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Behavioral characterization of mouse models for Smith–Magenis syndrome and dup(17)(p11.2p11.2)

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Contiguous gene syndromes (CGS) refer to a group of disorders associated with chromosomal rearrangements in which the phenotype is thought to result from altered copy number of physically linked dosage-sensitive genes. Smith–Magenis syndrome and [dup(17)(p11.2p11.2)] are CGS associated with a heterozygous deletion or duplication of band p11.2 of chromosome 17, respectively. We previously constructed animal models for these CGSs by engineering rearranged chromosomes carrying a deletion/deficiency [*Df(11)17*] (Del mutant) or a duplication [*Dp(11)17*] (Dup mutant) of the syntenic region on mouse chromosome 11. Here we present a behavioral analysis of these models indicating that heterozygous male mice carrying the engineered deletion or the duplication are hypoactive or hyperactive, respectively. In addition, male Dup mutant mice, but not Del mutant mice, have impaired contextual fear conditioning. Circadian rhythm studies revealed period length differences in Del mutant mice, but not Dup mutant mice. These results indicate that some of the behavioral abnormalities are gene dosage sensitive, whereas other behavioral abnormalities are specific to mice carrying the deletion or the duplication and can be observed in a sex preferential manner. Our findings suggest that there is a gene(s) present in this defined genomic interval that is responsible for behavioral abnormalities in the mouse, as has been shown for the human syntenic region.

INTRODUCTION

The genetic bases of the human behavioral traits remain largely unknown (1). Contiguous gene syndromes (CGS) refer to a group of disorders associated with chromosomal rearrangements (deletions or duplications) in which the phenotype is thought to result from altered copy number of physically linked dosage-sensitive genes. Several CGS have been described, each of them presenting a complex and specific phenotype, and many of them having behavioral abnormalities as part of their complex phenotype (2). Owing to the restricted genomic interval involved in CGS, they may represent a useful tool to identify genes responsible for behavioral traits.

Smith–Magenis syndrome (SMS) is a CGS associated with a deletion within band p11.2 of chromosome 17 and a prevalence estimated at 1/25 000 (3). The clinical phenotype has been well described and includes craniofacial abnormalities, brachydactyly, congenital heart defects, seizures, hearing impairment and urinary tract anomalies (4). Up to 75% of these patients have symptoms associated with peripheral neuropathy (decreased deep tendon reflexes, decreased sensitivity to pain or temperature, pes cavus or planus), although nerve conduction velocities are normal (4). Mental retardation, learning disabilities and attention deficits have been reported, and are prominent features of this syndrome (3–7). Head banging, self-hugging, onychotillomania and polyembolokoilmania (3), are several of the self-injurious behaviors reported in SMS, and some of these behaviors appear specific and distinctive for this syndrome. Other behaviors include a spasmodic upper body response elicited when excited (8).

Another major neurobehavioral characteristic is sleep disturbance (4,9). The specific sleep disturbances include reduced REM sleep, early sleep onset, frequent awakenings, early waking and 'sleep attacks' at the end of the day. Melatonin, a pineal gland hormone with light–dark cycles, undergoes a phase shift of its circadian secretion in SMS patients (10,11). One report indicates that treatment of SMS patients with β1-adrenergic antagonists improves their sleep, theoretically by

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suppressing the abnormal rhythm of melatonin secretion, and the behavioral disturbances (12).

Molecular studies revealed a common deleted region of ~4 Mb in 80–90% of SMS patients (4,13). Three copies of a low-copy repeat (SMS-REPs) were identified in the common deletion region (13,14). The flanking low-copy repeat (LCR) copies provide substrates for homologous recombination and mediate non-allelic homologous recombination (NAHR) resulting in rearrangement of this chromosomal segment. Recently, the predicted reciprocal duplication product [dup(17)(p11.2p11.2)] has been described for humans (15). The clinical phenotype associated with the duplication rearrangement seems to be less severe than SMS in the limited number of patients reported to date ($n = 7$). However, mild to borderline mental retardation, attention deficit disorder, hyperactivity, autistic features and behavioral difficulties were consistently observed.

Human chromosome 17p11.2 is syntenic to the 32–34 cM region of murine chromosome 11. The number and order of the genes are highly conserved (16). By chromosome engineering, and taking advantage of the significant conserved synteny between humans and mice in this genomic interval, we were able to construct an animal model containing a deletion or deficiency [*Df11(17)*] and reciprocal duplication [*Dp11(17)*] in the mouse chromosomal region syntenic to the SMS critical interval (17).

To assess phenotypic consequences of gene deletion and duplication in this region of the mouse genome and determine whether *Df(11)17/+* (Del mutant) and *Dp(11)17/+* (Dup mutant) mice exhibit aspects of the SMS and dup(17)(p11.2p11.2) neurobehavioral phenotype, respectively, we tested Del mutant and Dup mutant mice in a variety of experimental paradigms that evaluate different domains of central nervous system functioning. The tests included an open field exploration test that assesses exploratory activity and anxiety related responses, a light–dark test that provides insights into anxiety-related responses, an acoustic startle response and prepulse inhibition of the startle response (PPI) that is a measure of sensorimotor gating, the Pavlovian conditioned fear test that assesses learning and memory, and wheel running activity to assess circadian rhythms. Our data indicate that several of the neurobehavioral aspects of the human del/dup 17p11.2 syndromes can be reproduced in mice, and reveal important new findings that should prompt the reevaluation of the human patients.

RESULTS

Chromosome engineered mouse models for two independent human syndromes, Smith–Magenis syndrome associated with del(17)(p11.2p11.2) and the dup(17)(p11.2p11.2), were subjected to a battery of behavioral assays, that included tests for locomotor activity, anxiety-related responses, sensorimotor gating, conditioned fear, analgesic-related responses, and circadian activity. The mouse models were heterozygous for either a chromosomal deletion (Del mutant) or a chromosomal duplication (Dup mutant) in chromosome 11 (17). For each independent behavioral test we performed an analysis to examine the overall effect of the genotype wherein we included the data from both the male and female mice. In addition, we performed follow-up statistical analyses on the two genders separately. For the Dup mice there were no significant genotype × batch effects ($P > 0.05$), indicating that impact of the duplication on behavior was present in mice tested either before or after the flood of 2001 in which much of the mouse colony was lost. Therefore, these interaction terms are not presented.

Previously Walz *et al.* (17) documented that ~25% of Del mutant, but not Dup mutant, mice experienced seizures. In the present study six female mice and one male Del mutant mouse were observed having seizures at some point during the experiment. Most of the seizures were observed during cage changing. We analyzed the Del data to determine if there was a significant impact of having seizures on the behavioral responses of Del mutant mice. Performing separate analyses that either included or excluded those mice that had seizures did not change the statistical significance of the main effects of genotype or the genotype × gender interactions. Therefore, the data for mice having had a seizure during the study were not excluded from the analysis with the exception of those that had seizures immediately before, during or immediately after the test.

As self-injuring behavior is one common behavior phenotype found in SMS patients, we observed mice for the presence of self-injurious behaviors in Del and Dup mice. There was no evidence of self-injurious behavior in either line of mutant animals, when single housed.

Locomotor activity in the open-field

The open-field test can be used to assess exploratory activity and anxiety-related responses in a novel arena. Various measures assessed during the open-field test are presented in Figure 1.

Deletion mice. Del mutant mice were significantly less active in the open-field compared with their wild-type littermates as assessed by both the total distance [$F(1,53) = 5.992$, $P = 0.018$] and movement time data [$F(1,53) = 6.657$, $P = 0.013$; Fig. 1A and B]. Although the genotype × gender interactions were not significant for either the total distance or movement time measures ($P > 0.05$), follow-up analyses for each gender clearly demonstrated that male, but not female, Del mice had lower activity scores in the open field. Male Del mice traveled less distance [$F(1,27) = 8.686$, $P = 0.007$], and spent less time moving [$F(1,27) = 5.836$, $P = 0.023$] than wild-type male mice. Although there was no overall difference in the amount of rearing behavior between the two genotypes [$F(1,53) = 1.527$, $P = 0.222$], male Del mice reared less frequently in the open-field [$F(1,27) = 5.23$, $P = 0.03$] than wild-type male mice (data not shown). There was no significant difference in total distance, movement time or rearing responses between female Del mutant and female wild-type mice ($P > 0.05$; Fig. 1C and D and data not shown, respectively). There was no overall difference in movement speed or the center distance ratio between the two genotypes, or differences between the genotypes when the genders were analyzed separately ($P > 0.05$). These findings indicate that male, but not female, Del mutant mice are hypoactive in the open field.

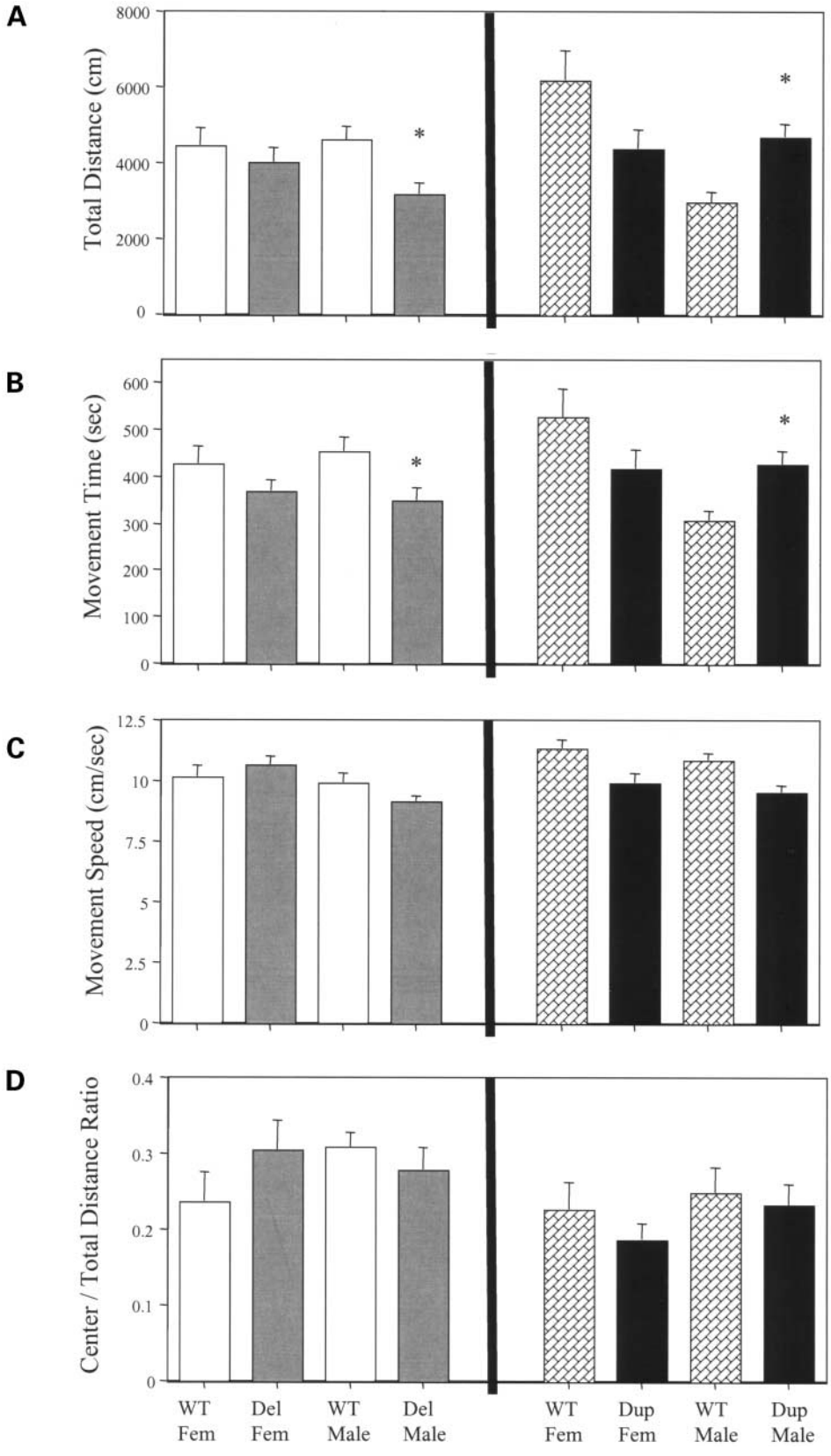

Figure 1. Locomotor activity in the open field. Total distance (cm), movement time (s), movement speed (cm/s) and center/total distance ratio are represented respectively in panels (**A**)–(**D**). Values represent mean ± SEM. Gray bars, Del mutant; white bars, wild-type (WT) littermate; black bars, Dup mutants; and hatched bars, wild-type (WT) littermate. The asterisk denotes significantly different from their respective wild-type littermate.

Duplication mice. In contrast to the Del mutant male mice, male Dup mutant mice were hyperactive compared to their wild-type littermates. The overall main effect of genotype was not different for either the total distance or movement time measures ($P > 0.05$), but there were significant gender × genotype interactions [distance: $F(1,53) = 16.055$, $P < 0.001$; movement time: $F(1,53) = 11.387$, $P = 0.001$]. Follow-up comparisons revealed that male, but not female, Dup mice traveled significantly farther [$F(1,25) = 13.301$, $P = 0.001$] and spent more time moving [$F(1,25) = 10.177$, $P = 0.004$] compared with their wild-type controls (Fig. 1A and B). There were no differences between Dup mutant and wild-type mice in the vertical activity, speed, or center ratio measures ($P > 0.05$; data not shown, and Fig. 1C and D, respectively).

Light–dark test

The light–dark exploration test is typically used to more directly assess anxiety-related responses. The number of light–dark transitions is presented as Figure 2.

Deletion mice. Data from one female Del mutant mouse was not used due to a seizure during testing. The time to enter the dark and number of transitions were not significantly different between Del mutant and wild-type mice ($P > 0.05$). The light–dark test and the center ratio data in the open field indicate that Del mutant and wild-type mice have similar anxiety-related responses.

Duplication mice. Similar to the open-field test, there was no overall main effect of genotype in the number of light–dark transitions between Dup mutant and wild-type mice ($P > 0.05$). However, there was a significant genotype × gender interaction [$F(1,53) = 5.197$, $P = 0.027$]. Male dup mutant, but not female mutant mice, had significantly fewer light–dark transitions compared with their wild-type controls [$F(1,25) = 6.529$, $P = 0.017$]. There were no differences between Dup and wild-type mice in the latency to enter the dark chamber ($P > 0.05$) (data not shown).

Prepulse inhibition of the acoustic startle response

Prepulse inhibition is used to assess sensorimotor gating by quantitating the normal suppression of a startle that is preceded by a weak, non-startling prestimulus. The maximum startle amplitude and prepulse inhibition data are presented in Figure 3.

Deletion mice. The acoustic startle response was similar in Del mutant and wild-type mice, when mice were grouped by sex. In addition, there were no differences between Del mutant and wild-type mice in levels of prepulse inhibition ($P > 0.05$).

Duplication mice. In contrast to the Del mutant mice, Dup mutant mice startled significantly less than their wild-type littermates [$F(1,47) = 4.216$, $P = 0.046$]. However, the levels of prepulse inhibition were similar between Dup mutant and wild-type mice ($P > 0.05$).

Conditioned fear

The conditioned fear test is utilized to assay a fear-based response using a Pavlovian learning and memory paradigm. Levels of freezing for the context and auditory cued conditioned fear tests are shown in Figure 4.

Deletion mice. Six female and one male Del mutant mice had to be excluded due to seizures during some part of the test. During the context and CS tests, Del mutant mice displayed similar levels of freezing to that seen in their wild-type littermates ($P > 0.05$).

Duplication mice. The overall main effect of genotype for the Context test was not significant ($P > 0.05$); however the genotype × gender interaction was significant [$F(1,47) = 4.896$, $P = 0.032$]. Follow-up comparisons indicated that male, but not female, Dup mutant mice displayed significantly less freezing during the context test 24 h after training [$F(1,22) = 4.7$, $P = 0.041$]. In contrast to the context test, male and female Dup mice showed similar levels of freezing during the CS test [$P > 0.05$].

An additional experiment was performed to determine if the impaired context conditioning in the male Dup mutant mice was delay-dependent. In this supplemental experiment, male Dup mutant and male wild-type littermates were tested for context conditioning after a 1 h delay (Fig. 4C). Even after only a 1 h delay interval between training and testing, male Dup mice display significantly less freezing than wild-type mice to the contextual cues associated with training [$F(1,18) = 5.36$, $P = 0.033$]. These findings suggest that Dup mutant mice have a selective impairment in fear conditioning that is associated with the context or environment where the shock occurred, but not to a single cue that is associated with the footshock, and this contextual fear impairment is present even after a short delay.

Hotplate

The hotplate test is an indicator of an animal's sensitivity to painful stimuli. The time to the first hind-limb response is shown as Figure 5.

Deletion mice. The latency to the first hindlimb response was not different between Del and wild-type mice ($P > 0.05$).

Duplication mice. The latency to the first hindlimb response was not different between Dup and wild-type mice ($P > 0.05$).

Circadian activity

Twenty-four hour monitoring of wheel running activity in constant darkness (D/D) after entrainment in a light dark (L/D) cycle is an indicator of circadian responses (Fig. 6) (18).

Deletion mice. Del mutant and wild-type mice were entrained to the 12 h light/12 h dark (L/D cycle). In constant darkness (D/D), however, the Del mice displayed an average circadian period of 23.60 ± 0.06 h, which was significantly shorter than their wild-types littermates 23.87 ± 0.02 h ($P < 0.0001$; Fig. 6E). Interestingly, the period length distribution among

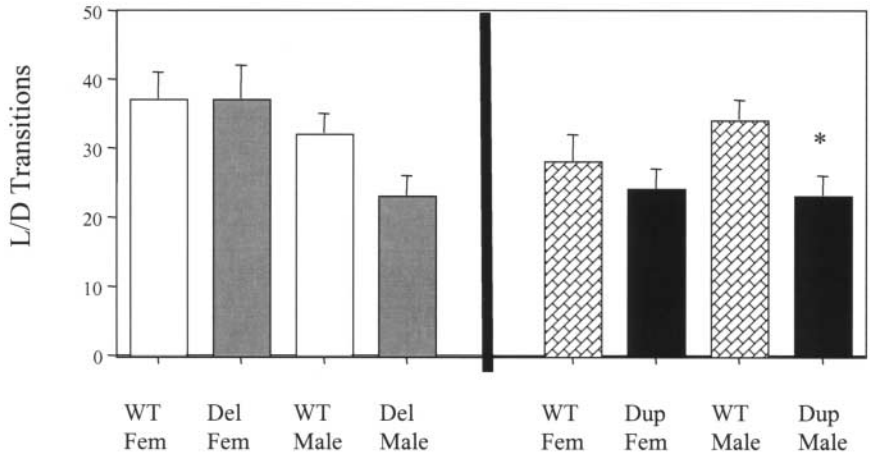

Figure 2. Total light/dark transitions in the light–dark exploration test are represented by vertical bars: gray bars, Del mutant; white bars, wild type littermate; black bars, Dup mutants; and hatched bars, wild-type littermate. The asterisk denotes significantly different from their respective wild-type. Values represent mean ± SEM.

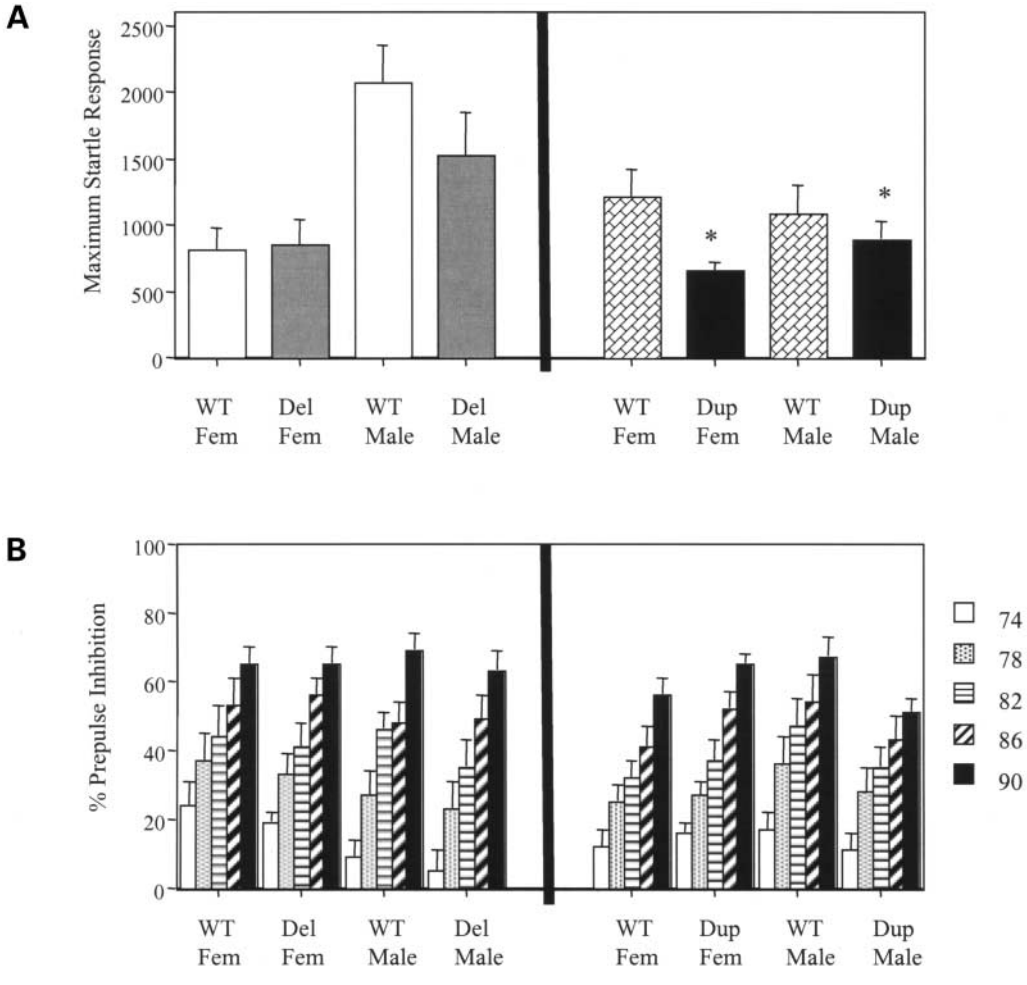

Figure 3. Maximum startle response (**A**) is represented by vertical bars: gray bars, Del mutant; white bars, wild-type littermate; black bars, Dup mutants; and hatched bars, wild-type littermate. The percentage of prepulse inhibition (**B**) with a 20 ms prepulse sound at 74, 78, 82, 86 and 90 dB (as indicated on the right) is represented for each group of animals. Values represent mean ± SEM (the asterisk denotes significantly different from wild-type littermate control).

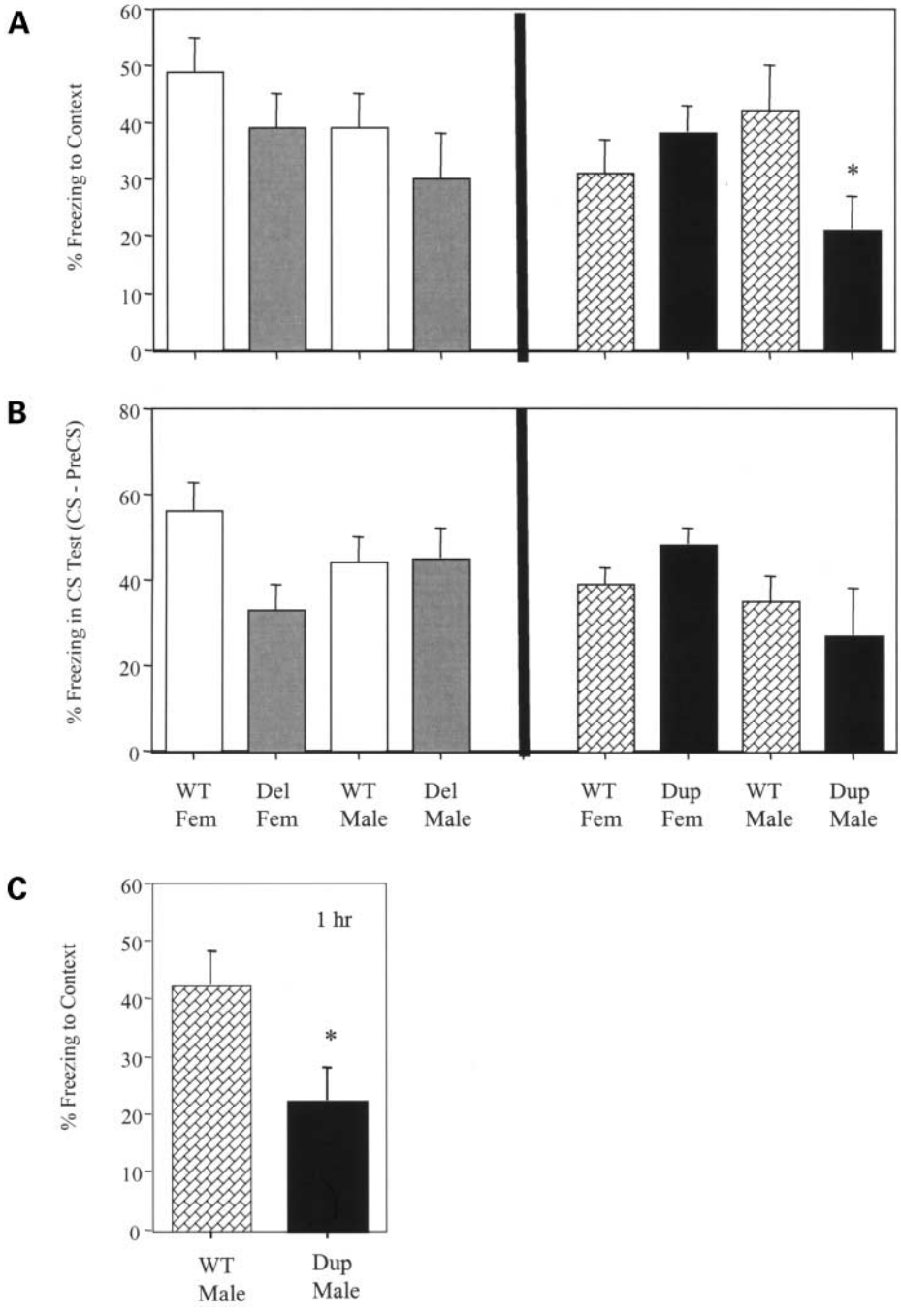

Figure 4. Conditioned fear in Del and Dup mutant mice. (**A**) Percentage of freezing to context, or (**B**) percentage to freezing in CS test (gray bars, Del mutant; white bars, wild-type littermate; black bars, Dup mutants; and hatched bars, wild-type littermate). (**C**) Percentage of freezing to context after 1 h for Dup mutants males compared to their wild-type dup male littermates. The mean ± SEM values are presented (the asterisk denotes significantly different).

Del mice was much more variable than among wild-type mice, which may indicate a reduced precision of the clock control of period length.

Duplication mice. Similar to the Del mice, Dup mutants and wild-type mice entrained to the 12 h L/D cycle. In contrast to the Del mice, there was no difference between Dup mutant and wild-type mice in the average circadian period during the 24 h D/D cycle ($P > 0.05$).

DISCUSSION

Individuals with SMS display a number of neurobehavioral abnormalities including mental retardation, learning disabilities, attention deficits, decreased sensitivity to pain or

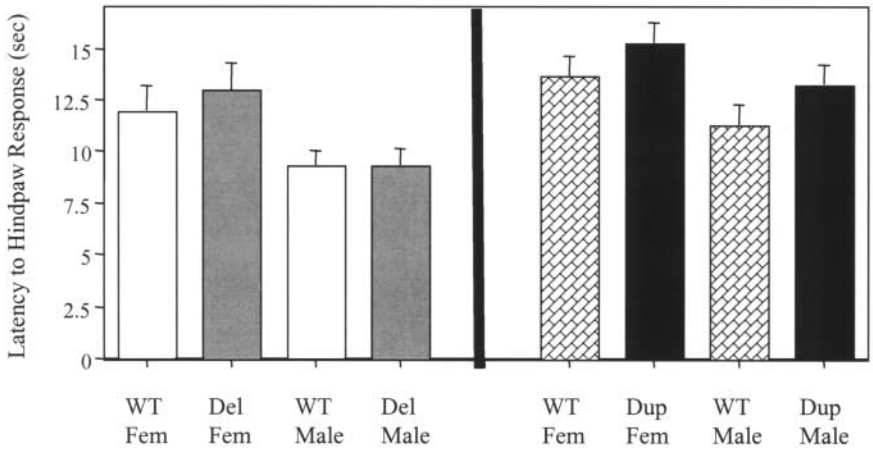

Figure 5. Latency of hindpaw response (s). Gray bars, Del mutant; white bars, wild-type littermate; black bars, Dup mutants; and hatched bars, wild-type littermate. The mean ± SEM values are presented.

temperature, sleep disturbances and several self-injurious behaviors (4–7). The predicted reciprocal duplication product [dup(17)(p11.2p11.2)] of SMS has also been described for humans (15). Duplication individuals have several behavioral abnormalities that are most commonly thought to be less severe than those present in SMS patients, and include mild to borderline mental retardation, attention deficit disorder, hyperactivity and autistic features. In order to assess phenotypic consequences of gene deletion and duplication in this region of the mouse genome, and determine whether Del mutant and Dup mutant mice exhibit aspects of the SMS and Dup17(p11.2p11.2) neurobehavioral phenotype respectively, we tested both mutant models in a variety of paradigms to evaluate different domains of central nervous system functioning.

In summary, there are several behavioral phenotypes in these two mouse models. Male mice, but not female mice, from both the Del and Dup mutant models displayed abnormal activity in the open field compared with their respective wild-type mice. Male Del mutants are hypoactive, while male Dup mutants are hyperactive. Del mutant mice also display a significant decrease in the circadian period during the D/D cycle of the wheel running test and a lack of clock precision. Del mutants appeared normal on several measures of the open-field including the movement speed and center distance ratio, light–dark test for anxiety, prepulse inhibition test for sensorimotor gating, the conditioned fear test for learning and memory, and the hotplate test for analgesia-related responses. In contrast, the male Dup mice displayed fewer light–dark transitions in the light–dark box, suggesting that in this assay they may have increase anxiety. Both male and female Dup mice had decreased startle responses, but normal prepulse inhibition. Male, but not female, Dup mutant mice also had impaired conditioned fear that was selective to the context test, and was present following both a 1 and 24 h delay interval. The level of freezing during the CS test was not significantly different between Dup mutant and wild-type mice. Finally, the Dup mutant mice had a normal circadian period, and showed similar responses on the hotplate test compared to their wild-type littermates.

Both Del and Dup male mutant mice displayed abnormal activity responses in the open-field assay. These findings are particularly interesting for several reasons. First, the fact that both mutant models showed positive phenotypes in this assay provides consistency and confidence that levels of activity are regulated by genes in this region. Second, there was increased activity in the Dup mutants and decreased activity in the Del mutants indicating that locomotor activity is related to gene number. Recently, a genomewide scan for attention-deficit/hyperactivity disorder (ADHD) suggest a linkage on 17p11, in the SMS deleted region (19), reinforcing the idea of a gene present in this area important in the regulation of levels of activity. In addition, some patients with dup(17)(p11.2p11.2) syndrome (15) are hyperactive. The combined results clearly indicate that there is a gene or combination of genes in this interval important for the regulation of levels of behavioral activity. Only male mice from both the Del and Dup mutant lines showed significant activity differences relative to their wild-type controls, indicating that there is some interaction between gender and activity. The nature of the gender specificity shown in this study is unknown and clearly will require further investigation; however, we do not believe that this gender specificity deters the current mutant lines from being useful animal models for the several of the behavioral abnormalities associated with SMS and dup(17)(p11.2p11.2). In the only report of dup(17)(p11.2p11.2) syndrome (15) out of seven patients in total, four males and three females, three males were hyperactive contrasting with no females presenting that phenotype. Also, ADHD is more frequently diagnosed in boys, with a male:female ratios between 3 : 1 and 4 : 1 (20,21).

Sleep disturbances have been extensively reported in SMS patients (4,9). Some of the abnormalities described for these patients include: early sleep onset, frequent awakenings, early waking, and ‘sleep attacks’ at the end of the day, all this suggesting a potential sleep phase advance. The normal circadian secretion of melatonin, a pineal gland hormone with light–dark cycles, is phase shifted in SMS patients (10,11). One report indicates that treatment of SMS patients with β1-adrenergic antagonists improves the sleep and the behavioral disturbances (12).

The mammalian circadian pacemaker resides in the paired suprachiasmatic nuclei (SCN) and influences several biological processes, including the sleep–awake rhythm (22). Although the timing of sleep is strongly influenced by the circadian

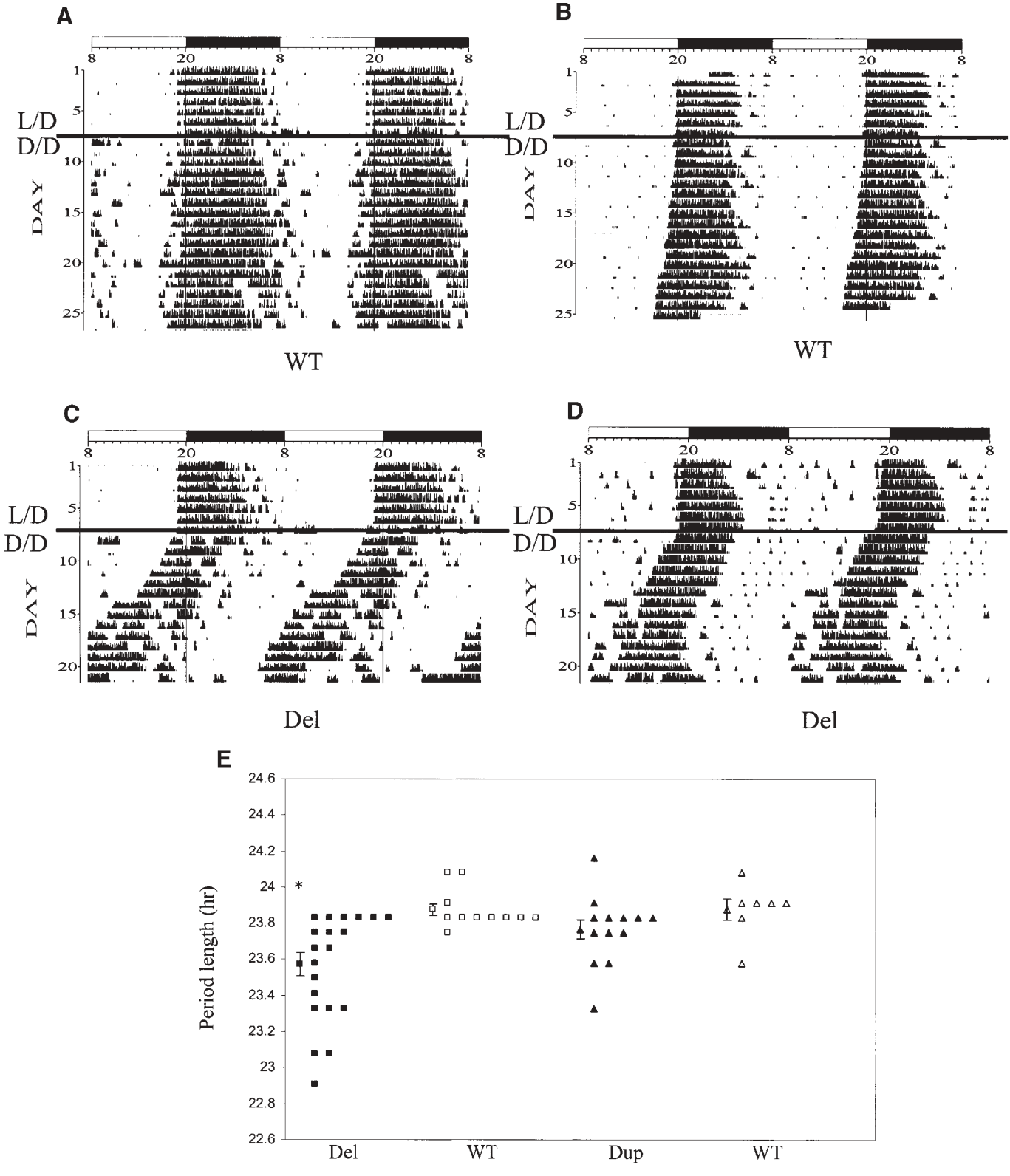

Figure 6. Locomotor activities recorded in the wheel running test for circadian rhythm. Representative locomotor activity records of wild type mice (**A**, **B**) and Del mutant mice (**C**, **D**). L/D: 12 h light–12 h dark; D/D, total darkness. The horizontal bar superimposed above the records indicates the light, L (open bar) and dark, D (solid bars) cycles. The superimposed horizontal line indicates the time when switched from L/D to D/D cycles. (**E**) Average (±SEM) and individual period length data are plotted for each genotype; Del mutant = solid square; Del wild-type = open square; Dup mutant = solid triangle; and Dup wild-type = open triangle. The first point for each series represents the average for each genotype with respective error bars (the asterisk significantly different).

system, social factors may predominate. The data presented here suggest that haploinsufficiency of one or several genes included in this region may be involved in the regulation and precision of the period. Our Del mice presented a shorter period length when compared with their wild-type littermates, but, more important, they presented a lack of clock precision, as shown by the great variation in period length between the animals. This lack of clock precision may potentially explain the sleep disturbance observed in SMS patients.

Male Dup, but not Del, mice displayed significantly less conditioned fear to the training context, but not to the auditory cue. The conditioned fear impairment in the Dup mice was observed even with a short 1 h delay interval between training and testing. This pattern of results suggest that the Dup mice have an impaired process necessary for learning about the fear-related stimuli associated with the training context. Alternatively, it could be that Dup mice have an overall reduction in fear and/or anxiety related behavioral responses to novel environments. We believe this is unlikely because the Dup mice displayed normal levels of anxiety-related responses in the open-field, and if anything showed an increase in 'anxiety' in the light–dark test. In addition, the Dup mice displayed normal levels of fear during the CS test. This latter finding also indicates that it is unlikely that the Dup mice had an overall reduction in sensitivity to the footshock because if they had they would have showed significantly less freezing during the CS. It is unclear why the Dup male mice showed impaired contextual fear conditioning while the Del mice were normal. Future investigations will be required to understand this apparent paradox by studying the learning and memory responses in both Del and Dup mice on a wide variety of learning and memory tests. However, the findings with the Dup mice do suggest that perhaps more systematic evaluation of the cognitive abilities of individuals with Dup 17 (p11.2p11.2) is warranted.

It is important to point out that Del mice display other physical problems compared with the wild-type mice, including craniofacial anomalies, weight differences and seizures. For the current study we have excluded data from those animals that had seizures immediately before, during or immediately after testing to help ensure that the results are obtained from mice not having a seizure around the time of testing. Although it is possible that having a seizure at some point during life could affect future behavioral responses, we found no obvious relationship between mice having seizures immediately, during or after one test and their responses on subsequent tests. We verified this by analyzing the results of all the tests with and without the data from mice that had seizures and there were no significant differences (data not shown). However, it is interesting to note that most of the mice that displayed seizures were female Del mice. On several of the behavioral tests we only observed behavioral differences in male mutants and not females. Finally, it is possible that craniofacial anomalies and weight differences could have contributed in some way to the behavioral differences in the current study; however, it is unclear how these physical abnormalities could have caused the very specific behavioral differences seen in the Del and Dup mice. In addition we have examined mutant mice that weigh significantly more or less than their wild-type mice and they did not show the same behavioral phenotypes as the Del or Dup mice.

Extensive analysis of the genes present in the deletion/duplication region has been reported by us and others (16,23). Dexamethasone-induced RAS protein 1 (*Dexras*1), a GTP-binding protein is highly expressed in brain. Mouse *Dexras*1 gene was found to be expressed in a circadian rhythmic manner in the suprachiasmatic nucleus (24). Retinoic acid inducible-1 (*Rai*1) has neuron-specific expression in the brain and is induced by retinoic acid. *Rai*1 was found to be mutated in SMS patients without a deletion (25). These patients present almost all the phenotypes described for SMS patients, including sleep disturbances and developmental delay. *Drg*2, a developmentally regulated GTP binding protein predominantly expressed in the embryonic brain and down-regulated during development (26) is potentially a strong candidate due to the expression pattern. *Zfp179*, a RING finger protein predominantly expressed in brain and testis (27), was a good candidate, but because of the methodology used for the creation of these mouse models, in the Del animals this gene is presenting the normal dosage, excluding it as the responsible gene for the phenotype observed in the Del mice.

Today it is unknown if the neurobehavioral phenotype observed in the SMS and Dup17(11.2p11.2p) patients are due to one or several dosage sensitive genes in the region; however, the mouse models presented here are the first step in recognizing genes and pathways regulating locomotor activities, learning impairment and circadian abnormalities. Future investigations will include exploring the role of background strain, and exploring the possibility of treating the mutant lines with agents such as beta1 antagonists (see Introduction) to rescue the behavioral and circadian phenotypes. Although β1-adrenergic antagonists have been used in SMS patients, there is no data to date indicating that the adrenergic system is preferentially affected. The current mouse models will be very useful to begin to determine what underlying mechanisms are responsible for the behavioral abnormalities in SMS and for evaluating potential drug treatments.

MATERIALS AND METHODS

Animals

Heterozygous mice carrying a deletion [*Df(11)17/+* = 'Del' mutant] or a duplication [*Dp(11)17/+* = 'Dup' mutant] were analyzed in a mixed genetic background C57BL/6-*Tyr*$^{c\text{-}Brd}$ × 129S5/SvEvBrd. The animals were genotyped by Southern blot analysis as described in Walz *et al.* (17). All the mice were 3 months of age at the beginning of the testing.

Deletion mice. Twenty-nine (15 female and 14 male) Del mutant and 28 (13 female and 15 male) wild-type littermate control mice were evaluated in the open-field, light–dark, prepulse inhibition of the startle response, conditioned fear, and hot-plate tests. However, for each test data from some of the Del mutant animals were not used because of the presence of a seizure immediately before, during or after the test. The number of mice excluded from statistical analyses due to seizures at the time of testing is described in the results section for each behavioral test. Circadian behavior was recorded using an independent batch of 21 Del mutant and 11 wild-type littermate male mice.

Duplication mice. Thirty-four (21 female and 13 male) Dup mutant and 27 (13 female and 14 male) wild-type littermate control mice were evaluated in the open-field, light–dark, prepulse inhibition of the startle response, conditioned fear and hot-plate tests. Approximately half of the Dup mice from each gender and genotype were evaluated prior to a flood in June 2001. A second batch of Dup mice were evaluated after they were embryo re-derived following the June 2001 flood. An independent batch of Dup mutant and wild-type littermate male mice were used for the 1 h context test (see below). Circadian behavior was recorded using an independent batch of 13 Dup mutant and seven wild-type littermate male mice.

With the exception of the circadian behavior experiments, mice were run in multiple batches of five to 10 mice from each genotype.

With the exception of the circadian behavior experiments (see below) mice were housed two to five per cage in a room with a 12 h light–dark cycle (lights on at 6 a.m., off at 6 p.m.) with access to food and water *ad libitum*. In general, behavioral testing was performed between 9 a.m. and 2 p.m. Experiments were conducted by an experimenter blind to the genotypes of the mice. All behavioral testing procedures were approved by the Baylor Institutional Animal Care and Use Committee and followed NIH Guidelines.

Locomotor activity in the open field

Locomotor activity was evaluated by placing a mouse into the center of a clear Plexiglas (40 × 40 × 30 cm) open-field arena and allowed to explore for 30 min. Overhead incandescent lights provided room lighting that measured ~800 lx inside the test arenas. In addition, white noise was present at ~55 dB inside the test arenas. Activity in the open-field was quantitated by a computer-operated Digiscan optical animal activity system [RXYZCM (16), Accuscan Electronics] containing 16 photoreceptor beams on each side of the arena, which divides the arena into 256 equally sized squares. Total distance (locomotor activity), movement time (in seconds), movement speed (cm/s), vertical activity (rearing measured by number of photobeam interruptions), and center distance (the distance traveled in the center of the arena) were recorded. The center distance was also divided by the total distance to obtain a center distance–total distance ratio. The center distance–total distance ratio can be used as an index of anxiety-related responses (28). Data were collected in 2 min intervals over the 30 min test session. Open-field activity data for the total 30 min test were analyzed using two-way (genotype × gender) analysis of variance (ANOVA) for the Del mice and three-way (genotype × gender × batch) ANOVA for the Dup mice.

Light–dark exploration

One to three days later mice were then tested in the light–dark exploration test, which consists of a polypropylene chamber (44 × 21 × 21 cm) unequally divided into two chambers by a black partition containing a small opening. The large chamber is open and brightly illuminated (800 lx), while the small chamber is closed and dark. White noise is present in the room at ~55 dB in the test chamber. Mice were placed into the illuminated side and allowed to move freely between the two chambers for 10 min. The time to enter the dark and the total number of transitions were recorded. Data were analyzed using two-way (genotype × gender) ANOVA for the Del mice and three-way (genotype × gender × batch) ANOVA for the Dup mice.

Startle and prepulse inhibition of the startle

One to three days later mice were tested for prepulse inhibition of acoustic startle responses using the SR-Lab System (San Diego Instruments, San Diego, CA, USA), as previously described (29). 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 trial types. The prepulse sound was presented 100 ms before the startle stimulus. The 20 ms prepulse sounds were at 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 % prepulse inhibition of a startle response: 100 − [(startle response on acoustic prepulse plus startle stimulus trials/startle response alone trials) × 100]. Thus, a high percentage prepulse inhibition value indicates good prepulse inhibition, i.e. the subject showed a reduced startle response when a prepulse stimulus was presented compared with when the startle stimulus was presented alone. Conversely, a low percentage prepulse inhibition value indicates poor prepulse inhibition, i.e. the startle response was similar with and without the prepulse. Acoustic response amplitude data were analyzed using two-way (genotype × gender) ANOVA for the Del mice and three-way (genotype × gender × batch) ANOVA for the Dup mice. Prepulse inhibition data were analyzed using three-way (genotype × gender × prepulse sound level) ANOVA with repeated measures for the Del mice and four-way (genotype × gender × batch × prepulse sound level) ANOVA with repeated measures for the Dup mice.

Pavlovian conditioned fear

One to two weeks later performance in a conditioned fear paradigm was measured using a test chamber (26 × 22 × 18 cm high) made of clear Plexiglas (front wall) and stainless steel (back wall and two side walls). The bottom of the test chamber was a grid floor used to deliver a mild electric footshock. The test chamber was placed inside a sound attenuated chamber (Med Associates, internal dimensions: 56 × 38 × 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 two min. A white noise (80 dB), which served as the conditioned stimulus (CS), was then presented for 30 s followed by a mild (2 s, 1.0 mA) foot shock, which served as the unconditioned stimulus (US). Two min 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. Freezing was operationally defined as no movement with the exception of those movements associated with breathing. Responses (run, jump and vocalize) to the foot-shock were also recorded. If a mouse did not respond to the foot-shock it was excluded from the analysis.

Twenty-four hours 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). One to 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 percentage freezing value. Context and CS test data were analyzed using two-way (genotype × gender) ANOVA for the Del mice and three-way (genotype × gender × batch) ANOVA for the Dup mice.

Analgesic response using the hot plate test

One week later the hot-plate test was used to evaluate sensitivity to a painful stimulus. Mice were placed on a 55.0°C (±0.3) hot-plate, and the latency to the first hind-paw response was recorded. The hind-paw response was either a foot shake or a paw lick. Hot-plate data were analyzed using two-way (genotype × gender) ANOVA for the Del mice and three-way (genotype × gender × batch) ANOVA for the Dup mice.

Wheel running behavior for circadian activity

All animals were placed in individual cages equipped with a running wheel for entrainment, 10–15 days prior to beginning the recordings. Wheel-running cages were placed in a ventilated, light controlled chamber. Wheel running activity was recorded by an online PC computer equipped with the Chronobiology kit (Stanford Software Systems, Santa Cruz, CA, USA). Wheel running responses were recorded for 7 days in chambers that had a standard 12 : 12 light–dark (L/D) light cycle. At the end of the 7 days, the L/D cycle was switched from a 12 : 12 L/D to a 12 : 12 dark–dark (D/D) cycle. Mice were maintained in the D/D cycle for 10 days. To determine the period length, activity data during the D/D conditions were analyzed with a χ^2 periodogram (30) using the Chronobiology Kit.

Statistical analyses

All data from SMS Del and Dup mice were analyzed individually due to the fact that some of the Dup mice were tested before the flood of June 2001, see above in section describing 'animals'. To reduce type I errors, two sets of Del and Dup mice were evaluated, but data for the two sets were analyzed together since there were no differences between the two batches of subjects.

Data for the open-field, light–dark, acoustic startle, conditioned fear, hot-plate, and circadian activity were analyzed using two-way (genotype × gender) analysis of variance (ANOVA) for the Del mice and three-way (genotype × gender × batch) ANOVA for the Dup mice. Prepulse inhibition data were analyzed using three-way (genotype × gender × prepulse sound level) ANOVA with repeated measures for the Del mice and four-way (genotype × gender × batch × prepulse sound level) ANOVA for the Dup mice.

In addition, we performed follow-up statistical analyses on the two genders separately even when the gender × genotype interaction was not statistically significant. This decision was made based on the fact that six of the seven Del mice that had seizures were female (see Results section).

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