Mice deleted for the DiGeorge/velocardiofacial syndrome region show abnormal sensorimotor gating and learning and memory impairments

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Del22q11 syndrome is caused by heterozygous deletion of an ∼3 Mb segment of chromosome 22q11.2. Children diagnosed with del22q11 syndrome commonly have learning difficulties, deficits of motor development, cognitive defects and attention deficit disorder. They also have a higher than normal risk for developing psychiatric disorders, mainly schizophrenia, schizoaffective disorder and bipolar disorder. Here, we show that mice that are heterozygously deleted for a subset of the genes that are deleted in patients have deficits in sensorimotor gating and learning and memory. The finding of sensorimotor gating deficits is particularly significant because patients with schizophrenia and schizotypal personality disorder show similar deficits. Thus, our deletion mouse models at least two major features of the del22q11-associated neurobehavioral phenotype, and as such, represents an animal model of this complex behavioral phenotype. These findings not only open the way to pharmacological analyses that may lead to improved treatments, but also to the identification of gene/s that modulate these specific behaviors in humans.

INTRODUCTION

Del22q11 syndrome, which includes DiGeorge syndrome (OMIM no. 188400) and velocardiofacial syndrome (OMIM no. 192430), is caused by haploinsufficiency of one or more genes in an ∼3 Mb region of chromosome 22q11.2 that is heterozygously deleted in patients (1–4). Del22q11 syndrome is the most common chromosomal deletion disorder in humans, having an estimated prevalence of approximately 1:4000 livebirths (5). Most cases are sporadic, but the deletion can also be inherited from a mildly affected parent (6). The del22q11 syndrome phenotype has key components that are thought to derive from abnormal development of the pharyngeal system (7,8), namely, cardiovascular, thymic, parathyroid and craniofacial abnormalities (9,10). In addition, patients have neurobehavioral problems (10,11) of unknown etiology. These include impaired cognitive development in 70–80% of patients (12–14), though severe mental retardation is rare (5,12), attention deficit disorder (11,15,16), deficits in speech and language development (5,12,13) and deficits in fine and gross motor development (13,17,18). In addition, several studies of del22q11 syndrome patients have reported an increased prevalence of neuropsychiatric disorders, especially schizophrenia, schizoaffective disorder and bipolar disorder (16,19,20). Current estimates are that ∼35% of patients develop psychiatric disorders in adolescence or adulthood (21). However, since diagnostic testing has become available only relatively recently, many patients are still children. Therefore, the overall risk to patients for developing psychiatric disorders is not known.

The neurobehavioral and psychiatric aspects of del22q11 syndrome are a major challenge to the clinical management of these patients, and they remain a lasting burden to patients and caregivers alike, after physical problems have been corrected (heart defects, cleft palate), or have ameliorated with treatment and time (hypocalcemia and immune defects).

We have modeled the 22q11 deletion in the mouse (22). Using chromosome engineering techniques, we created a heterozygous deletion of ∼1 Mb (Df1) in a region of mouse chromosome 16 that is homologous to del22q11. The Df1 deletion encompasses 18 of the estimated 30 genes that are deleted in the majority of del22q11 syndrome patients. Heterozygously deleted mice (Df1/+) have cardiovascular defects that are similar to those seen in patients (22). We and others have recently shown that Tbx1 haploinsufficiency causes the cardiovascular phenotype in Df1/+ mice (23–25).

To ascertain whether Df1/+ mice exhibit aspects of the del22q11 syndrome neurobehavioral phenotype, we tested Df1/+ mice in a variety of experimental settings that assess different domains of central nervous system functioning. The tests included prepulse inhibition (PPI) of the acoustic startle response, which is a measure of sensorimotor gating, and the Pavlovian conditioned fear test, which assesses learning and memory. Additionally, simple tests of sensory/motor function,

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exploratory activity, anxiety-related traits and analgesia-related responses were evaluated.

RESULTS

Identification of sensorimotor gating deficits in Df1/+ mice

Sensorimotor gating is a neural process that filters and inhibits rapidly incoming sensory, cognitive and motor information to facilitate proper integration of complex stimuli (26). Sensorimotor gating can be operationally measured using the PPI of the acoustic startle response assay. PPI is the phenomenon in which a weak prestimulus, or prepulse, suppresses the response to a subsequent startle stimulus (27,28). Reduced PPI of the startle response is indicative of impaired sensorimotor gating.

To establish whether the Df1 heterozygous deletion affects sensorimotor gating, we measured PPI of the acoustic startle response. Nineteen Df1/+ mice (nine female, 10 male) and 21 wild-type littermates (10 female, 11 male) were tested. The behavioral studies were performed using two batches of sibling mice of a mixed C57BL/6–c–:129S5/SvEvBrd background. During the startle stimulus alone trials (Materials and Methods) we found that the overall startle response of Df1/+ mice was significantly greater than the startle response of wild-type mice (P = 0.008; Fig. 1A). In addition, for both genotypes, males startled more than females (P = 0.00003; Fig. 1B). Finally, there was a significant interaction between genotype and gender (P = 0.004), due to the fact that Df1/+ males startled significantly more than wild-type males (P = 0.0003), but Df1/+ and wild-type females had similar startle responses (P = 0.86). Therefore, Df1/+ mice have an altered startle response, but it is only seen in mutant males.

For both Df1/+ and wild-type mice, levels of PPI increased as the prepulse sound levels increased (P < 0.00001; Fig. 1C). However, PPI levels were significantly reduced in Df1/+ mice compared to wild-type mice (P = 0.009). There was no overall difference in PPI responses between males and females (P = 0.557), or in the interaction between gender and genotype (P = 0.269). Simple effect analyses of a triple interaction between genotype, gender and prepulse sound level (P = 0.022) revealed that Df1/+ mice of both sexes had significantly lower levels of PPI than wild-type mice at both the 74 and 78 dB prepulse sound levels (P < 0.03). At the 82 dB sound level, the difference was significant in males (P = 0.003) but not females (P = 0.64) and, at the two highest prepulse sound levels, the two genotypes were not significantly different (P > 0.16). Overall, these results indicate that Df1/+ mice have impaired sensorimotor gating, especially at lower prepulse sound levels.

The finding of lower levels of PPI in Df1/+ mice compared with wild-type mice, could indicate that their overall sensitivity to low level sounds is impaired. Therefore, we performed a startle threshold experiment with the second batch of mice to further explore these differences. If Df1/+ mice have generalized impaired sound sensitivity, we would expect that the sound intensity required to produce a startle response would be higher in Df1/+ mice than in wild-type mice. The startle threshold experiment revealed that the sound level required to elicit a significant startle response [i.e. startle magnitude significantly (P < 0.05) above response to background sound level] was in fact significantly lower in Df1/+ mice (90 dB) than in wild-type mice (94 dB). This suggests that PPI impairments in Df1/+ mice are not due to a reduced sensitivity to low level sounds.

Df1/+ mice have specific learning and memory defects

As del22q11 syndrome patients have learning difficulties and cognitive deficits, we have studied Pavlovian conditioned fear paradigm to assess learning and memory in Df1/+ mice. Two different batches of mice were tested for a total 32 Df1/+ (20 female and 12 male) and 32 wild-type (20 female and 13 male) mice. The results showed that Df1/+ mice displayed significantly less freezing than wild-type mice when they were returned to the context (context test) where they had received the shock 24 h after training (P = 0.012; Fig. 2A). However, their level of freezing in the presence of the auditory...
conditioned stimulus (CS) test was similar for both the wild-type and Df1/+ mice (P = 0.195; Fig. 2B). These results indicate that following a 24 h delay, Df1/+ mice have impaired contextual fear conditioning, but normal auditory-cued conditioned fear. There were no gender differences or gender × genotype interactions for either the context or CS tests (P > 0.1), and there were no significant differences between the two batches of mice and no batch × genotype interactions (P > 0.1). To determine whether poor contextual freezing in Df1/+ mice was delay-dependent, a separate batch of mice was given the same training, but the context test was given 1 h after training. Under these conditions, Df1/+ mice (four female, three male) displayed similar levels of freezing as wild-type mice (three female, five male, P = 0.853; Fig. 2C) indicating that the contextual fear impairment is delay dependent.

In additional behavioral tests that are listed in Table 1, there were no differences between Df1/+ and wild-type mice (data not shown).

### DISCUSSION

The purpose of the present study was to characterize the behavioral phenotypes of Df1/+ mice to determine whether these mutants could be a useful model in which to investigate the genetic and cellular basis for the neurobehavioral abnormalities associated with del22q11 syndrome. The PPI paradigm has become a widely used assay to study sensorimotor gating, because it is one of the few neuropsychological tests that can be similarly evaluated in humans and rodents, thus making it a particularly relevant test for mouse models of human diseases.

PPI responses are reduced in patients with del22q11 syndrome, despite their predisposition to schizophrenia. The PPI assay relies on the ability to hear. Therefore, hearing loss could affect or even abolish the PPI response. There are several reasons why we think that the PPI deficits in Df1/+ mice are not directly related to potential hearing deficits. First, Df1/+ mice show inhibition of the startle response even at low PPI sound levels (74 dB is only 4 dB above background noise), indicating that they hear and process this level of sound, so they are not deaf. Second, the results of the startle threshold experiment suggest that Df1/+ mice may actually be more sensitive to low level sounds, which is inconsistent with the notion that the poor PPI response is related to an inability to detect the sounds. Third, the same sound level (80 dB) that results in impaired PPI, elicits normal levels of freezing during the CS test phase of the conditioned fear test. If Df1/+ mice could not detect this sound level in the PPI paradigm, then they should not have been able to detect it during the CS test, which would have resulted in abnormal levels of freezing. It is also

### Table 1. Normal responses of Df1/+ mice on assays in a behavioral test battery

<table>
<thead>
<tr>
<th>Test/assay</th>
<th>CNS trait(s)</th>
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<tr>
<td>Neurological screen</td>
<td>Simple sensory/motor function</td>
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<tr>
<td>Open-field</td>
<td>Exploratory activity and anxiety-related responses</td>
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<td>Light-dark</td>
<td>Anxiety-related responses</td>
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<tr>
<td>Rotarod</td>
<td>Motor coordination and skill learning</td>
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<tr>
<td>Acoustic startle habituation</td>
<td>Sensorimotor adaptation</td>
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<tr>
<td>Hotplate</td>
<td>Analgesia-related responses</td>
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unlikely that the PPI deficits are attributable to generalized neurological problems, because in other tests of neurological function (Table 1), Df1/+ mice behaved like wild-type mice. Rather, it suggests that one or more genes in the Df1 deletion modulates sensorimotor gating and acoustic startle responses in mice, and that heterozygous loss of this gene (or genes) is responsible for reduced PPI responses in Df1/+ mutants.

The responses of Df1/+ mice in the Pavlovian conditioned fear test suggest that they have difficulty remembering the types of cues associated with a complex training environment for long (24 h), but not short (1 h) periods. Df1/+ mice successfully learn and remember that a single auditory cue was paired with a footshock, suggesting that the conditioned fear impairment is selective. Similar selective deficits have been seen in rodents with hippocampal damage (30,31), suggesting that Df1/+ mice may have impaired hippocampal function.

Overall, the results of the behavioral tests show the Df1/+ mice have specific deficits that are related to those found in del22q11 syndrome patients and patients with schizophrenia, particularly in the areas of processing of complex information such as the learning and remembering of complex cues and sensorimotor gating.

Amongst the genes that are heterozygously deleted in Df1/+ mice, there are two candidate behavioral genes, Prodh, which encodes proline dehydrogenase and Comt, which encodes catechol-O-methyltransferase. Mice homozygous for a missense mutation in Prodh are hyperprolinemic and have sensorimotor gating deficits (32), but the behavioral phenotype of heterozygous mice has not been reported. The amount by which PPI is reduced in mutant versus wild-type animals is greater in Df1/+ mice (~15% reduction) than in the Prodh mutants (~9% reduction) (32) at similar startle/prepulse sound levels, indicating that Df1/+ mice are more severely impaired. However, Df1/+ mice have normal serum proline levels (data not shown), suggesting that sensorimotor gating deficits in Df1/+ mice do not result from defective proline metabolism due to Prodh haploinsufficiency. Mice heterozygous for Comt have normal sensorimotor gating (33), and we did not observe the increased aggressivity in that was reported in heterozygous males.

We recently studied the same behavioral responses of mice with a 150 kb deletion that partially overlaps with Df1 (34). In contrast to our findings in Df1/+ mice, mice with the 150 kb deletion mice had significantly enhanced PPI. We do not know the reason for this unexpected finding, but it may relate to heterozygosity of one of the four genes not shared with Df1, as Df1/+ mice do not have this behavior. Alternatively, the 150 kb deletion may have an effect on expression of a distant gene that affects sensorimotor gating. In any case, we believe that Df1/+ mice may be a more appropriate animal model for studying the neurobehavioral dysfunctions that underly some of the behavioral abnormalities associated with del22q11 syndrome.

Whether the behavioral phenotype in patients is caused by heterozygous loss of a single gene or multiple genes is unknown. However, a genetic dissection of the homologous mouse region, similar to that used to identify that Tbx1 haploinsufficiency causes cardiovascular defects in Df1/+ mice (23), is likely to identify the genetic basis of the behavioral phenotype. This in turn may point to genetic pathways that are relevant to schizophrenia and learning disorders.

**MATERIALS AND METHODS**

**Mouse breeding and genotyping**

Behavioral studies were performed on mice of a mixed C57BL/6–/–;129S5/SvEvBrd background that had been generated by backcrossing Df1/+ males with wild-type C57BL/6–/– females for four or five generations. There is no evidence of imprinting in the human syndrome (3), and in Df1/+ mice, the cardiovascular phenotype at least is the same whether the deletion is inherited paternally or maternally (35). Male and female mice were housed in groups of three to five per cage throughout the study period and were genotyped by PCR using DNA prepared from tail biopsies.

**Behavioral studies**

Behavioral studies were carried out on male and female littermates that were 2 months old at the beginning of testing. In order to improve reliability of test results, mice were divided into batches and tested on different days. The experimenter performing the tests was blind to the genotype of the mice. The neurological screen, open-field exploration test, light–dark test, rotarod test, startle habituation test and hotplate test were performed as described in McIlwain et al. (36).

**Prepulse inhibition.** Sensorimotor gating can be measured by PPI of the startle response, which is modulation of the startle by a weak prepulse (28). PPI of acoustic startle responses was measured using the SR-Lab System (San Diego Instruments, San Diego, CA) as described previously (37). A test session began by placing a mouse in the Plexiglas cylinder where it was left undisturbed for 5 min. A test session consisted of seven trial types. One trial type was a 40 ms, 120 dB sound burst used as the startle stimulus. There were five different acoustic prepulse plus acoustic startle stimulus trials. The prepulse sound was presented 100 ms before the startle stimulus. The 20 ms prepulse sounds were 74, 78, 82, 86 or 90 dB. Finally, there were trials where no stimulus was presented to measure baseline movement in the cylinders. Six blocks of the seven trial types were presented in pseudorandom order such that each trial type was presented once within a block of seven trials. The average intertrial interval was 15 s (ranged from 10 to 20 s). The startle response was recorded for 65 ms (measuring the response every 1 ms) starting with the onset of the startle stimulus. The background noise level in each chamber was 70 dB. The maximum startle amplitude recorded during the 65 ms sampling window was used as the dependent variable.

The following formula was used to calculate percent PPI of a startle response: $100 - \left( \frac{\text{[startle response on acoustic prepulse + startle stimulus trials / startle response alone trials]} \times 100} {\text{[startle response alone trials]}} \right)$. Thus, a high percent PPI value indicates good PPI, i.e. the subject showed a reduced startle response when a prepulse stimulus was presented compared to when the startle stimulus was presented alone. Conversely, a low percent PPI value indicates poor PPI, i.e. the startle response was similar with and without the prepulse.

We conducted separate experiments to optimize PPI assays for future studies and found the 78 dB prepulse to be the optimal sound level. At this sound level, PPI levels in mutant and wild-type mice were not significantly different from the
original experiments, demonstrating the reproducibility of the PPI test under these conditions (data not shown).

Startle threshold. The startle threshold was determined in the second batch of mice (16 DfJ/+ mutants (seven female, nine male) and 20 wild-type (10 female and 10 male) using the procedures outlined previously (36). The startle response threshold test session began by placing a subject in the startle chamber where it was left undisturbed for 5 min. Each subject was then presented with 65 trials over the 16 min test session. There were 13 different sound levels (dB) presented: 70, 74, 78, 82, 86, 90, 94, 98, 102, 106, 110, 114 and 118. Each stimulus was 40 ms and was presented five times in a pseudorandom order, such that each sound level was presented within a block of 13 trials. The average interstimulus interval was 15 s. The background noise level in each chamber was 70 dB. The maximum startle amplitude recorded during the 65 ms sampling window was used as the dependent variable.

Pavlovian conditioned fear. Two to 3 weeks later performance in a conditioned fear task was measured as described in Paylor et al. (38), using the Freeze Monitor system (San Diego Instruments). The test chamber (26 \( \times \) 22 \( \times \) 18 cm high) was made of clear Plexiglass and surrounded by a photobeam detection system (12 \( \times \) 10 beams). The bottom of the test chamber was a grid floor used to deliver a mild electric shock. The test chamber was placed inside a sound attenuated chamber (Med Associates; internal dimensions, 56 \( \times \) 38 \( \times \) 36 cm). Mice were observed through windows in the front of the sound-attenuated chamber. A mouse was placed in the test chamber (house lights ‘ON’) and allowed to explore freely for 2 min. A white noise (80 dB), which served as the CS, was then presented for 30 s followed by a mild (2 s, 0.5 mA) foot-shock, which served as the unconditioned stimulus (US). Two minutes later another CS–US pairing was presented. The mouse was removed from the chamber 15–30 s later and returned to its home cage. Freezing behavior was recorded using the standard interval sampling procedure every 10 s. Responses (run, jump, squeak) to the foot-shock were recorded. If a mouse did not respond to the foot-shock it was excluded from the analysis. All mice used in both experiments responded to the foot-shock. Twenty-four hours or 1 h later, the mouse was placed back into the test chamber for 5 min and the presence of freezing behavior was recorded every 10 s (context test). Two hours later, the mouse was tested for its freezing to the auditory CS. Environmental and contextual cues were changed for the auditory CS test: a black Plexiglass triangular insert was placed in the chamber to alter its shape and spatial cues, red house lights replaced the white house lights, the wire grid floor was covered with black Plexiglass and vanilla extract was placed in the chamber to alter the smell. Finally, the sound attenuated chamber was illuminated with red house lights. There were two phases during the auditory CS test. In the first phase (pre-CS), freezing was recorded for 3 min without the auditory CS. In the second phase, the auditory CS was turned on and freezing was recorded for another 3 min. The number of freezing intervals was converted to a percent freezing value.

Data analyses. Acoustic response amplitude data were analyzed using two-way (genotype \( \times \) gender) ANOVAs. PPI data were analyzed using a three-way (genotype \( \times \) gender \( \times \) prepulse sound level) ANOVA with repeated measures. For the startle threshold study, the DfJ/+ and wild-type data were analyzed individually using an ANOVA with repeated measures, followed by planned contrast comparisons in which the response to each stimulus intensity was compared to the response following the presentation of the 70 dB (baseline, background noise level) stimulus. The threshold response was defined as the stimulus level which produced a significantly higher response (\( P < 0.05 \)) compared to the baseline response measured to the 70 dB sound. To ensure that this was a stable response, the response to each of the remaining stimuli also had to be significantly different from the baseline. Context and CS test data were analyzed using a two-way (genotype \( \times \) gender) ANOVA. Follow-up comparisons were made using Newman–Keuls and simple effects analysis.

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