crusio et al., 1987), and can be used to test working and reference memory (Olton and Samuelson, 1976). In this task, rodents are trained to visit a pattern of arms in an eight-arm maze to receive a food reward, which is located at the end of the arms. In order to perform well, the animal must retain the egocentric and allocentric spatial information, that is which of the maze arms it has visited during the course of the task, both between and within trials. Working memory (WM) performance is indicated within each trial by the animal's avoidance of entering arms it has already visited. Reference memory (RM) is demonstrated between trials by the animal selectively visiting previously baited arms.

An eight-arm radial arm maze was used for RAM testing (Pathfinder maze system model 89000B: Lafayette Instrument, Sagamore Parkway North, USA—walls of arms were red-stained—illumination on arms: 70 lx). The maze was located in a room with different visual cues on the walls. At the outer end of each arm was a shallow sunken food cup, which was baited with a drop of sweetened condensed milk (Nestlé Australia Ltd., Rhodes, Australia). Test animals' access to food was limited during testing in order to improve the value of the food rewards. Food restriction was commenced 2 weeks before starting the experiment; mice were kept at 80–90% of the free-feeding pre-

test body weight by restricting their access to food to 2 h/day, directly after completing the last trial of the day during the experiment.

Following 3 days of habituation to the maze (10 min of free exploration twice a day with all eight arms baited), mice were tested for 13 days. Animals were tested three times per day (intertrial interval of at least 1 h) for 5 min or until all food rewards had been located (day 1–13). A distinct pattern of four arms was baited (quasi-randomized between genotypes) and test mice were placed into a different quasi-randomized non-baited start arm each trial. 48 h after the last session, animals were retested twice to examine RM after a 48 h-delay (day 15). Parameters recorded were latency and number of arm entries to complete the task (i.e. to find all four food rewards), order of arm entries, and rearing frequency. WM errors were scored whenever a mouse re-entered an arm already visited during that trial, whereas RM errors were defined as entries into unbaited arms.

Novel object recognition task (NORT). Cognitive performance in the NORT is demonstrated by an animal's recognition of and response to novelty. After being habituated to two identical objects in an open arena, the animal is presented with one of these now familiar objects as well as a novel object. The task therefore investigates both memory and emotionality as indicated by recognition of and response to the new object in a familiar environment, respectively (Misslin and Ropartz, 1981). On day 1, test mice were habituated to the test arena (open field activity test chamber: MED Associates Inc., Vt., USA –43.2×43.2 cm^2). On day 2, animals were placed in the test arena, in which two identical objects had been placed opposite each other along the central line of the arena, and were allowed to explore freely for 10 min. After an intertrial interval of 10 min, mice were placed back into the arena for the 10 min test trial and again exposed to two objects, one from the previous trial (familiar object) and a differently-shaped new object (novel object). The position of the two objects was counterbalanced and randomly permuted for both genotypes.

Animals' behaviour was recorded online and using the automated MED Associates Inc. tracking software (software settings: box size: 3; ambulatory trigger: 2; resting delay: 1000 ms; resolution: 100 ms) whereby activity in two independent zones around the two objects was measured (software coordinates: zone 1=1/6, 1/10, 5/6, 5/10 and zone 2=11/6, 11/10, 15/6, 15/10). Time spent in and motor activity (i.e. overall distance travelled: ODT) within the object zones as well as frequency of nosing towards the objects were analysed. Ratios of ODT and frequencies were calculated to avoid any confound of these spatiotemporal measures/results by the hyperactive phenotype of *Nrg1* TM HET mice (Karl et al., 2007).

Fear conditioning—contextual and cued version (FC). Fear conditioning is a form of associative learning that occurs when a previously neutral stimulus (e.g. tone) elicits fear responses after it has been paired with an aversive stimulus [e.g. electric foot-shock; Sigurdsson et al., 2007]. The FC task can be used to measure emotional learning/memory and emotionality. We used a basic FC task that evaluated both cued and contextual conditioning (Owen et al., 1997). On day 1 test animals were placed into the test chamber (shuttle box–MED Associates Inc.) for 2 min to explore and habituate to the environment, after which the conditioned stimulus (CS: 30 s, 80 dB tone stimulus on a 60 dB white noise background) was paired with a co-terminating unconditioned stimulus (US: electric foot shock of 0.4 mA for 2 s) twice with an inter-pairing interval of 120 s. The test mouse was returned to its home cage 120 s after the second CS–US pairing. 24 h later on day 2 (context test), the mouse was returned to the testing chamber and its percentage freezing response was measured automatically for the following 7 min (FreezeFrame software: Actimetrics, Wilmette, USA; settings: bouts of freezing≥1250 ms; threshold=20), whereby freezing is a fear response characterized by complete behavioural immobility except for natural respiratory motions (Stiedl and Spiess, 1997). On day 3 (cued test), animals were placed in an altered context (i.e. grid floor replaced by a flat plastic floor, presence of cinnamon odour, and a grey PVC divider placed into the chamber). Following the first 120 s, during which no auditory stimulus was presented (pre-CS), the CS was then presented continuously for 5 min. The experiment was terminated after another 120 s without CS. Again, percentage freezing behaviour was examined (van Gaalen and Steckler, 2000).

Statistical analysis

Results were analysed using two- and one-way analysis of variance (ANOVA: between factor: "genotype" and "treatment"), with the exception of PA, FC and RAM, which were also analysed using Repeated Measures ANOVA (within factor: "time"). Fisher-PLSD was used for post hoc comparisons, if appropriate. Differences were regarded as significant if $P<.05$. All data are presented as means±standard error of the mean (SEM). Figures show means+SEM and significant post-hoc effects are indicated by asterisks (* $P<.05$, ** $P<.01$ and *** $P<.001$).

RESULTS

The comprehensive phenotyping strategy revealed task-specific impairments in behaviours reflecting learning and memory processes of *Nrg1* TM mutant mice.

Passive avoidance

A significant increase in latency to enter the dark chamber on the second day indicated that mice had learned to associate foot shock and dark chamber [Repeated Measures ANOVA latency to enter dark chamber: $F(1,20)=55.0$, $P<.001$]. There were no overall baseline differences between wild type-like and mutant mice to enter the dark chamber on day 1 [one-way ANOVA for "genotype" effect on latency: $F(1,20)=1.1$, $P=.3$], nor any evidence of impaired learning in *Nrg1* TM HET mice on day 2, as there were no genotype differences in the increased latency to enter the dark chamber [Repeated Measures ANOVA latency to enter dark chamber, "genotype" over "time": $F(1/20)=.009$, $P=.9$; Table 1].

Baseline and DEX-induced Y-maze performance

Animals of both genotypes showed similar exploration of the unfamiliar novel arm of the YM under baseline conditions, as measured by the time spent in this arm [one-way ANOVA for factor "genotype": $F(1/11)=2.1$, $P=.2$] and entry ratio into this arm [$F(1/11)=.8$, $P=.4$—Table 2]. Similarly, no genotype differences were seen in the novel arm exploration after DEX treatment [time spent in novel arm: $F(1/16)=2.8$, $P=.1$—entry ratio into novel arm: $F(1/16)=7.5$; $P=.7$, Table 2], despite DEX treatment having a

Table 1. Passive avoidance performance during training (day 1) and test trial (day 2)—measured as latency to enter dark chamber (latency) [s]

Parameter	WT	*Nrg1* TM HET
Latency day 1	120.4±24.1	90.1±16.9
Latency day 2	266.5±15.7	240.0±35.6

Table 2. Y-maze memory—measured as time spent in unfamiliar arm (novel time) [s] and ratio of entries into the unfamiliar arm compared to total arm entries (novel ratio) [%] at baseline and after i.p. treatment with dexamphetamine (5 mg/kg body weight–15 min prior to test)

Baseline	WT	*Nrg1* TM HET
Novel time	56.1±16.4	86.8±27.8
Novel ratio	43±4	34±4
After DEX treatment	WT	*Nrg1* TM HET
Novel time	123.4±44.4	182.5±14.6
Novel ratio	32.8±2.3	33.7±1.2

significant main effect on both parameters, increasing novel arm exploration ["treatment": time spent in novel arm: $F(1/28)=12.6$, $P=.001$—entry ratio into novel arm: $F(1/28)=533.1$; $P<.0001$].

Spatial memory in the radial arm maze

The significant overall reduction in WM and RM errors as well as total number of entries until the task was completed over time demonstrate that mice learned successfully to complete the task by locating the food rewards [Repeated Measures ANOVA for "time": WM errors: $F(12,20)=13.2$, $P<.0001$—RM errors: $F(12,20)=31.0$, $P<.0001$—total number of entries: $F(12,20)=17.2$, $P<.0001$; Fig. 1A–C]. The apparent increase in WM errors in *Nrg1* TM HET compared to WT mice was not significant [one-way ANOVA for factor "genotype": $F(1,20)=1.2$, $P=.3$—Repeated Measures ANOVA for "genotype" over "time": $F(12,20)=1.0$, $P=.5$; Fig. 1A]. Similarly, there were no differences between genotypes in the number of entries to find all baited arms [one-way ANOVA for "genotype": $F(1,20)=.3$, $P=.6$—Repeated Measures ANOVA for "genotype" over "time": $F(12,20)=.7$, $P=.8$] or in RM errors ["genotype": $F(1,20)=.9$, $P=.4$—"genotype" over "time": $F(12,20)=.9$; $P=.6$; Fig. 1B, C]. Furthermore, spatial memory performance remained intact in mutant mice after a 48 h delay, as there were no significant genotype differences in any of the parameters investigated (Fig. 1A–C).

Novel object recognition task

Impaired recognition memory was seen in *Nrg1* TM HET mice in the NORT, as mutant mice showed a significant reduction in the ratio of nosing towards the novel object compared to the overall nosing frequency, relative to WT mice [one-way ANOVA for "genotype": $F(1/16)=5.8$, $P=.03$; Fig. 2A]. No genotype differences were seen, however, in the ratio of time spent or motor activity ratio in the zone containing the novel object [ratio of time spent: $F(1/16)=.16$, $P=.7$—motor activity ratio: $F(1/16)=1.0$, $P=.3$; Fig. 2B]. The apparent increase in overall nosing frequency in *Nrg1* TM HET mice was not significant ["genotype": $F(1,16)=.7$; $P=.4$; WT=10.8±4.4 vs. *Nrg1* TM HET=17.7±4.9].

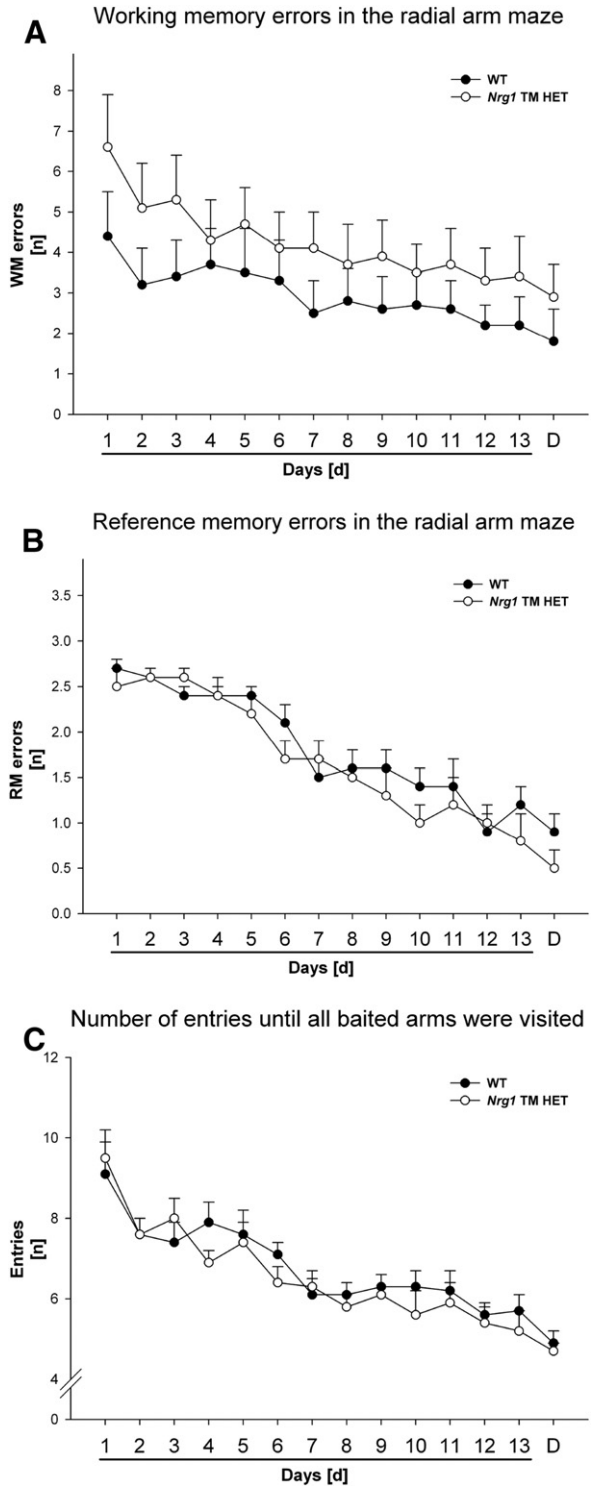

Fig. 1. (A–C). Spatial memory in the radial arm maze: (A) frequency of working memory errors (i.e. re-entry [*n*] into a previously visited arm), (B) reference memory error (i.e. entry [*n*] into a non-baited arm) frequency, and (C) arm entries [*n*] until task completed (i.e. all baited arms visited once). Mean±SEM are shown for the 13 d of testing and the 48 h delay trials D. Repeated Measures ANOVA revealed a significant effect over time for all parameters but not a factor "genotype" or "genotype" over "time" interaction.

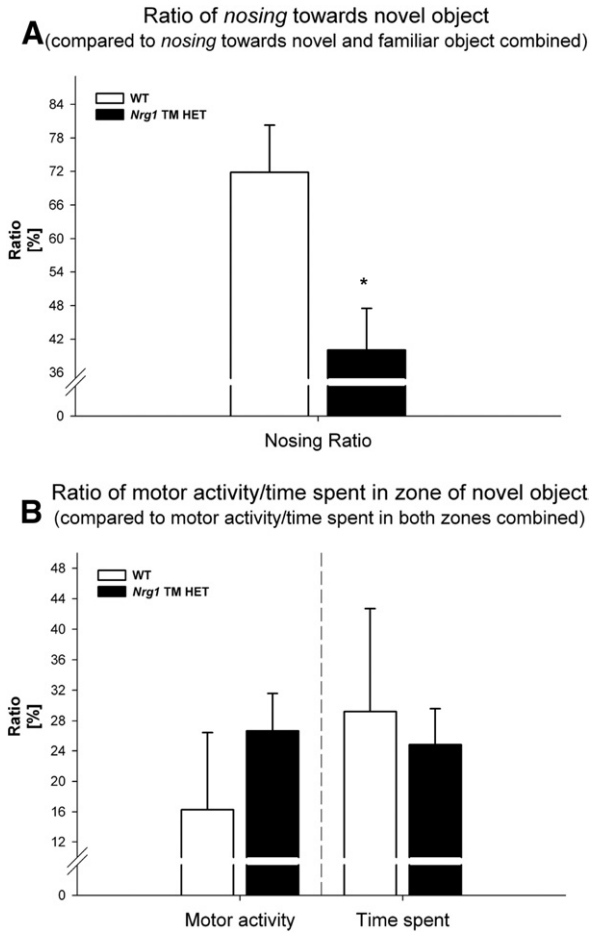

Fig. 2. (A, B) Novel object recognition: (A) ratio [%] of novel object nosing frequency to overall nosing frequency; and (B) ratio [%] of motor activity (i.e. overall distance travelled)/of time spent in the zone of the novel object compared to overall motor activity/time spent in the zone of novel and familiar object. Mean±SEM are shown. Significant post-hoc effects versus WT mice are indicated by asterisks (* $P<.05$).

Fear conditioning

Nrg1 TM HET mice demonstrated impaired conditioned fear to a context but not discrete auditory cue conditioning. Conditioning to the auditory cue (i.e. percentage of freezing) was not significantly reduced in *Nrg1* TM HET mice, when tested on the third day [one-way ANOVA for "genotype": $F(1/12)=2.6$, $P=.1$; Fig. 3A]. In contrast, *Nrg1* TM HET mice showed a reduced fear response to the context in which they had been conditioned, compared to their WT counterparts [$F(1/12)=11.1$, $P=.006$; Fig. 3B], with lower freezing scores in *Nrg1* TM HETs during the test period. The reduced freezing behaviour seen in mutant mice when placed in the conditioned context was not evident prior to conditioning, as there were no significant baseline genotype differences during the first 120 s of the conditioning phase prior to the first tone-shock presentation (percentage of baseline freezing: WT=6.9±3.3 vs. *Nrg1* TM HET=3.4±1.6).

DISCUSSION

Domain-specific cognitive deficits were found in transmembrane domain neuregulin 1 heterozygous mice in selected behavioural paradigms. *Nrg1* mutant mice demonstrated reduced novel object recognition and impaired FC to the context. However, learning impairments were not universal, as *Nrg1* TM HETs showed intact spatial learning and memory abilities in the RAM and wild type-like conditioning in the PA task as well as the cued FC task. Similarly, intact working memory was seen in these mice in the YM.

In the NORT, memory of a familiar object is demonstrated by an increased or dishabituated interest in a novel object, when it is presented simultaneously together with a familiar object. The two-trial recognition task applied in this study is a useful tool to screen for basic learning and memory abilities, as it does not require spatial learning, reinforcement or exposure to highly stressful situations (Dere et al., 2007). *Nrg1* TM HET mice demonstrated reduced exploration of a novel object compared to WT mice, in the absence of any overall changes in object exploration. Hippocampal glutamatergic activity appears to

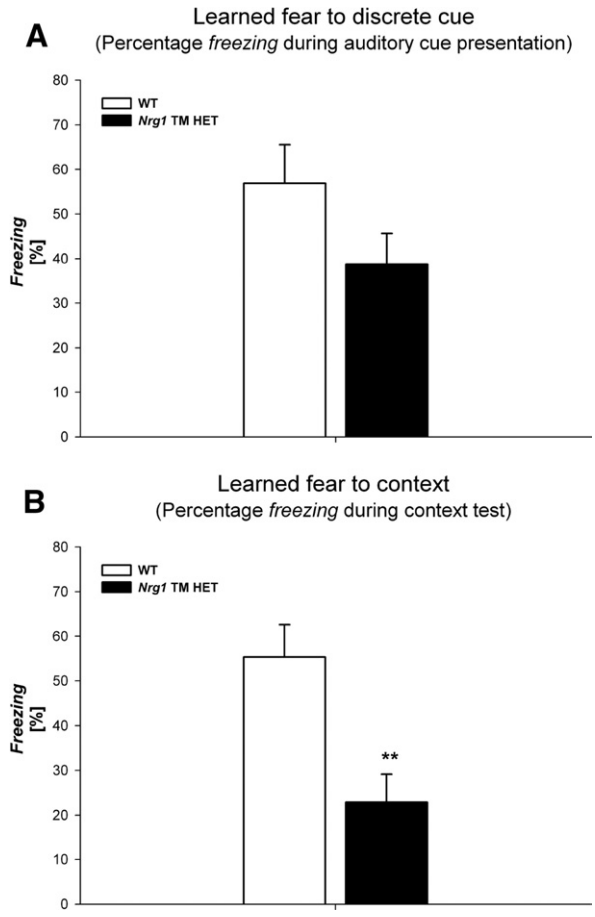

Fig. 3. (A, B) Fear conditioning: (A) percentage freezing in the cue trial over the 5 min presentation of the auditory cue; and (B) percentage freezing in the context test over 7 min. Mean±SEM are shown. Significant post-hoc effects versus WT mice are indicated by asterisks (** $P<.01$).

be involved in object recognition memory, particularly for intertrial intervals longer than 10 min (Clark et al., 2000), although other brain regions including the perirhinal cortex have also been implicated (Winters and Bussey, 2005a,b), and NMDA receptor antagonism has been shown to reduce recognition of a familiar object in the novel object task (Nilsson et al., 2007). Importantly, Nrg1 has been shown to modulate glutamatergic neurotransmission (Li et al., 2007) and can also affect NMDA receptor activity without altering NMDA receptor expression (Gu et al., 2005; Hahn et al., 2006). Dean and colleagues (2008) reported wild type-like levels of NMDA receptors in *Nrg1* TM HETs and similarly, lack of Nrg1-ErbB signaling during development had no discernible effect on brain morphology or the development/function of NMDA $NR2_C$ receptors (Gajendran et al., 2009). However, other studies have demonstrated reduced phosphorylation of $NR2_B$ receptor subunits in the *Nrg1* TM HET mouse (Bjarnadottir et al., 2007), and a down-regulation of NMDA receptors in the forebrain of epidermal growth factor (EGF)-like domain *Nrg1* HET mice (Stefansson et al., 2002). Therefore, the extent to which impaired glutamatergic neurotransmission subsequent to dysfunctional Nrg1 signalling is involved in the NORT deficits remains to be clarified. In addition to the glutamatergic system, the serotonergic system also appears to influence recognition memory, as 5-HT inactivation impairs the performance of object recognition in the rat (Lieben et al., 2006). Importantly, mutant *Nrg1* TM HET mice exhibit an imbalanced serotonergic system, characterized by increased 5-HT_{2A} receptor and 5-HT transporter (Dean et al., 2008), which could have been involved in the observed memory deficits. No differences have been identified in serotonin 5-HT_{1A} receptor density in the forebrain of *Nrg1* HET compared to WT mice (van den Buuse et al., 2009).

The reduced interest in the novel object seen in mutant mice could also indicate neophobia (Hughes, 2007), although it is anticipated that this would also manifest in other paradigms such as the YM, which was not the case. Interestingly, the observed reduction in novelty preference is consistent with the reduced interest in a novel conspecific that has previously been seen in TM domain *Nrg1* HET mice (O'Tuathaigh et al., 2007). It should be noted that the reduced interest in the novel object was specific to nosing behaviour, as ratios of time spent and motor activity in the vicinity of the novel object were not altered in mutant mice. *Nrg1* TM HET mice showed reduced conditioned fear to the context in which they were conditioned, but not to a discrete conditioned auditory cue. Importantly, no baseline differences were seen between mutant and wild type-like mice in freezing behaviour, indicating that baseline differences in mobility/freezing were not responsible for the differences observed. Extensive evidence indicates that the amygdala is a key brain region involved in fear learning [for review see; Kim and Jung, 2006]. It is understood that the CS-US association is formed within the amygdala, via LTP or other synaptic plasticity mechanisms [for details see; Sigurdsson et al., 2007]. Subsequent CS presentations consequently lead to the activation of amygdalar projections to various downstream structures, eliciting a preparatory fear response. The reduced fear learning to the background context that was seen in *Nrg1* mutant mice suggests a disruption of this mechanism. Importantly, contextual FC is dissimilar to discrete cue conditioning in that it requires an intact hippocampal formation (Paylor et al., 1994). Unlike auditory tone conditioning, context conditioning requires LTP in the hippocampus for the formation of long-term memory (Stiedl et al., 1999). *Nrg1*–Erb signalling in the hippocampus is necessary for LTP (Li et al., 2007), and Nrg1 deficiency is known to impair both short-term plasticity and LTP in mice (Bjarnadottir et al., 2007). The mutation to the mouse *Nrg1* gene might therefore affect the neural plasticity underlying LTP, thereby impairing the formation or consolidation of context conditioning memory. Contextual conditioning can also be seen as a more complex task, as it requires the configuration of multisensory stimuli into a unitary representation (Rudy et al., 2004). Therefore, impaired context conditioning could also reflect a differential response to the inherently more complex nature of the task, as *Nrg1* TM HET mice have previously demonstrated increased sensitivity to a more complex environment (Karl et al., 2007). Importantly, *Nrg1* TM HET mice showed intact performance in the PA task indicating that not all fear learning is impaired.

The cognitive impairments seen in *Nrg1* TM HET mice were not universal, as no genotype differences in either reference or working memory were seen in the hippocampus-dependent spatial learning task, the RAM. *Nrg1* TM HET mice also showed wild type-like exploration of the novel arm of the YM. The intact spatial learning and memory in the RAM is consistent with previous investigation of *Nrg1* TM HET mice in the Barnes maze (O'Tuathaigh et al., 2007). Similarly, the intact WM of *Nrg1* TM HET mice is consistent with the WT-like spontaneous alternation previously observed in EGF-like and TM domain *Nrg1* HET mice and intact T-maze performance in Immunglobulin-like domain *Nrg1* HET mice.

As mentioned above, the learning and memory impairments seen in *Nrg1* TM HET mice could be due to altered synaptic plasticity, which is known to be impaired in these mice (Bjarnadottir et al., 2007). The cognitive deficits observed in *Nrg1* mutant mice could be linked to impaired dendritic spine maturation, which can be caused by deficient Nrg1-ErbB2/B4 neurotransmission (Barros et al., 2009) and is involved in the short- and long-term neural plasticity necessary for learning and memory (Kasai et al., 2010). Nrg1's impact on the development of GABA-mediated circuits (Fazzari et al., 2010) might also be involved, as GABAergic function has been indicated in schizophrenia-related cognitive deficits (Lewis et al., 2005).

When administered prior to encoding, DEX has been shown to reduce learning and memory at subsequent test trials (Wood and Anagnostaras, 2009), as the dopamine system is involved in learning and memory (Myhrer, 2003). Importantly, animal models for schizophrenia can show increased sensitivity to DEX treatment. However, there was no difference in the effects of treatment with DEX on the cognitive performance of *Nrg1* TM HET mice when compared to WT mice, confirming the results of recent

studies demonstrating WT-like responses to psychotropic drugs of *Nrg1* TM HET mice in the open field and prepulse inhibition task and WT-like dopamine D_2 receptor density in the forebrain of *Nrg1* TM HET mice (Ehrlichman et al., 2009; van den Buuse et al., 2009).

It is interesting to speculate whether the cognitive deficits of *Nrg1* mutants could be rescued by antipsychotic drugs (APDs). Clozapine has been shown to rescue the hyper locomotive phenotype of *Nrg1* TM HET mice without affecting WT mice (Stefansson et al., 2002). Clozapine acts on dopamine D_2 receptors, but also has strong affinity for 5-HT_{2A} receptors, in addition to muscarinic, histaminergic, and glutamatergic receptors (Miyamoto et al., 2005). Although there is little evidence for altered dopamine in *Nrg1* TM HET mice, alterations to the serotonergic system have been identified (Dean et al., 2008) and could present a potential mechanism to improve the cognitive performance of mutant mice. Further studies will be necessary to clarify the effectiveness of clozapine and other APDs on the cognitive phenotype of *Nrg1* mutant mice.

CONCLUSION

In conclusion, *Nrg1* TM HET mice demonstrated task-specific impairments in cognition, including deficits in fear conditioning and recognition of a previously encountered object. These findings suggest that neuregulin 1 plays a moderate role in learning and memory performance, and provide further evidence that *Nrg1* TM HET mice represent a model for investigating the gene's impact on schizophrenia-relevant behavioural domains. Given the complexity of the neural mechanisms by which Nrg1 affects the neurotransmitter systems, including glutamate, 5-HT and GABA (Woo et al., 2007; Dean et al., 2008), further investigations are necessary to delineate the task-specific learning and memory deficits in mutant mice and their underlying causal mechanisms in more detail.

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