# Sociability Deficits and Altered Amygdala Circuits in Mice Lacking *Pcdh10*, an Autism Associated Gene

## *Supplemental Information*

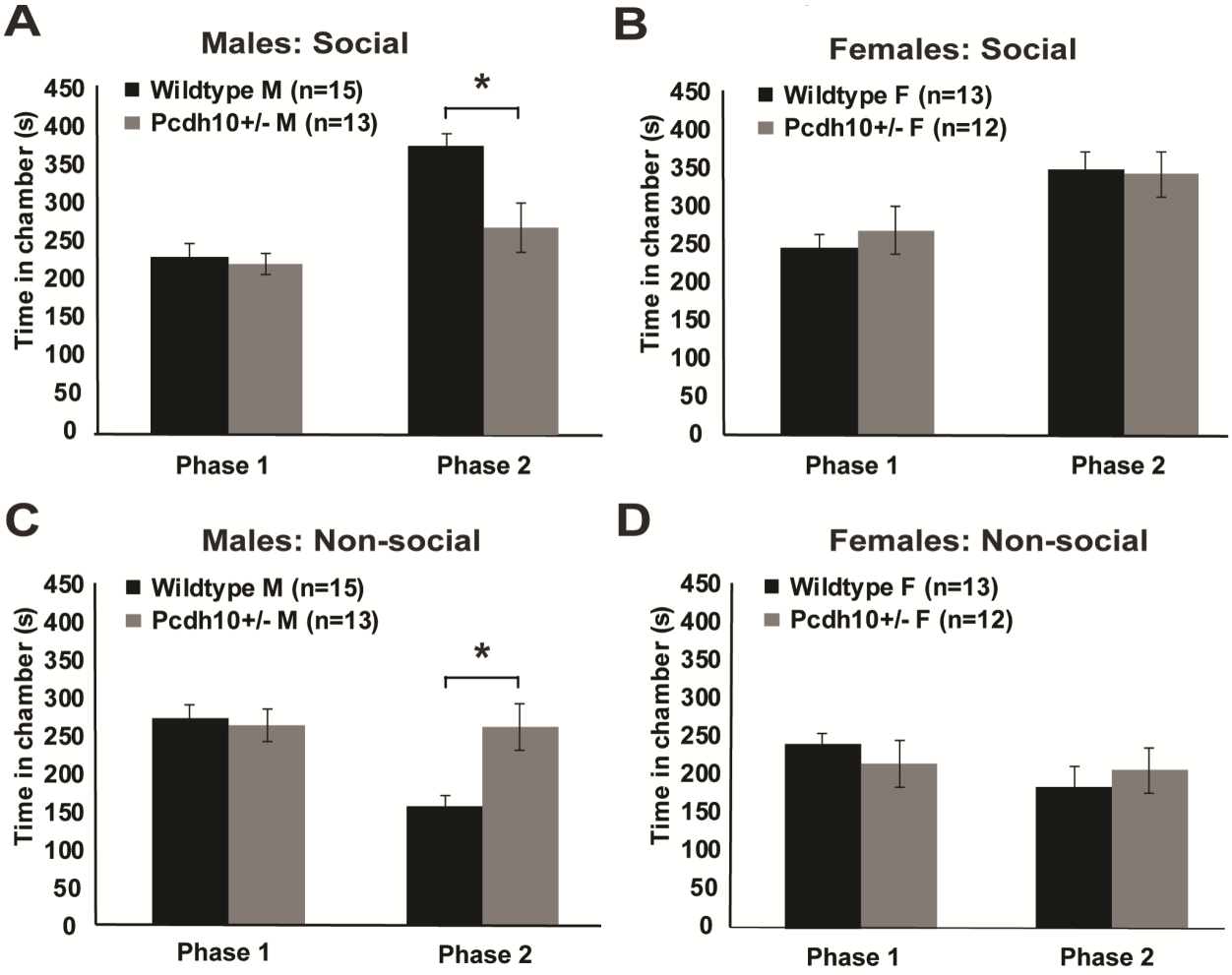


**Figure S1. Reduced time spent in the social chamber and increased time spent in the nonsocial chamber in Phase 2 in juvenile male Pcdh10$^{+/-}$ mice.**
The lower social approach of male *Pcdh10*$^{+/-}$ mice was also demonstrated by time spent in the social and nonsocial chambers: **(A)** Time spent in the social chamber in *Pcdh10*$^{+/-}$ and WT males. *Pcdh10*$^{+/-}$ males showed significantly less increase in time spent in the social chamber from Phase 1 to Phase 2 than did male WT littermates (phase by genotype interaction, $p = .003$; also, a significant main effect of phase ($p = .019$) but not of genotype). **(B)** Time spent in the social chamber in *Pcdh10*$^{+/-}$ and WT females. There was no significant phase by genotype interaction; there was a significant main effect of phase ($p < .001$); but there was no main effect of genotype. **(C)** Time spent in the nonsocial chamber in *Pcdh10*$^{+/-}$ and WT males. *Pcdh10*$^{+/-}$ males showed significantly less decrease in nonsocial chamber time from Phase 1 to Phase 2

than did male WT littermates (phase by genotype interaction, $p$ = .002; no significant main effects of phase or genotype). **(D)** Time spent in the nonsocial chamber in *Pcdh10*$^{+/-}$ and WT females. There was no significant phase by genotype interaction, and there were no significant main effects of phase or genotype.

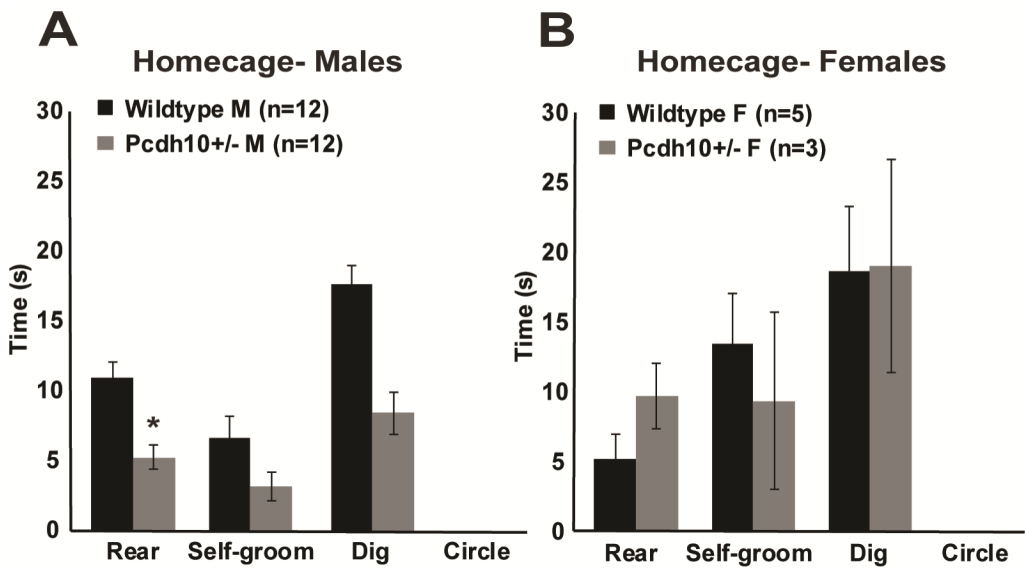


**Figure S2. Home cage behaviors repetitive in juvenile *Pcdh10*$^{+/-}$ and WT littermates.** Home cage repetitive behaviors in juvenile *Pcdh10*$^{+/-}$ and WT littermate mice. Most mice were tested during the first half of the dark cycle. Mice were allowed to acclimate to the behavioral testing room for 30 minutes. Then, cage tops were replaced with a flat, transparent Plexiglas top with air holes. The cameras (Sony DCR-DVD508 Handycam Camcorder, Sony Corporation, Tokyo, Japan) over the cages emitted and detected infrared light, and so were able to record the mice in complete darkness. During acclimation and testing, all visible light sources in the room were switched off. The cameras began recording and the experimenter left the room for about 60 min. The cameras were then switched off, and the mice were returned to the colony housing room. The home cage behaviors were scored from videos of a 30-min period beginning 20 min after the experimenter started the camera and left the room. The behaviors of both mice in each cage were scored simultaneously once every 30 sec. for a total of 61 data points over the 30-min. period for each mouse. If a mouse was not engaged in any of the behaviors at a given scoring time, the mouse was marked as having “no behavior” at that time. A mixed effects poisson model was used to analyze this count data. **(A)** In males, there was a significant effect of genotype on rearing, but not on self-grooming, digging, or circling. **(B)** In females, there was no significant genotype effect on rearing, self-grooming, digging, or circling.

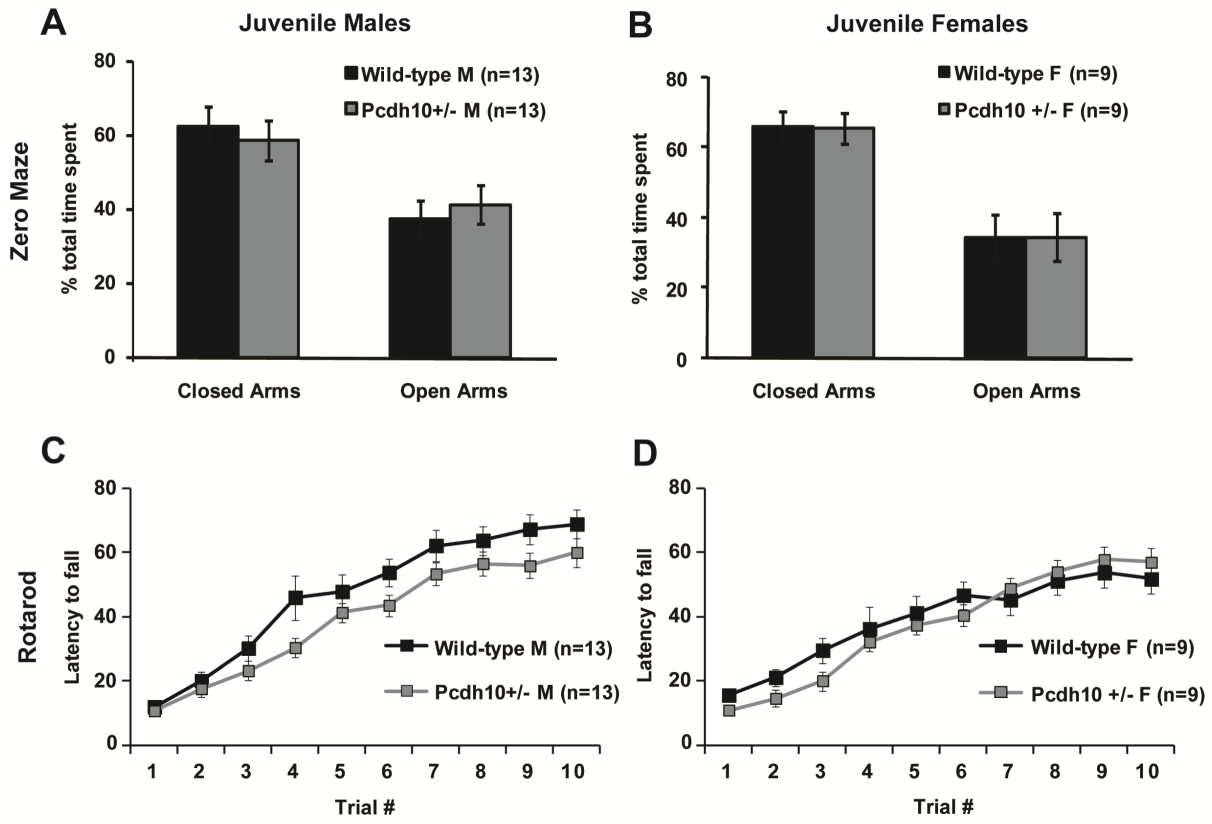


**Figure S3. Juvenile *Pcdh10*$^{+/-}$ mice showed no significant differences from WT littermates in anxiety-like behavior. Juvenile female, but not male, *Pcdh10*$^{+/-}$ mice showed differences in motor function.**

*Elevated Zero Maze*. The Plexiglas zero maze had a width of 2 inches and outside circumference of 79 inches. It consisted of two open quadrants and two closed quadrants. In this test of anxiety-like behavior (1), each mouse was allowed to investigate the apparatus for 5 minutes. The Viewpoint Tracking System (Viewpoint Life Sciences, France) was used to calculate time spent in the open quadrants vs. in the closed quadrants and numbers of transitions between the quadrants. **(A)** Percentage of time spent in the closed arms and open arms of an elevated zero maze in male *Pcdh10*$^{+/-}$ ($n$ = 13) and WT littermates ($n$ = 13). There was no significant effect of genotype on percentage of time that males spent in the closed arms (linear regression model, $p$ = .604). **(B)** Percentage of time spent in the closed arms and open arms of an elevated zero maze in female *Pcdh10*$^{+/-}$ ($n$ = 9) and WT littermates ($n$ = 9). There was no significant effect of genotype on percentage of time that females spent in the closed arms (linear regression model, $p$ = .960).

*Accelerating Rotarod.* To assess motor coordination and balance, mice were placed on a turning rotarod that accelerated from 0 to 40 revolutions per minute over the course of 5 minutes (1). Each trial ended when the mouse fell off of the rotarod to a table top several inches below or when 5 minutes elapsed. There were 10 consecutive trials, with 5 minute inter-trial intervals. Time that each mouse remained on the rotarod in each trial was measured. **(C)** Latency to fall during 10 trials in the accelerating rotarod in male *Pcdh10*$^{+/-}$ ($n$ = 13) and WT littermates ($n$ = 13). A mixed model was fit with trial (values 1-10 treated as continuous to examine impact of

trial) and genotype. The main effect of trial was significant ($p < .001$), but there was no significant main effect of genotype ($p = .055$) and no significant trial by genotype interaction ($p = .119$). **(D)** Latency to fall during 10 trials in the accelerating rotarod in female *Pcdh10*$^{+/-}$ ($n = 9$) and WT littermates ($n = 9$). A mixed model was fit with trial (values 1-10 treated as continuous to examine impact of trial) and genotype. The main effects of trial ($p < .001$) and genotype ($p = .016$) were significant, and there was a significant trial by genotype interaction ($p < .001$).

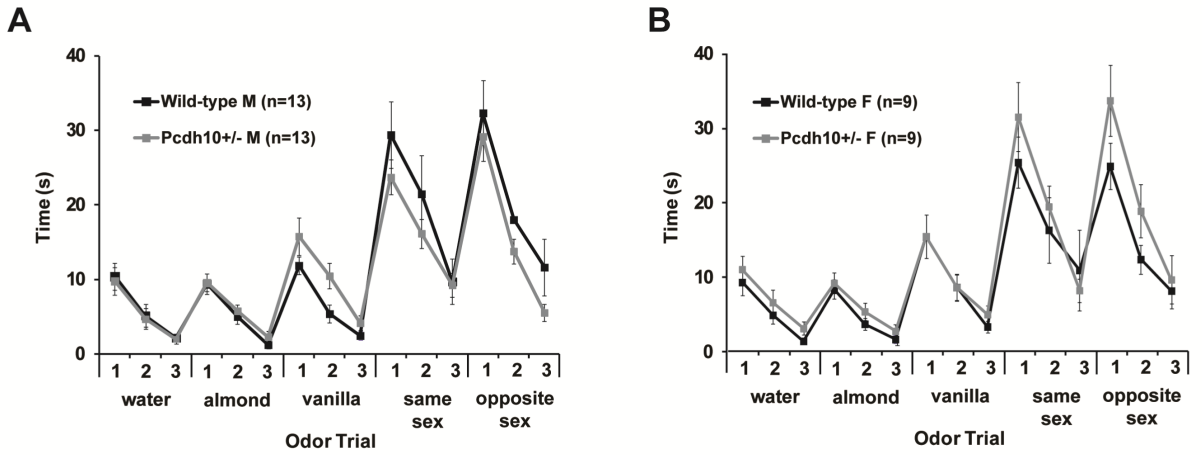


**Figure S4. Olfactory habituation dishabituation in juvenile *Pcdh10*$^{+/-}$ and wild-type mice.** This test of ability to detect and differentiate odors (2) was performed under infrared lighting in an otherwise darkened room. Mice were allowed to acclimate for 30 minutes to a clean testing cage, and then were presented with a sequence of different odorant-dipped cotton swabs that were lowered into the cage for 2 min each, with 1 min intervals between presentations of swabs. Each odorant was presented 3 consecutive times, followed by another odorant in the following order: water, almond, vanilla, soiled bedding from an unfamiliar same-sex mouse, soiled bedding from an unfamiliar opposite sex mouse. The time that the test mouse spent sniffing each swab was measured. **(A)** Time spent sniffing odorants in male *Pcdh10*$^{+/-}$ ($n = 13$) and WT littermates ($n = 13$). A mixed model was fit to assess the effects of odorant (5 odorants), period (1,2,3), and genotype. There were no interactions present. There were significant main effects of period ($p < .001$) and odorant ($p < .001$) but not of genotype ($p = .867$). Thus, there was no effect of genotype on olfactory habituation dishabituation in males. **(B)** Time spent sniffing odorants in female *Pcdh10*$^{+/-}$ ($n = 9$) and WT littermates ($n = 9$). A mixed model was fit to assess the effects of odorant (5 odorants), period (1,2,3), and genotype. There were no interactions present. There were significant main effects of period ($p < .001$) and odorant ($p < .001$) but not of genotype ($p = .795$). Thus, there was no effect of genotype on olfactory habituation dishabituation in females.

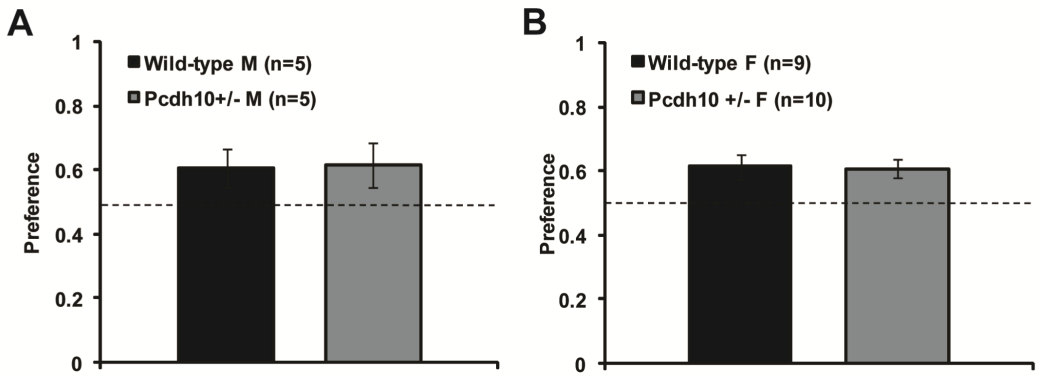


**Figure S5. Preference for non-social novel objects is not altered in *Pcdh10*$^{+/-}$ mice.** Group-housed male mice and group-housed female mice were trained and tested as previously reported (3). Briefly, mice were handled for 3 days, and habituated to the empty arena for 5 days. Animals were exposed to two identical objects for 15 min, and tested at 24 h with one familiar and one novel object for 15 min. Preference for the novel object was expressed as sniffing on the novel object divided by total exploration time for both objects. Preference scores on the y-axis represent time spent sniffing the novel object divided by total sniffing time spent sniffing both the novel object and the familiar object. The Mann-Whitney test was used to compare genotypes in preference for non-social novel objects. **(A)** There was no significant difference between male *Pcdh10*$^{+/-}$ mice vs. male WT littermates in novel object recognition. **(B)** There was no significant difference between female *Pcdh10*$^{+/-}$ mice vs. female WT littermates in novel object recognition.

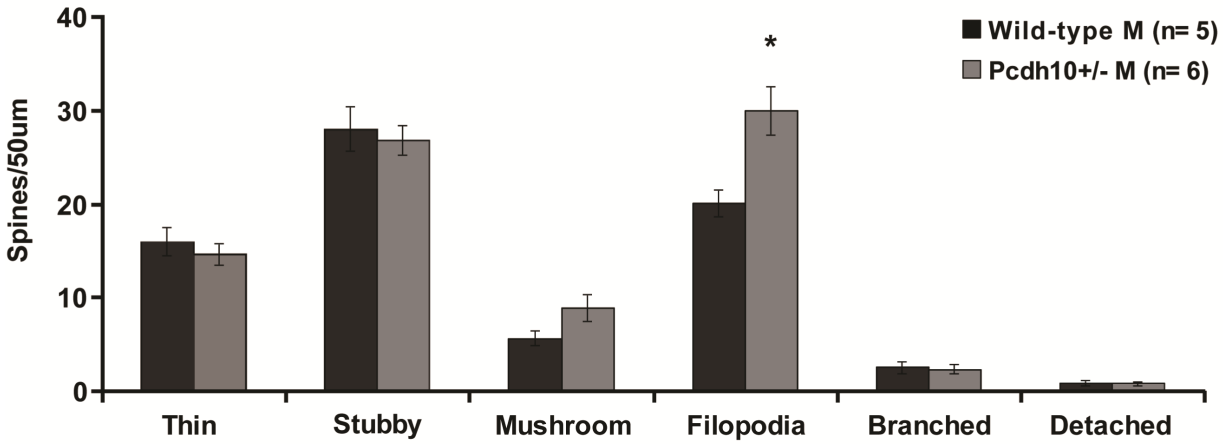


**Figure S6. Change in spine density in LA/BLA is driven by increases in filopodia type spines.**

Average number of spines counted in each morphological category in 50 mm regions of wild-type and *Pcdh10*$^{+/-}$ LA/BLA amygdala neurons. A mixed model was fit separately for each spine type with a one-sided test, based on the a priori hypothesis of higher spine counts in *Pcdh10*$^{+/-}$ mice (4). Relative to WT, *Pcdh10*$^{+/-}$ amygdala neurons showed significantly higher levels of filopodia spines ($p$ = .030), but there were no significant differences in any of the other spine types.

## Supplemental References

1. Tarantino LM, Gould TJ, Druhan JP, Bucan M (2000): Behavior and mutagenesis screens: The importance of baseline analysis of inbred strains. *Mamm Genome*. 11: 555–564.

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3. Oliveira AMM, Hawk JD, Abel T, Havekes R (2010): Post-training reversible inactivation of the hippocampus enhances novel object recognition memory. *Learn Mem*. 17: 155–60.

4. Tsai N-P, Wilkerson JR, Guo W, Maksimova M a, DeMartino GN, Cowan CW, Huber KM (2012): Multiple autism-linked genes mediate synapse elimination via proteasomal degradation of a synaptic scaffold PSD-95. *Cell*. 151: 1581–94.