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Abnormal social behaviors in mice lacking Fgf17

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The fibroblast growth factor family of secreted signaling molecules is essential for patterning in the central nervous system. Fibroblast growth factor 17 (Fgf17) has been shown to contribute to regionalization of the rodent frontal cortex. To determine how Fgf17 signaling modulates behavior, both during development and in adulthood, we studied mice lacking one or two copies of the *Fgf17* gene. Fgf17-deficient mice showed no abnormalities in overall physical growth, activity level, exploration, anxiety-like behaviors, motor co-ordination, motor learning, acoustic startle, prepulse inhibition, feeding, fear conditioning, aggression and olfactory exploration. However, they displayed striking deficits in several behaviors involving specific social interactions. Fgf17-deficient pups vocalized less than wild-type controls when separated from their mother and siblings. Elimination of Fgf17 also decreased the interaction of adult males with a novel ovariectomized female in a social recognition test and reduced the amount of time opposite-sex pairs spent engaged in prolonged, affiliative interactions during exploration of a novel environment. After social exploration of a novel environment, Fgf17-deficient mice showed less activation of the immediate-early gene *Fos* in the frontal cortex than wild-type controls. Our findings show that Fgf17 is required for several complex social behaviors and suggest that disturbances in Fgf17 signaling may contribute to neuropsychiatric diseases that affect such behaviors.

Keywords: Autism, cortical development, fibroblast growth factor, neuropsychiatric disease, schizophrenia, social behavior

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Despite the devastating consequences of common human neuropsychiatric diseases such as schizophrenia and autism, little is known about the neural circuits and signaling events contributing to the social deficits that characterize these diseases. The analysis of experimental animal models could shed light on the pathophysiological mechanisms, but the development of such models has been challenging. Although it is unlikely that a single animal model could ever capture the full complexity of neuropsychiatric diseases, a fruitful approach may be to model individual aspects of behavioral dysfunction. Unraveling the intricate interplay of genetic, developmental, structural and molecular factors that contribute to such dysfunction might provide insights into the neural basis of schizophrenia, autism spectrum disorders and related conditions.

Notably, recent studies have linked changes in rodent social behaviors to neurodevelopmental abnormalities (Cheh *et al.* 2006; Clapcote *et al.* 2007; Gemelli *et al.* 2006; Kwon *et al.* 2006; Mineur *et al.* 2006; Moretti *et al.* 2005; Shu *et al.* 2005; Spencer *et al.* 2005). Fibroblast growth factors (Fgf) are particularly interesting in this regard. *Fgf* genes encode a family of 22 signaling molecules that play important roles in development and tissue homeostasis (Dailey *et al.* 2005). Blocking Fgf receptor signaling by expressing a dominant negative Fgf1R receptor during embryonic development resulted in decreased cortical thickness (Shin *et al.* 2004). Expressing the dominant negative Fgf1R in dopamine (DA) neurons reduced the number of DA neurons, increased DA levels in the striatum and impaired prepulse inhibition (Klejbor *et al.* 2006), changes that may have relevance to the neuropathology and sensorimotor gating deficits in schizophrenia (Meyer-Lindenberg *et al.* 2002; Swerdlow & Geyer 1998).

Recent evidence suggests that Fgf17 is important in neural patterning. During embryonic development, Fgf17 is expressed in the patterning centers for the rostral forebrain and the midbrain/hindbrain. Studies of Fgf17-deficient ($Fgf17^{-/-}$) mice showed that Fgf17 is required for development of the cerebellar vermis and inferior colliculus (Xu *et al.* 2000) and for the regionalization of frontal cortex (Cholfin & Rubenstein 2007). Fgf17 ablation reduced the size of the dorsal frontal cortex and the extent of frontal cortex projections to subcortical targets (Cholfin & Rubenstein 2007).

The physiological and behavioral consequences of Fgf17 deficiency have not yet been reported. The viability of $Fgf17^{-/-}$ mice provides an opportunity to investigate the role of this molecule in regulating behavior, both during development and in adulthood. In the present study, we show that removal of Fgf17 alters specific social behaviors in mice, without affecting olfaction, pheromone responses, aggression or an array of non-social behaviors. The importance of Fgf17 in neural development and complex social behaviors suggests that $Fgf17^{-/-}$ mice may model key aspects of neuropsychiatric disorders.

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Materials and methods

Animals

All mice were housed and handled in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals, and all studies were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco. For behavioral analysis, Fgf17$^{+/-}$ males and females (129X1/SvJ background) that had been backcrossed one generation to C57BL/6NCrl were mated to generate Fgf17$^{+/+}$, Fgf17$^{+/-}$ and Fgf17$^{-/-}$ progeny. This mating strategy was used for all tested mice to avoid generational differences between cohorts. Mice were housed in a pathogen-free barrier facility on a 12-h light–dark cycle. Group housing was maintained, except where noted. Food and water were freely available. Behavioral testing occurred under normal indoor lighting conditions (approximately 350 lux) between 0800 and 1700 h, except where noted. Experimenters were blind to mouse genotype during testing. For most adult behavioral testing, data presented were pooled from three different cohorts of 4 to 8-month-old mice. Cohort A comprised 14 Fgf17$^{+/+}$ mice (3 male, 11 female), 22 Fgf$^{+/-}$ mice (13 male, 9 female) and 10 Fgf17$^{-/-}$ mice (5 male and 5 female). Cohort B was all male and comprised 13 Fgf17$^{+/+}$ and 8 Fgf17$^{-/-}$mice. Cohort C comprised 13 Fgf17$^{+/+}$ mice (7 male, 6 female), 15 Fgf$^{+/-}$ mice (6 male, 9 female) and 21 Fgf17$^{-/-}$ mice (14 male and 7 female). Cohorts A and B went through the same tests in the same order: elevated plus maze, open field, social recognition, prepulse inhibition of startle, olfactory recognition, Y maze, rotarod, novel object recognition, novelty-suppressed feeding, fear conditioning and social exploration of novel environment. Only males were tested in social recognition, olfactory recognition, novel object recognition and novelty-suppressed feeding. Cohort C underwent novel object recognition, olfactory recognition, fear conditioning, pheromone recognition and social interaction. Only males were tested in novel object recognition, olfactory recognition and fear conditioning. Testing for each cohort was completed within 2–3 months. Aggression tests were conducted in a separate cohort of 4 to 5-month-old singly housed male Fgf17$^{-/-}$ and Fgf17$^{+/+}$ mice. Developmental assessment occurred in a separate group of animals that were not tested as adults. All equipment was thoroughly cleaned with 70% ethanol between testing of individual mice to standardize odors, except where noted.

Developmental assessment

Fgf17$^{+/-}$ males and females were mated, and plugged females were separated until the litters were weaned. Beginning on P2, pups were individually numbered (using non-toxic ink on their stomachs) and monitored daily. During testing, all pups were transferred to a cage filled with clean bedding placed over a heating pad to maintain the temperature at 22–24°C. Individual pups were removed, checked for physical abnormalities and weighed. Unless otherwise noted, absence of a milestone was scored if the mouse did not exhibit the behavior within 60 s. To assess surface righting, each pup was placed on its back and monitored until it successfully righted itself. To assess negative geotaxis, the pup was placed facing downward on a sheet of textured plastic inclined at a 30° angle and monitored until the pup reoriented itself with its head and forelimbs higher up the plane than its hindlimbs. To assess cliff avoidance, the pup was placed with its hindlimbs resting on a circular Styrofoam platform mounted on a 30-cm high stand. The pup was positioned so its forepaws and nose were suspended over the edge of the platform and monitored until the pup moved away from the edge. To assess grasp reflex, each forepaw was gently stroked with the wooden end of a swab. If the pup immediately curved its paw to grasp the swab, the grasp reflex was considered present. The date when both eyes were open was recorded. Visual placing was assessed by suspending the pup by its tail and gently lowering it toward the tabletop. If the pup raised its head and extended forelimbs toward the surface, visual placing was scored as present. To assess air righting, the pup was held with its ventral side facing upward 30 cm above a chamber filled with soft bedding. The pup was released, and air righting was considered present if the pup turned while falling so that it landed on its feet. To assess bar hanging, the pup was allowed to grasp a small wire bar and then released so that it was hanging by its forelimbs. Once the pup was able to hang suspended for 10 s, bar holding was scored as present.

Isolation-induced ultrasonic vocalization

On day P8, ultrasonic vocalizations (USV) were assessed in pups of Fgf17$^{+/-}$ mothers. The pups were removed from their home cage and placed in a cage on top of a heating pad that maintained the bedding temperature at 22–24°C. If the litter contained six or more pups, the pups were randomly divided into two equally sized groups that were assessed separately to minimize length of time away from the mother. After a 10-min maternal separation, each pup was removed from the litter individually, taken to a different room and placed in a warmed, clean cage with no bedding. Its USV were counted for 3 min using the UltraVox detector (Noldus Information Technology, Wageningen, The Netherlands) tuned to 65 kHz. An observer listened to signals through headphones to confirm the accuracy of detection. After the isolation task, the mother was placed in the test chamber for 3 min and vocalizations from both mother and pup were recorded. Then, the mother was removed again and pup vocalizations were recorded for 3 min. The test chamber was cleaned with lukewarm water between pups.

Social recognition

Male mice were singly housed at least 5 days prior to testing. The home cage, without lid or food hopper, was moved to the testing area for a 3-min habituation period. A novel ovariectomized C57BL/6J female (Jackson Lab, Bar Harbor, ME, USA) was introduced into the cage. The amount of time spent interacting was recorded. After 90 seconds, the female was returned to her home cage, while the male mouse rested in his home cage for 3 min. Then the same female was reintroduced for another 90-s interaction period. Interaction periods were repeated 10 times with 3-min intertrial intervals. On the 11th and final trial, a different ovariectomized C57BL/6J female was introduced and the time spent interacting was recorded.

Novel object recognition

Mice were habituated to the 16″ × 16″ testing chamber during three 15-min sessions on three consecutive days. For training, mice were placed in the chamber with a single object (either a die or marble) for 10 min. Four h later, the mouse was returned to the chamber and exposed to an exact duplicate of the first object and a novel object. The time spent exploring each object was recorded during both the training and test.

Olfactory or pheromone habituation and dishabituation

Protocol 1 used singly housed male mice and was similar to social recognition testing. The home cage, without lid or food hopper, was moved to the testing area for a 3-min habituation period. A cotton ball soaked in a novel odorant (cineole or limonene; Sigma Chemicals, St Louis, MO, USA) was placed inside a perforated plastic tube. This tube was introduced to the mouse's home cage, and the time the mouse spent actively exploring the tube (touching, licking or sniffing from a distance of <1 cm) was recorded for 3 min. Then, the tube was removed, and the mouse rested for 3 min. The odor tube was then reintroduced to the cage for another 3-min trial. This procedure was repeated for a total of five trials with 3-min intertrial intervals. On the sixth and final trial, a new tube with a new smell was introduced. The same protocol was used to assess pheromone recognition. The pheromone stimuli were 1-cm squares of filter paper soaked in urine from novel, opposite-sex FVB/N mice.

Protocol 2 was designed to increase dishabituation to novel odors. Pair-housed male mice were tested in their home cages after the cagemate was moved to a clean holding cage. A cotton swab soaked in vehicle (mineral oil) was suspended from a wire top over the cage for 10 min. Then, the swab was replaced by a swab soaked in a novel odorant (cineole, limonene or isoamyl acetate), and the number and length of explorations (mouse rearing up to bring his nose within 1 cm of swab and sniffing) were scored for 3 min. Then, the swab was removed and immediately replaced by a swab soaked in the same odorant. This was repeated for a total of three presentations of the same odor with no intertrial intervals. On the fourth trial, a new odor was introduced, and the sequence of three presentations was repeated. A third odor was introduced on the final trial.

Social exploration of a novel environment

We adapted the novel environment task (Palop *et al.* 2005). Same-genotype opposite-sex pairs of mice explored a cage containing a new bedding type and novel olfactory, pheromone, tactile and visual stimuli for 2 hours prior to killing. The paired mice had never interacted with each other before. The mice were videotaped and scored by a genotype-blind observer. Four discrete time intervals were scored: 0–10 min, 30–40 min, 60–70 min and 90–100 min after introduction to the environment. The total time spent interacting was recorded, and the number of social interactions was counted. Social interactions included any physical contact, including sniffing, allo-grooming, huddling, chasing, mounting and fighting. Tail rattling was also scored as social interaction, but no other non-contact interactions were included. Each interaction was classified as either brief exploratory (lasting $<$10 s), extended affiliative ($>$ 10 s, with no aggression or mating behaviors), aggressive (chasing, fighting, tail rattling) or mounting.

Social interaction – video analysis

Male mice were tested in their home cages. The lids were removed, and a novel, same-genotype female mouse was introduced into the cage. A video analysis program (Social Scan; Clever Sys Inc., Reston, VA, USA) recorded digital video of the mice for 2 h and determined activity counts and the number and length of social contacts.

Resident–intruder aggression test

Adult mice were housed under a reversed 12:12 h light–dark cycle, with the lights switched on at 0100 h. Fgf17$^{+/+}$ and Fgf17$^{-/-}$ males tested as residents were 10 to 14 weeks old at the time of testing. The animals were group housed by sex after weaning, and then moved to individual housing 1–2 weeks prior to the first aggression assay. They remained in individual housing until the assays were completed. All mice were sexually naïve. All testing occurred after the lights had been switched off for at least 1 h. The intruder was an 8- to 12-week-old gonadally intact 129SvEv wild-type male, group housed since weaning. The intruder was placed in the home cage of the resident, and the interactions were recorded for 15 min in the dark, using a Sony camcorder. Each of the 11 Fgf17$^{+/+}$ mice and 7 Fgf17$^{-/-}$ males used as residents was tested three times in this assay. No mice were tested on consecutive days or more than twice a week. Each resident was exposed to a different intruder in each assay. The assays were scored for sniffing, grooming, attacking, chasing, mounting and tail rattles displayed by either the resident or the intruder. An episode of attack included one or more instances of biting, tumbling or wrestling. The behaviors were scored using customizable settings in Observer v. 5.0.31 (Noldus).

Open field, Y maze and elevated plus maze

Testing was conducted as described (Cheng *et al.* 2007). The arms of the elevated plus maze were 2″ wide and 15″ long, with 4″ high walls on the closed arms. Plus maze testing was conducted in low light (120 lux).

Startle reactivity and prepulse inhibition

Acoustic startle reactivity and prepulse inhibition was measured as described (Esposito *et al.* 2006).

Rotarod

Motor coordination and balance were assessed by placing mice on a rotating drum 3.2 cm in diameter (Rota-Rod 5-station mouse treadmill; MED Associates, St Albans, VT, USA). The speed of the rod was gradually accelerated from 4 to 40 rpm over 5 min, and the latency to fall was recorded. Each mouse received four trials with an intertrial interval of at least 1 h.

Fear conditioning

After 3-min acclimation to a conditioning chamber (Freeze Monitor; San Diego Instruments, San Diego, CA, USA), a 3-kHz auditory tone was activated for 30 s, coterminating with a 1-s foot shock (0.3 mA). This was followed by a 90-s rest period, after which the same tone/shock/rest sequence was repeated once; 48 h later, the mouse returned to the chamber for 5 min, and freezing was monitored by beam breaks. Data were analyzed using Freeze Detector software (San Diego Instruments).

Novelty-suppressed feeding

Mice were singly housed at least 5 days prior to testing; 18 h before testing, food was removed from the cage. The next day, the mouse was placed in a brightly lit novel arena (16″ × 16″, 820 lux) that contained a small dish of food pellets at the center of the field. The mouse was allowed to explore the field freely for 15 min or until it began to eat the food. At the end of the test, the food was placed with the mouse in its home cage, and the mouse was allowed to eat for 10 min. To measure the food consumed by each mouse, the dish was weighed before beginning the test and again after the 10-min feeding period.

Fos induction after novel environment exposure

Tissue preparation

After novel environment exploration (see above), mice were taken to an adjacent room, deeply anesthetized and perfused transcardially with normal saline. Control mice remained singly housed and undisturbed in their home cages for 3 days prior to killing. The brains were removed and drop fixed with 4% phosphate-buffered paraformaldehyde at 4°C for 48 h. After rinsing with phosphate-buffered saline (PBS), brains were transferred to 30% sucrose in PBS at 4°C for 24 h and sectioned with a sliding microtome. Ten subseries of coronal floating sections (30 μm) were collected from one hemibrain; eight subseries of 30-μm sagittal sections were collected from the opposite hemibrain.

Immunohistochemistry

After quenching endogenous peroxidase activity and blocking non-specific binding, sections were incubated with rabbit anti-Fos (Ab-5, 1:10 000; Calbiochem, San Diego, CA, USA) and detected with biotinylated goat anti-rabbit (1:200; Vector Laboratories, Burlingame, CA, USA) and ABC kit (Vector). Diaminobenzidine was the chromagen.

Fos analysis

Quantification and analysis were performed blind to genotype and experimental condition. The two most medial sagittal sections from each brain were photographed at high resolution using

a microscope-mounted camera (Axiocam HRC on a Bx60 Microscope; Olympus, Melville, NY, USA), and cells were counted on the digital images. For frontal cortex analysis, each image was overlaid with a standard three-box grid using the rhinal sulcus and corpus callosum/ striatum as landmarks. The number of Fos$^+$ cells in each bin was recorded. For the main olfactory bulb, sagittal sections were photographed and aligned, and Fos$^+$ cells in the ventral side of the glomerular layer were counted and averaged for two medial sections. For the accessory olfactory bulb (AOB), all Fos$^+$ cells in two sagittal sections in which the AOB was clearly defined were counted and averaged. For hippocampus, Fos$^+$ cells in the granule cell layer of the dentate gyrus were counted and averaged for five sections.

Statistical analysis

After determining that there were no statistical differences between mice of the same genotype in different cohorts on each test, data from those cohorts were pooled. Data were analyzed by ANOVA with genotype as a main factor with three levels: Fgf17$^{+/+}$, Fgf17$^{+/-}$ and Fgf17$^{-/-}$. When mice of both sexes were tested, sex was also included as a factor in the ANOVA. In experiments where the genotype effect was significant, a *post hoc* Tukey–Kramer or Scheffé test was used to identify significant differences between genotypes. Repeated-measures ANOVA with trial or time bin as the repeated measure was used to compare the time course for USV, postnatal growth, social recognition, olfactory habituation and dishabituation, pheromone recognition, social exploration, prepulse inhibition of acoustic startle, open field and rotarod. Statistical analyses were performed using StatView v. 5.0.1 (SAS Institute, Cary, NC, USA). Except where noted, all data are expressed as means ± SEM.

Results

Reduced USV in Fgf17$^{-/-}$ pups

Ultrasonic vocalizations are an important component of rodent mother–infant social interaction. When 8-day-old pups were initially isolated from their Fgf17$^{+/-}$ mother and mixed-genotype litter, Fgf17$^{-/-}$ and Fgf17$^{+/-}$ pups vocalized less than Fgf17$^{+/+}$ littermates (Fig. 1a; genotype effect, $F_{2,50} = 4.6$, $P < 0.05$). When the pups were subsequently allowed 3-min contact with the mother and when the mother was again removed from the cage for a second 3-min isolation period, differences among genotypes were less prominent, primarily because of reduced vocalization in Fgf17$^{+/+}$ mice, although there was a genotype effect when all three phases of testing were considered (repeated-measures ANOVA, genotype effect, $F_{2,47} = 4.7$, $P < 0.05$ and test session effect, $F_{2,94} = 4.6$, $P < 0.05$). Ablation or reduction of Fgf17 significantly decreased the total vocalizations emitted during all three phases of testing (Fig. 1b; $F_{2,50} = 6.3$, $P < 0.005$). The reduced vocalizations could not be explained by abnormal physical growth or motor development because Fgf17 genotype did not affect weight gain (Fig. 1c) or attainment of developmental milestones (Fig. 1d). Instead, the decrease in vocalizations suggests abnormal interactions between pups and their mothers, which we hypothesized

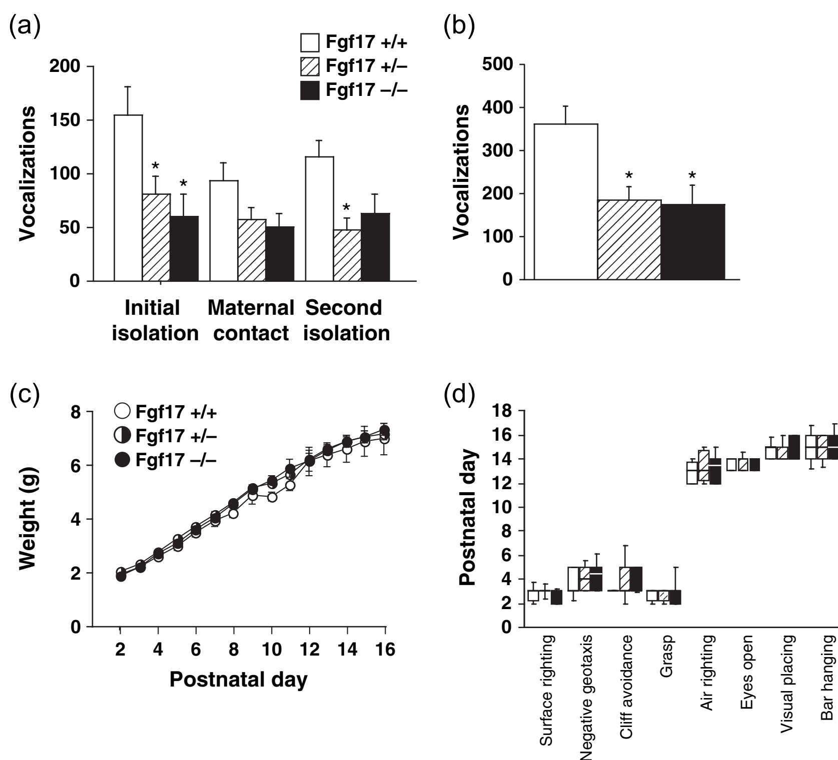

Figure 1: Fgf17 reduction impairs social vocalization behavior in mouse pups. (a) Number of vocalizations per 3-minute testing period. (b) Total number of vocalizations during all three testing phases. (c) Weight during the first 16 postnatal days. (d) Postnatal day on which developmental milestones were attained for each genotype. Box plot shows the 10th, 25th, 50th, 75th and 90th percentile for each measure. Twelve Fgf17$^{+/+}$, 26 Fgf17$^{+/-}$ and 15 Fgf17$^{-/-}$ mice were analyzed in (a) and (b); and 7 Fgf17$^{+/+}$, 19 Fgf17$^{+/-}$, and 14 Fgf17$^{-/-}$ mice in (c) and (d). *$P < 0.05$ vs. Fgf17$^{+/+}$ mice by Tukey–Kramer test.

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to be a harbinger of broader social deficits in adult Fgf17$^{-/-}$ mice.

Fgf17 genotype does not affect activity level, emotional behaviors, sensorimotor gating, motor control, fear conditioning or feeding

We began our analysis of adult mice with a broad screen for differences in various fundamental behavioral domains. The results of all behavioral tests are summarized in Table S1. Fgf17 reduction did not affect locomotor activity (Fig. 2a) or rearings (Fig. 2b) in the open field or the proportion of locomotion occurring in the center of the field (Fig. 2c), a measure related to anxiety. These data agreed with other activity measures, including arm entries in the Y maze (Fig. 2d) and distance traveled in the elevated plus maze (Fig. 2e). In the elevated plus maze, a tendency to spend more time in the open arms is thought to indicate decreased anxiety or emotionality (Dawson & Tricklebank 1995). Fgf17$^{-/-}$ mice explored the closed arms of the maze slightly less than Fgf17$^{+/+}$ littermates (Fig. 2f; $F_{2,26} = 3.7$, $P < 0.05$). However, this behavioral difference was not particularly robust, as reflected by the lack of significant corresponding increases in exploration of the open arms or intersection of the maze. Another measure of emotionality and reactivity, the whole-body acoustic startle, did not show any genotype differences (Fig. 2g). Prepulse inhibition of acoustic startle, an indicator of sensorimotor gating, did not differ between genotypes (Fig. 2h). On the accelerating rotarod, Fgf17$^{+/-}$ mice showed a decreased latency to fall (Fig. 2i; $F_{2,61} = 3.9$, $P < 0.05$). As Fgf17$^{-/-}$ mice showed no deficits on this task ($P = .97$ vs. Fgf17$^{+/+}$), and Fgf17$^{+/-}$ mice showed no other evidence of motor impairments (Figs 1d and 2a,e and Figure S1d), the decreased fall latency of Fgf17$^{+/-}$ mice may reflect a multiple comparisons error or other testing artifact. Fgf17$^{+/-}$ mice and Fgf17$^{-/-}$ mice exhibited no learning deficits in a contextual fear-conditioning task (Fig. 2j). Novelty-suppressed feeding, a test that is thought to reflect serotonin function and that correlates with anxiety- (Gross *et al.* 2002) and depression-related behaviors (Santarelli *et al.* 2003), also showed no genotype differences (Fig. 2k,l). The feeding data suggest that reduction in Fgf17 has no major effects on metabolism or appetite.

Fgf17$^{-/-}$ mice interact less with novel female during social recognition test

To investigate complex social behaviors in adult mice, we performed a social recognition test (Ferguson *et al.* 2000). We measured the amount of time male mice interacted with the same ovariectomized Fgf17$^{+/+}$ female mouse during 10 sequential 90-second exposures. For all genotypes, males showed the same initial interest in the female, followed by a progressive decrease in social interaction time (Fig. 3a). When a new ovariectomized Fgf17$^{+/+}$ female was introduced at the end of the test, Fgf17$^{+/+}$ and Fgf17$^{+/-}$ males responded with a resurgence of interest, interacting as much with the new female as they had on the first trial with the prior female (Fig. 3a,b). In contrast, Fgf17$^{-/-}$ males exposed to a new female showed little change from the final trial with the familiar female. Fgf17$^{-/-}$ males spent significantly less time than their Fgf17$^{+/+}$ or Fgf17$^{+/-}$ littermates interacting with the novel female (Fig. 3b; $F_{2,39} = 6.4$, $P < 0.005$).

This abnormality in Fgf17$^{-/-}$ males could result from a circumscribed social deficit or from more global abnormalities in their novelty response or olfactory processing. To evaluate the general response to novelty, we used the novel object recognition test with non-olfactory, inanimate stimuli. There were no genotype differences in the preference for exploring a novel object over a familiar object (Fig. 3c), indicating that Fgf17$^{-/-}$ mice can recall prior exposure to a stimulus, can distinguish familiar from novel stimuli and have the normal tendency to interact more with a novel stimulus. Similarly, we found no evidence of olfactory impairments on two different tests of olfactory habituation and dishabituation.

The first task (Fig. 3d) was designed to parallel the conditions of the social recognition test, with 3-min intertrial intervals and a single exposure to a novel odor. All mice habituated quickly under these conditions, reaching almost no exploration after five trials. Dishabituation was equal in all three genotypes (Fig. 3d). We then eliminated intertrial intervals in a second protocol to enhance dishabituation (Fig. 3e). All mice showed normal patterns of habituation and dishabituation to two different odors. While there was a trend for Fgf17$^{+/+}$ mice to spend more time interacting with the initial odor, this difference was not statistically significant, and there were no genotype differences in the response to the novel odor. Pheromone perception also appeared normal in Fgf17$^{-/-}$ mice, based on habituation and dishabituation to urine taken from opposite-sex mice from a different strain (Fig. 3f). These findings are consistent with observations of normal morphology within the olfactory bulb and AOB (Cholfin & Rubenstein 2007). Therefore, the deficit observed in the social recognition task cannot be explained by learning impairments, reduced preference for novelty, or olfactory dysfunction, suggesting that it reflects social dysfunction.

Decreased social interaction in Fgf17$^{-/-}$ mice

To further investigate the effect of Fgf17 reduction on social behavior, we allowed same-genotype, opposite-sex pairs of mice to explore an environment filled with novel stimuli for 2 h. Initially, all mice spent similar amounts of time interacting with their partners (Fig. 4a). However, in the second hour of the test, Fgf17$^{+/+}$ mice increased the amount of time they spent interacting with each other, whereas Fgf17$^{-/-}$ mice decreased it (Fig. 4a; interaction of genotype and time, $F_{6,39} = 2.3$, $P < 0.05$). During the second hour, Fgf17$^{-/-}$ mice spent significantly less time interacting than Fgf17$^{+/+}$ mice (Fig. 4b; $F_{2,13} = 4.0$, $P < 0.05$). While the total time spent interacting decreased in Fgf17$^{-/-}$ mice, the total number of interactions did not differ between genotypes throughout the test.

To better understand this dissociation between the number of interactions and the time spent interacting, a genotype-blind observer categorized the types of interactions occurring. During the second hour, Fgf17$^{-/-}$ mice showed significantly

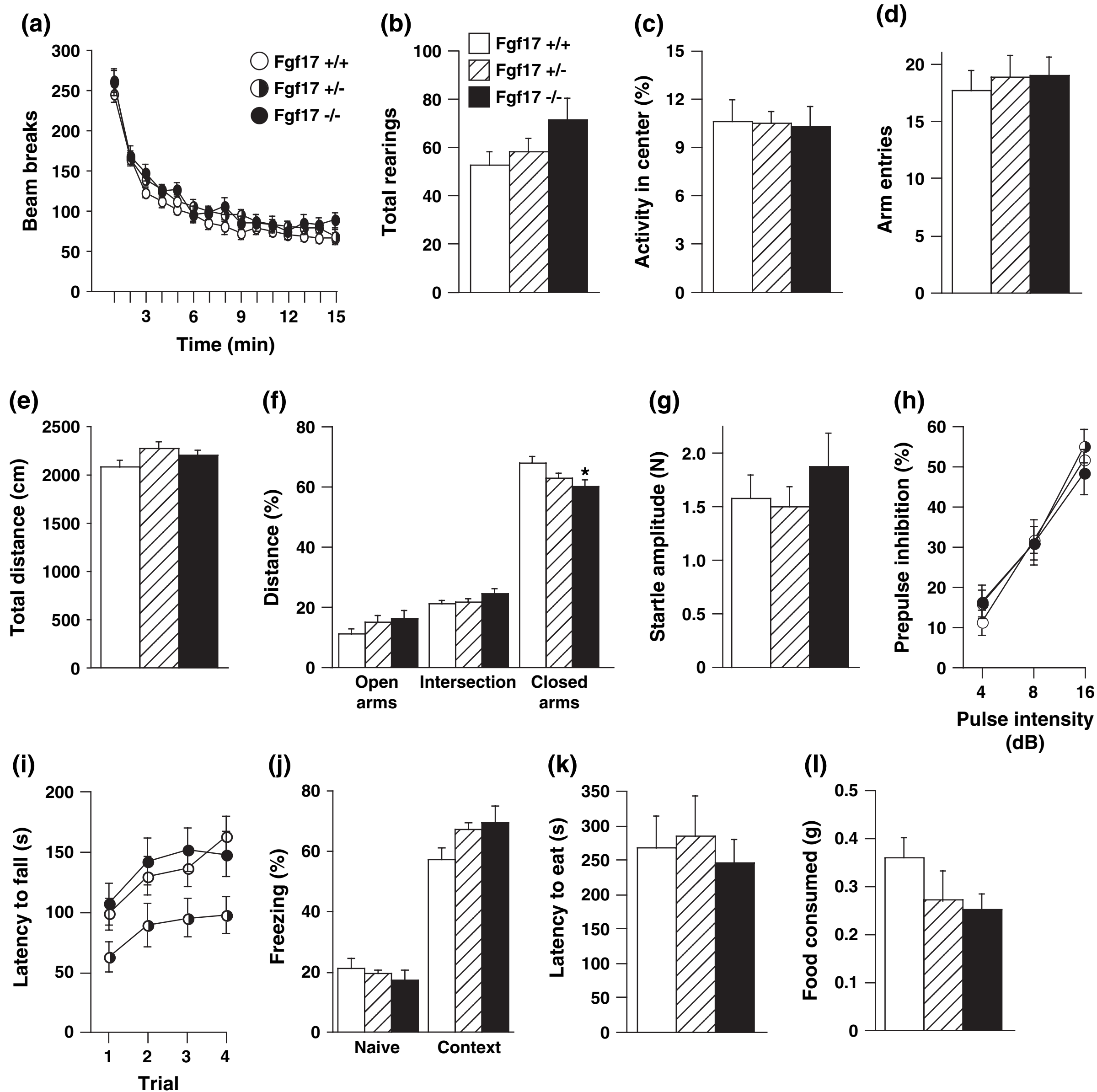

Figure 2: Fgf17 reduction does not affect most non-social behaviors. (a) Locomotion in novel open field during 15 min. (b) Total rearings during open-field test. (c) Per cent of locomotor activity in center portion of open field. (d) Total number of entries into arms of a Y-shaped maze during 6 min. (e) Total distance moved in the elevated plus maze during 10 min. (f) Percentage of distance moved in different areas of the plus maze. (g) Mean startle amplitude in response to a 120-dB acoustic stimulus without prepulse. (h) Inhibition of startle response when startle stimulus was preceded by prepulses of differing intensity. (i) Latency to fall off an accelerating rotating rod over four consecutive trials. (j) Contextual fear conditioning. Increased freezing behavior in mice when exposed to an environment where the mice had previously received 2 foot shocks. (k) Latency to begin eating in a brightly lit novel environment after an 18-h fast. (l) Amount of food consumed in home cage during 10 min after mouse began eating in novelty-suppressed feeding test. Twenty-five Fgf17$^{+/+}$, 25 Fgf17$^{+/-}$ and 17 Fgf17$^{-/-}$ mice were analyzed for (a)–(i); 13 Fgf17$^{+/+}$, 2 Fgf17$^{+/-}$ and 11 Fgf17$^{-/-}$ male mice for (j); and 11 Fgf17$^{+/+}$, 3 Fgf17$^{+/-}$ and 7 Fgf17$^{-/-}$ male mice for (k)–(l). $*P < 0.05$ vs. Fgf17$^{+/+}$ mice by Tukey–Kramer test.

fewer extended (>10 s) affiliative interactions than Fgf17$^{+/+}$ or Fgf17$^{+/-}$ mice (Fig. 4c; $F_{2,13} = 4.3$, $P < 0.05$). There were no genotype differences in brief exploratory interactions (<10 second), aggressive bouts, or mounting (Fig. 4c). This finding is consistent with a separate resident–intruder aggression assay that showed no differences between Fgf17$^{-/-}$ and Fgf17$^{+/+}$ mice in the latencies or lengths of attacks, chasing or sniffing behaviors (Fig. 4d).

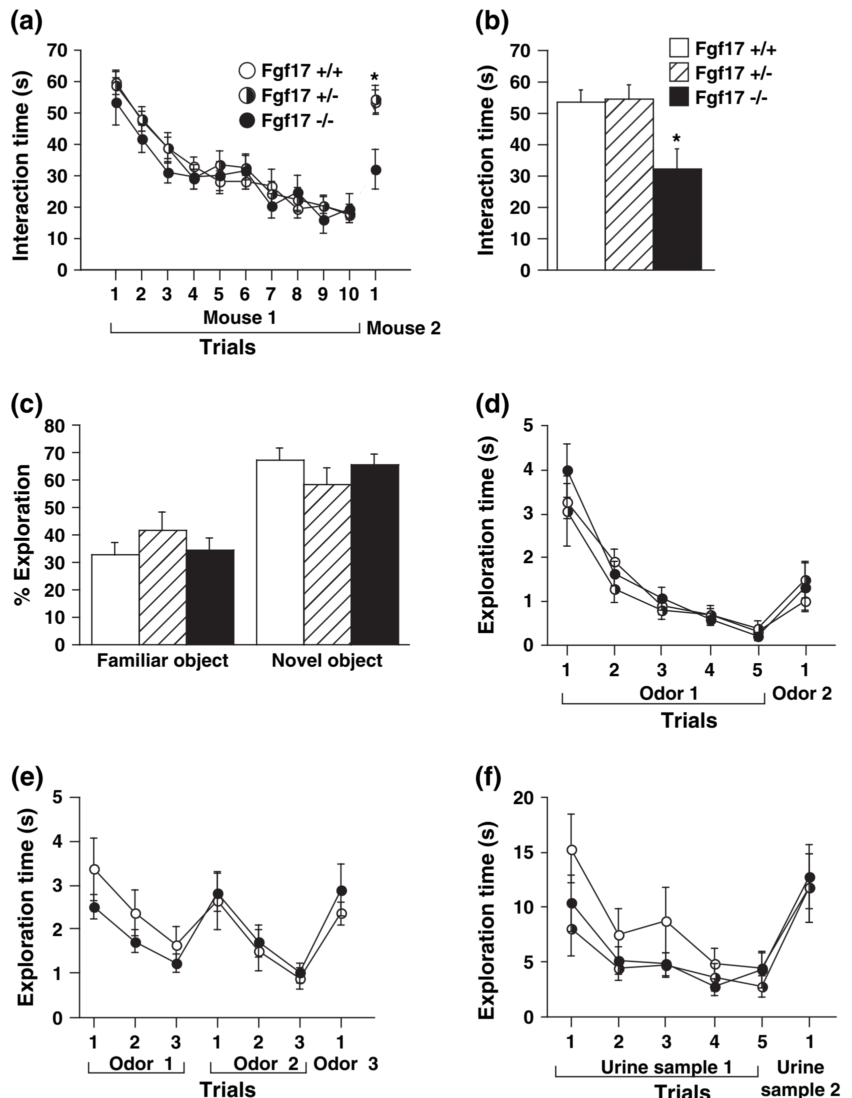

Figure 3: Fgf17 ablation reduces interaction with novel female in social recognition task. (a) Time spent interacting with the other mouse during successive 90-second trials and (b) total time spent interacting with novel mouse during final trial; 14 Fgf17$^{+/+}$, 16 Fgf17$^{+/-}$ and 12 Fgf17$^{-/-}$ male mice were analyzed in (a) and (b). (c) No genotype difference in percentage of a 10-min trial spent exploring a novel object; 18 Fgf17$^{+/+}$, 9 Fgf17$^{+/-}$ and 21 Fgf17$^{-/-}$ male mice were analyzed. (d) Normal habituation and dishabituation to odor stimuli presented for 3 min with 3-min intertrial intervals; 21 Fgf17$^{+/+}$, 7 Fgf17$^{+/-}$ and 23 Fgf17$^{-/-}$ male mice were analyzed. (e) Normal habituation and dishabituation to odor stimuli presented for 3 min with no intertrial intervals; 7 Fgf17$^{+/+}$ and 14 Fgf17$^{-/-}$ male mice were analyzed. (f) Normal habituation and dishabituation to pheromone stimuli (urine samples from opposite-sex FVB/N mice); 14 Fgf17$^{+/+}$, 15 Fgf17$^{+/-}$ and 21 Fgf17$^{-/-}$ mice were analyzed. *$P < 0.05$ vs. Fgf17$^{+/+}$ and Fgf17$^{+/-}$ mice by Tukey–Kramer test.

This social phenotype was confirmed in a separate cohort of mice using an automated video analysis system that continuously monitored social interaction between same-genotype pairs. Fgf17$^{+/+}$ mice increased interaction times during the second hour, but Fgf17$^{-/-}$ mice showed a steady decrease in time interacting over the 2 h (Figure S1a,b; interaction of genotype and time, $F_{46,437} = 1.7$, $P < 0.005$). Fgf17$^{+/-}$ had intermediate behavior, with interaction time not differing significantly from either Fgf17$^{-/-}$ or Fgf17$^{+/+}$ mice. Interaction time decreased in Fgf17$^{-/-}$ mice despite a slight increase in the total number of interactions (Figure S1c; $F_{2,19} = 4.4$, $P < 0.05$). No significant genotype differences were identified in total activity levels during the test (Figure S1d).

Reduced prefrontal cortex activation in Fgf17$^{-/-}$ mice during social exploration of novel environment

Fgf17$^{+/+}$ mice and Fgf17$^{-/-}$ mice were killed immediately after 2 h of exploration of a novel environment with a novel partner. The brains were processed and stained for Fos, a marker of neuronal activation (Morgan & Curran 1991). Control groups remained undisturbed in their home cages before killing. Few Fos^{+} neurons were detected in home cage controls of either genotype (Fig. 5a,c,d). In Fgf17$^{+/+}$ mice, exploration resulted in strong Fos induction in brain regions previously implicated in social behaviors and response to novelty, including the frontal cortex, olfactory bulb, AOB, hippocampus, septum, amygdala, bed nucleus of the stria terminalis, medial preoptic area and nucleus accumbens (Fig. 5a,b,d and data not shown). Fgf17$^{-/-}$ mice also had strong Fos induction in almost all of these areas, including ventromedial frontal cortex and orbital cortex (Fig. 5a,b and data not shown). As Fgf17 has been reported to affect development of the rostral cerebellum, we also examined Fos induction there (Fig. 5e). Cerebellar Fos induction was not intense, was most notable in the more caudal lobules VI–IX and did not differ between genotypes. In contrast, Fgf17$^{+/+}$

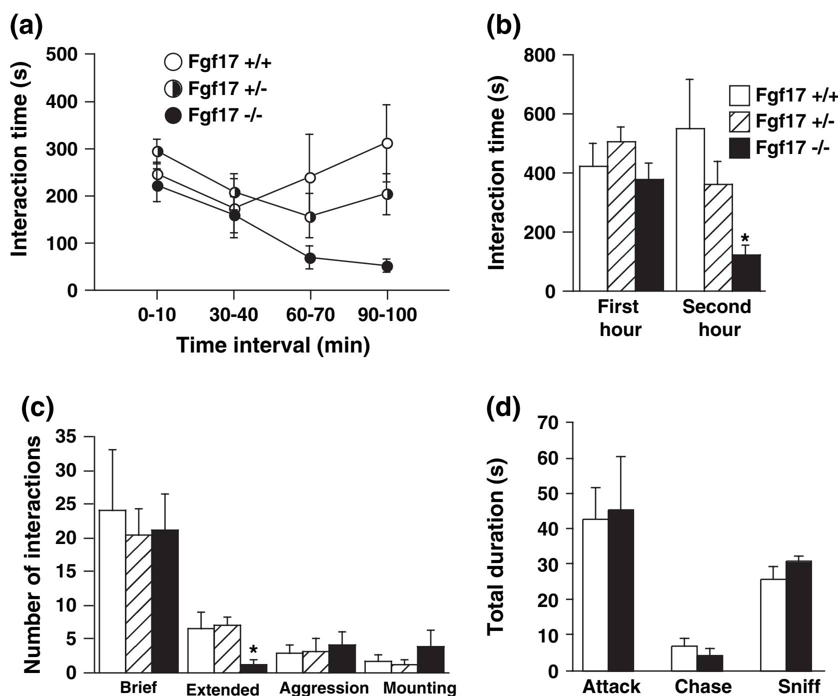

Figure 4: Ablation of Fgf17 reduces interaction with unfamiliar same-genotype, opposite-sex partner. (a) Total time spent engaged in social interactions during 10-min observation periods in a 2-h test. (b) Time spent engaged in social interactions during first and second hour. (c) Category of interactions during second hour. (d) Duration of attacks, chasing and sniffing in the resident–intruder test. Five Fgf17$^{+/+}$, 6 Fgf17$^{+/-}$ and 5 Fgf17$^{-/-}$ pairs of opposite-sex mice were tested in (a)–(c); and 11 Fgf17$^{+/+}$ and 7 Fgf17$^{-/-}$ male mice in (d). $^{*}P < 0.05$ vs. Fgf17$^{+/+}$ mice by Tukey–Kramer test.

and Fgf17$^{-/-}$ mice showed significant differences in Fos induction in the dorsomedial prefrontal cortex (Fig. 5a–c; $F_{1,8} = 37.0$, $P < 0.0005$). Overall, Fgf17$^{-/-}$ mice had 47% fewer Fos^{+} cells in the medial frontal cortex than Fgf17$^{+/+}$ mice after social exploration of a novel environment (Fig. 5c).

Discussion

This study shows that elimination of Fgf17 causes several behavioral alterations. Within the social domain, all of the observed alterations were surprisingly specific, as highlighted by social behaviors that remained intact. For example, aggressive behavior in the resident–intruder assay did not depend on Fgf17 genotype. Furthermore, when initially presented with a novel partner, either in tests of social recognition or social interaction, Fgf17$^{-/-}$ males showed normal levels of interaction, suggesting that they do not have increased social anxiety and do not find brief social interactions aversive. Social exploration appeared to be intact, as the number of interactions remained high in the social interaction paradigm even after the time they spent interacting fell sharply.

Heterozygous Fgf17-deficient mice were only impaired in some social assays. Fgf17$^{+/-}$ pups vocalized significantly less than Fgf17$^{+/+}$ pups, and no more than Fgf17$^{-/-}$ pups. In contrast, the behavior of Fgf17$^{+/-}$ adults was indistinguishable from Fgf17$^{+/+}$ controls on the social recognition task. In a novel environment, the social interactions of Fgf17$^{+/-}$ mice with a novel partner were intermediate between those of Fgf17$^{-/-}$ and Fgf17$^{+/+}$ mice, but showed no significant difference from either homozygous genotype. Embryonic Fgf17$^{+/-}$ mice have reduced expression of Fgf17 messenger RNA (Xu *et al.* 2000), but expression levels have not been carefully quantified at different ages for this developmentally regulated gene product. Conceivably, partial reductions in Fgf17 levels during development allow for the normal formation of neuroanatomical structures and physiological mechanisms important for certain social behaviors, but disrupt the formation of others. This would lead to disruptions in some, but not all, related behaviors in adulthood. Another possibility is that all of these social behaviors are modulated by the same neural circuits and activities, but some behaviors are especially sensitive to a partial reduction of Fgf17 signaling.

The deficits observed in adult Fgf17$^{-/-}$ mice primarily involved social interactions with unfamiliar mice. However, compared with Fgf17$^{+/+}$ pups, Fgf17$^{-/-}$ pups showed a marked decrease in vocalizations after isolation from their mother and littermates, suggesting that social abnormalities in Fgf17$^{-/-}$ mice are not restricted to interactions with novel individuals.

Many component behaviors contribute to normal social behavior, including sensory perception, exploratory activity and cognition. Fgf17$^{-/-}$ mice display normal exploration,

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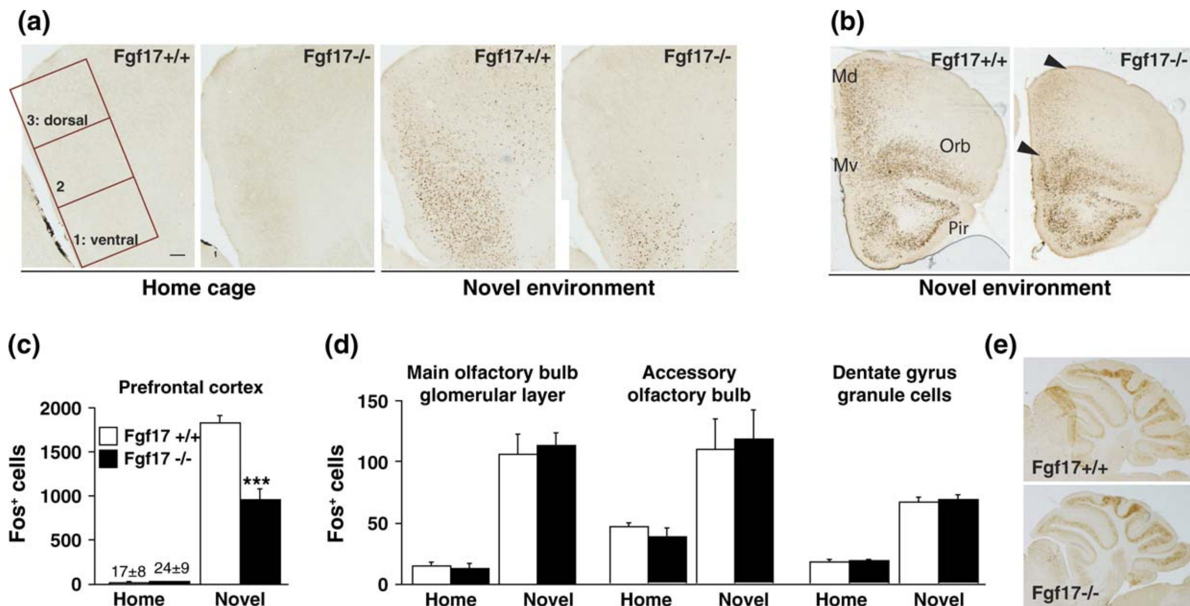

Figure 5: Fgf17 ablation decreases frontal cortex activation during exploration of novel environment with new same-genotype, opposite-sex partner. (a) Photomicrographs depict sagittal sections of medial prefrontal cortex stained for Fos. Overlay in Fgf17$^{+/+}$ home cage section illustrates placement of fields used to count Fos^{+} cells. Scale bar = 200 μm. (b) Coronal section of prefrontal cortex showing that Fos induction was prominent in dorsomedial regions of prefrontal cortex in Fgf17$^{+/+}$ mice but not Fgf17$^{-/-}$ mice. Arrowheads bracket region of reduced Fos induction in Fgf17$^{-/-}$ section. (c) Average number of Fos^{+} cells in the two most medial sagittal brain sections. (d) Total number of Fos^{+} cells counted in sagittal sections of olfactory bulb, AOB and dentate gyrus granular layer. Counts in (c)–(d) were obtained from five Fgf17$^{+/+}$ and five Fgf17$^{-/-}$ male mice. (e) Sagittal sections of medial cerebellum showing comparable levels of Fos^{+} expression in both genotypes after social exploration of a novel environment. ***$P < 0.0005$.

locomotion, emotionality and learning. However, other factors that contribute to complex social behaviors may be altered in these mice. Reductions in anxiety or stress response could reduce the drive for pups to vocalize or for adults to engage in affiliative behaviors. Although our battery of tests showed no evidence for altered anxiety in Fgf17$^{-/-}$ mice, more direct testing of stress response and social anxiety, both in adults and during development, could help refine our understanding of the observed social deficits.

Cognitive deficits may also alter social behaviors. The social recognition test requires attention, working memory and novelty recognition, as well as intact pheromone and odor perception. Mice lacking Fgf17 were able to distinguish between different odors and pheromones. Moreover, they had normal habituation to the first mouse, indicating that they can sense and retain social information. This is distinct from the social memory deficits observed in mice lacking estrogen receptors, vasopressin receptors or oxytocin (Bielsky *et al.* 2004; Choleris *et al.* 2003, 2006; Ferguson *et al.* 2000; Kavaliers *et al.* 2004). The reduced response to the second novel individual exhibited by Fgf17$^{-/-}$ mice suggests an impaired ability to compare and respond to new social information, an executive function that depends in part on the medial prefrontal cortex (Mitchell *et al.* 2006; Rudebeck *et al.* 2006). The intact performance in novel object recognition and other habituation/dishabituation tasks argues against deficits that are restricted to attention or memory, but does not exclude problems in assigning attention to social stimuli. Indeed, the emergence of deficits late in the testing period for both the social interaction and social recognition tests suggests a possible interaction of attention and social behavior. The anatomical abnormalities in the medial prefrontal cortex of mice lacking Fgf17 may disrupt attention and social behaviors (Cholfin & Rubenstein 2007). Tests of Fgf17$^{-/-}$ mice that challenge attention and working memory, both in social and non-social contexts, could shed light on this issue.

It is interesting that Fgf17 ablation selectively reduced Fos induction in the dorsomedial frontal cortex after social exploration of a novel environment. Fos induction in the ventromedial and orbital prefrontal cortex, areas that receive and integrate input from multiple sensory modalities and are involved in regulating reward and aggression (Heidbreder & Groenewegen 2003), appears to be maintained. While the reduction in dosomedial prefrontal cortex Fos induction corresponded with reduced social interaction, the mice were also exposed to new objects, odors, textures and tastes during this experiment. An impaired response to any of these stimuli could reduce Fos induction. It is unlikely that deficits in primary sensory function contributed to this deficit because we found normal Fos induction in the olfactory bulb, AOB, somatosensory cortex and auditory cortex (Fig. 5d and data not shown). This conclusion is further supported by our observation that Fgf17$^{-/-}$ mice responded normally to odors and pheromones. The impaired Fos induction in the dorso-

medial frontal cortex also cannot be explained by a reduced general response to novelty because Fgf17$^{-/-}$ mice showed normal Fos induction in granule cells of the dentate gyrus, which respond to novel stimuli (Montag-Sallaz *et al.* 1999).

The likeliest cause of the impaired Fos induction may be developmental abnormalities in the formation of the medial prefrontal cortex that have been identified in Fgf17$^{-/-}$ mice (Cholfin & Rubenstein 2007). It is interesting in this context that social isolation during early postnatal development in rats resulted in hypoplasia of the medial prefrontal cortex (Pascual *et al.* 2007) and reduced basal expression of *c-fos* and other immediate early genes (Levine *et al.* 2007) in this region. Furthermore, medial prefrontal cortex signaling modulates social behaviors in rodents (Heidbreder & Groenewegen 2003), nonhuman primates (Rudebeck *et al.* 2006) and humans (Iacoboni *et al.* 2004; Mitchell *et al.* 2006). However, we cannot exclude the possibility that loss of Fgf17 function in other brain regions also contributed to the observed deficits. For example, Fgf17$^{-/-}$ mice have mild hypolasia of the inferior colliculus and anterior vermis of the cerebellum (Xu *et al.* 2000), and En2-deficient mice with posterior cerebellar defects have abnormal social behavior (Cheh *et al.* 2006). Additional studies are needed to explore the interdependence of Fgf17-related anatomical abnormalities and the behavioral abnormalities identified in the current study.

A growing body of evidence suggests that genes involved in neurodevelopment are also essential for normal social behaviors. Null mouse mutants of genes involved in fragile X syndrome or Rett syndrome show developmental and social deficits (Gemelli *et al.* 2006; Mineur *et al.* 2006; Moretti *et al.* 2005; Spencer *et al.* 2005). Genes linked to other forms of mental retardation and autism spectrum disorders, including phosphatase and tensin homolog on chromosome ten (Pten) (Kwon *et al.* 2006), forkhead box P2 (FoxP2) (Shu *et al.* 2005) and En2 (Cheh *et al.* 2006), also disrupt social behavior when ablated in mice. Many of these mouse models also exhibit major deficits in other behavioral domains, including motor coordination, activity, anxiety and learning. These complex phenotypes relate closely to the range of deficits observed in the corresponding human disorders. However, changes in basic neurological functions and behaviors can affect performance in more complex social tasks, complicating the interpretation of social data. The current study complements these candidate-gene studies by pinpointing a molecular alteration that selectively alters social behavior. While we cannot exclude the possibility that Fgf17$^{-/-}$ mice have alterations in behaviors we have not yet explored, these mice provide the opportunity to analyze processes that impair social behaviors without confounding disruptions of locomotion, emotionality or novelty response.

The developmental expression of Fgf17 in the brain, the abnormalities in cortical patterning resulting from its deletion (Cholfin & Rubenstein 2007) and the social deficits observed during development and adulthood suggest that Fgf17$^{-/-}$ mice may model some of the social abnormalities found in complex human neuropsychiatric diseases. Interestingly, Fgf17 lies within the 8p22-21 chromosomal linkage region for schizophrenia (Katoh & Katoh 2005). The genes for FgfR2 receptor, a potential Fgf17 effector (Zhang *et al.* 2006), and Fgf1, the universal Fgf ligand, are also within schizophrenia linkage sites (Owen *et al.* 2005). A thorough investigation of the mechanisms linking Fgf17 signaling to social deficits may shed light on the disease processes underlying schizophrenia and other neuropsychiatric disorders.

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Supplementary Material

The following supplementary material is available for this article online from http://www.blackwell-synergy.com/doi/full/10.1111/j.1601-183X.2007.00357.x

Figure S1. Video analysis confirms reduced interactions of Fgf17$^{-/-}$ mice with unfamiliar same-genotype, opposite-sex partner. (a) Total time spent engaged in social interactions during 2-h test, divided into consecutive 5-min periods. (b) Total time spent in social interaction during first and second hour. (c) Number of discrete social interactions during 2-h test, divided into 5-min periods. (d) Total activity counts for pairs of mice, divided into 5-min periods. 7 Fgf17$^{+/+}$, 6 Fgf17$^{+/-}$ and 9 Fgf17$^{-/-}$ pairs of mice were analyzed. $*P < 0.05$ vs. Fgf17$^{+/+}$ mice by Tukey–Kramer test.

Table S1. Summary of behavior outcomes.

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