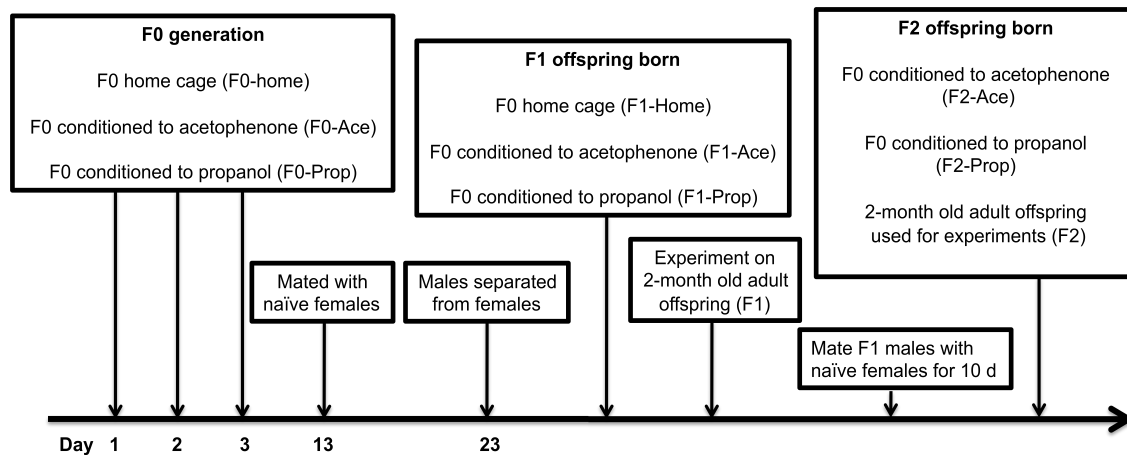
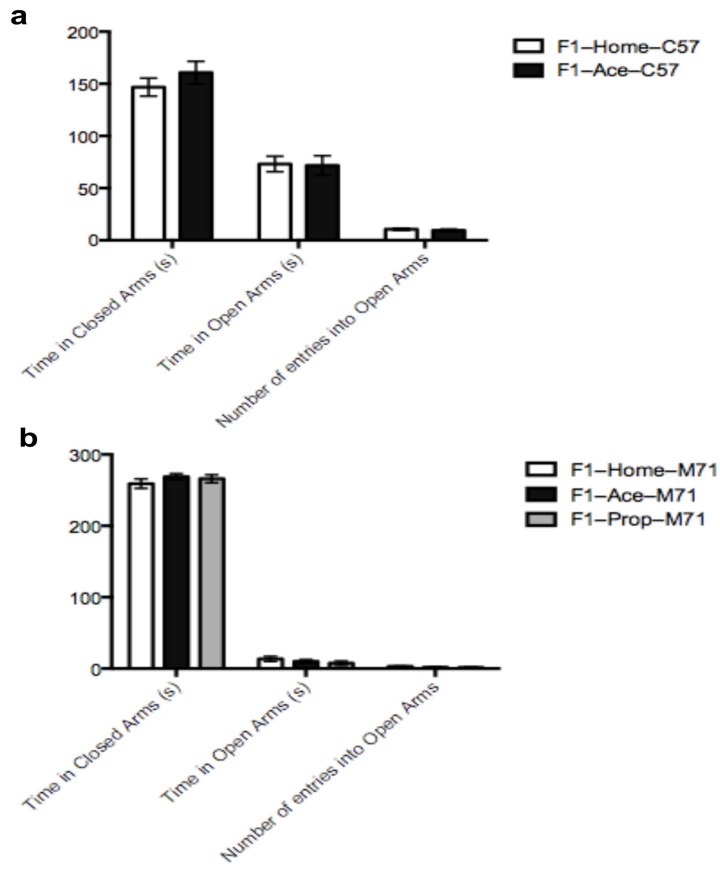


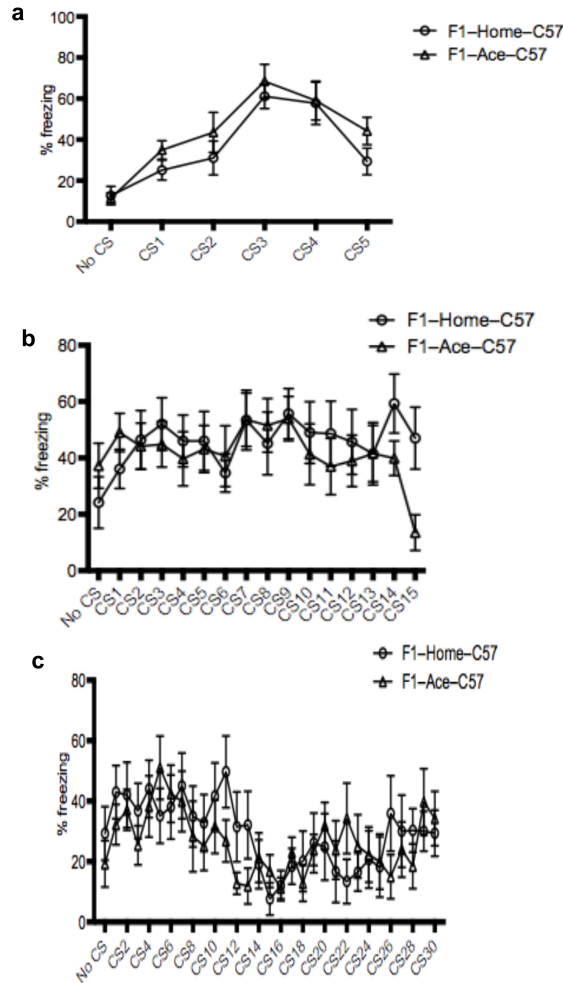
Supplementary Information:**Supplementary Figure 1. Experimental design to investigate the effect of cu-specific trauma to an F0 generation on subsequently conceived generations.**

Novel experimental paradigm that uses olfactory fear conditioning to examine the structural and functional changes in the nervous systems of adult mice (F1 and F2) conceived *after* the F0 generation was trained to associate specific odorant presentations with mild foot-shocks. Briefly, F0 adult male mice were trained to associate Acetophenone or Propanol presentation with mild-footshocks (5 odor-shock pairings/session, 3 sessions, 1 session/day). Ten days after this conditioning, these F0 males were mated with naïve females. Ten days after the mating was setup, the F0 males were separated from the females. F1 offspring born were tested at 2-months of age. For studies of the F2 generation, F1 males that had no previous exposure to either Acetophenone or Propanol were mated with naïve females for 10 days, and resulting F2 offspring were used for analyses. Our experimental design minimized the possibility of a “social transmission” mode of information transfer. Specifically, the F0 male has absolutely no contact with the F1 offspring, is placed with the female 10 days after the last conditioning day, should not have any trace of the conditioned odor on his skin or hair to transfer to the mother, and is separated from the female after a 10 day period to minimize any *in utero* exposure of the pups to the conditioned male.



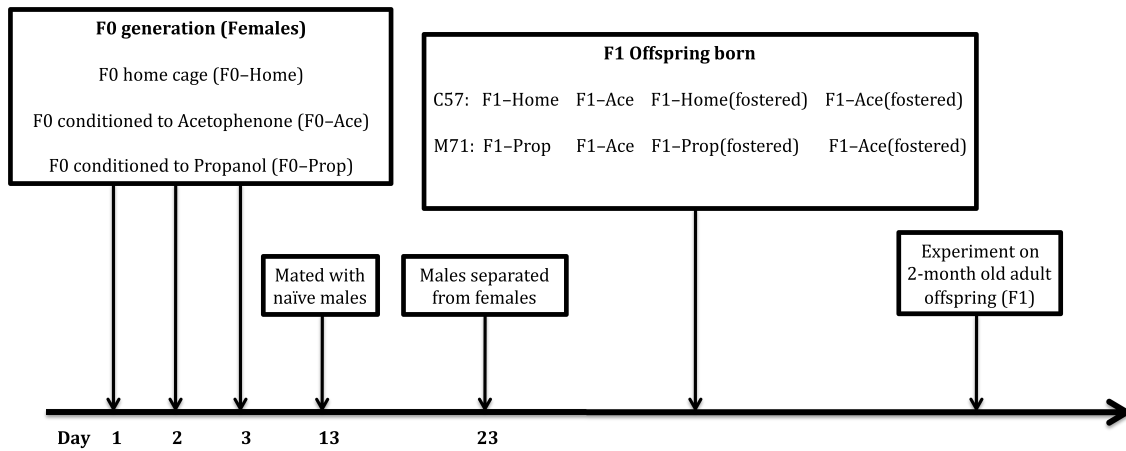
Supplementary Figure 2. No differences found in anxiety measures in adult male offspring that had been conceived after the F0 generation males had been subjected to olfactory fear conditioning with Acetophenone or Propanol.

C57Bl/6J (a) and M71-LacZ (b) adult male offspring (F1-Home, F1-Ace, F1-Prop) spend the same amount of time in the closed and open arms of an elevated plus maze, and make the same number of entries into the open arms. (C57Bl/6J: F1-Home-C57 $n = 9$ vs F1-Ace-C57 $n = 8$) (F1-Home-M71 $n = 11$ vs F1-Ace-M71 $n = 12$ vs F1-Prop-M71 $n = 11$) (Two-way ANOVA: $p > 0.05$ in both experiments).



Supplementary Figure 3. No differences in auditory fear conditioning in adult male offspring that had been conceived after the F0 generation males had been subjected to olfactory fear conditioning with Acetophenone or Propanol.

No significant differences were found between F1-Home-C57, and F1-Ace-C57 in the acquisition (a), consolidation (b), and extinction retention (c) of the memory of an aversive auditory cue after they were trained to associate 6kHz tone presentations with mild-footshocks. (C57Bl/6J: F1-Home-C57 n = 9 vs F1-Ace-C57 n = 9) $p > 0.05$ in all experiments.



Supplementary Figure 4. Cross-fostering study to determine transmission vs inheritance of observed effects.

Sexually naïve C57Bl/6J female mice were conditioned with Acetophenone or left in their Home Cage; they were then mated with C57Bl/6J males for 10 days. Offspring were then divided into the following groups: Offspring of Home Cage Mothers (F1-Home-C57), Offspring of Acetophenone Conditioned Mothers (F1-Ace-C57), O-MHC cross fostered starting at P1 by Mothers Conditioned to Acetophenone (F1-Home-C57fostered), O-MCA cross fostered by Home Cage Mothers (F1-Ace-C57fostered).

Olf6**Mouse chromosome 7: 114099198-114102748**

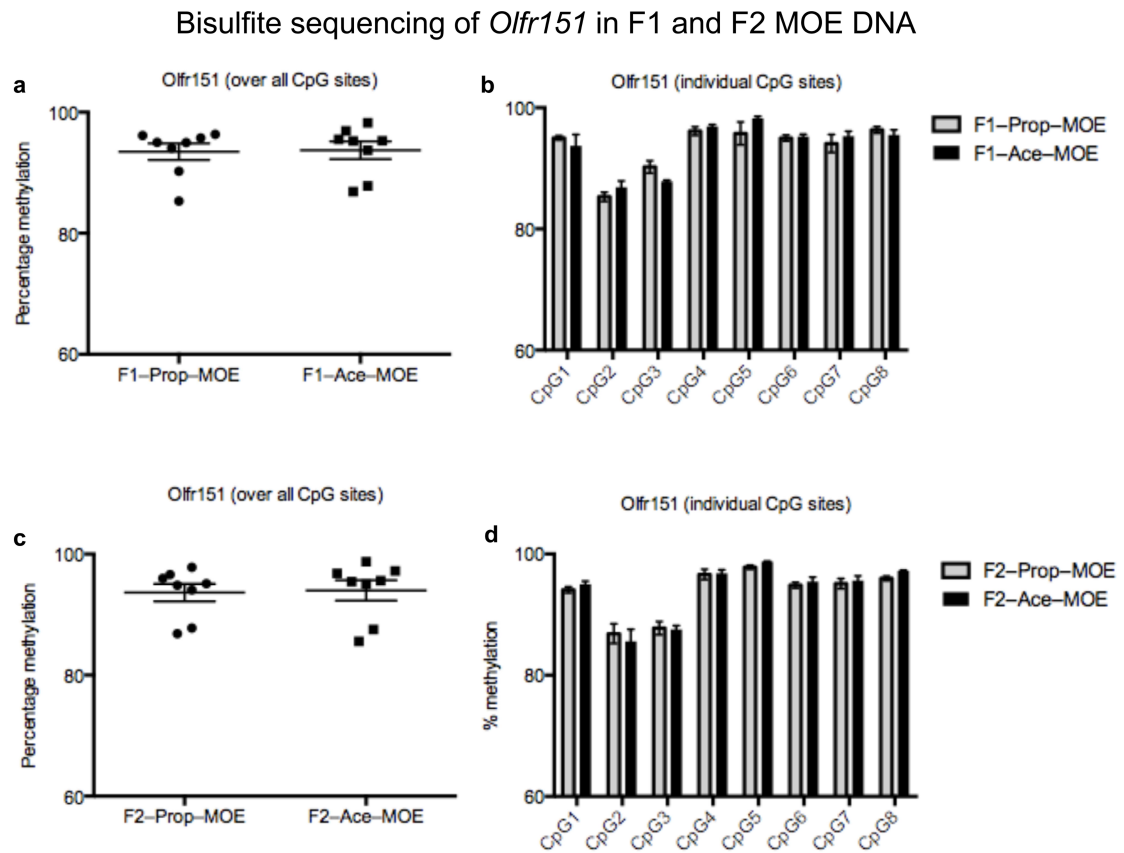
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Olf151**Mouse chromosome 9: 37537562-37541646**

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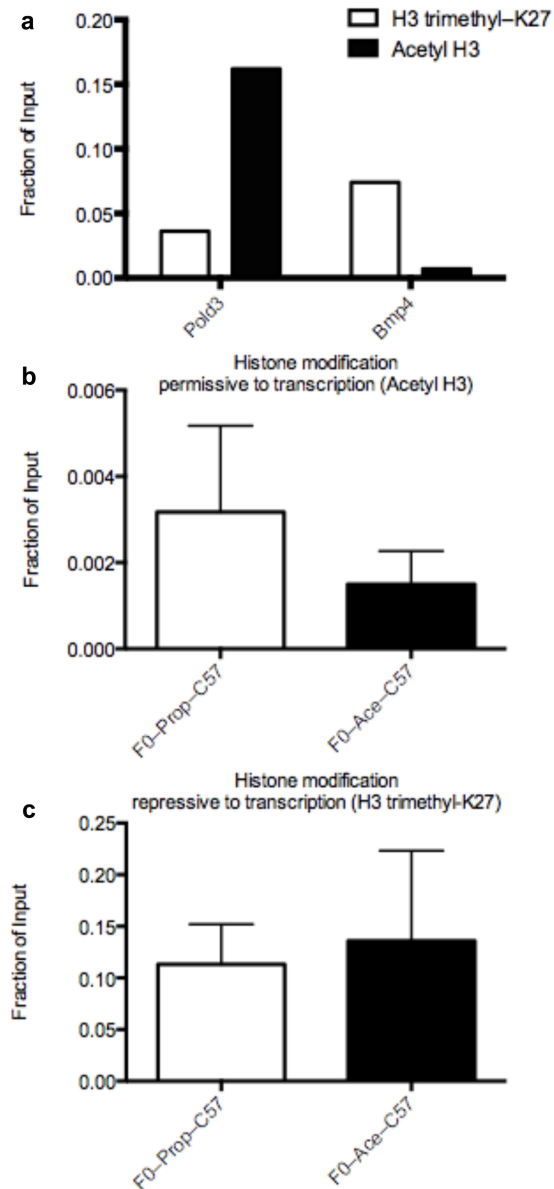
Supplementary Figure 5. Bisulfite sequencing around the *Olf151* and *Olf6* genes was conducted to query the methylation status of CpG di-nucleotides.

Coding sequences (red text) of *Olf6* and *Olf151* in reverse complement with primers used to generate amplicons highlighted in blue. CpG di-nucleotides shown in Fig. 6 are numbered. The CpG di-nucleotides not numbered in *Olf151* could not be queried due to technical issues.



Supplementary Figure 6. Methylation status of CpG di-nucleotides in the *Olfr151* (M71) gene in MOE DNA of the odor naïve F1 and F2 generations.

(a) Bisulfite sequencing data to query the methylation status of CpG di-nucleotides in the *Olfr151* (M71) gene in MOE of the F1 generation male reveals no differences in methylation between groups ($p > 0.05$) ($n = 4/\text{group}$). (b) Bisulfite sequencing data to query the methylation status of CpG di-nucleotides in the *Olfr151* (M71) gene in MOE of the F1 generation reveals no differences in methylation at individual CpG sites between groups. (c) Bisulfite sequencing data to query the methylation status of CpG di-nucleotides in the *Olfr151* gene in MOE of the F2 generation reveals no differences in methylation status between F2-Ace and F2-Prop ($n = 4/\text{group}$) across all CpG di-nucleotides queried ($p > 0.05$). (d) Bisulfite sequencing data to query the methylation status of CpG di-nucleotides in the *Olfr151* gene in MOE of the F2 generation reveals no differences in methylation status between F2-Ace and F2-Prop across specific CpG di-nucleotides queried (Bonferroni corrected for multiple comparisons). All graphs represent Mean \pm SEM.



Supplementary Figure 7. Validation of Sperm N-ChIP protocol, and histone modifications around the M71 locus in the sperm of F0 males (fathers) that had been subjected to olfactory fear conditioning.

(a) Sperm ChIP was validated by performing qPCR for Pold3 and Bmp4. As has been shown previously, Pold3 is associated with more of the “activating” mark (Acetyl H3), and less of the “repressive” mark (H3 trimethyl-K27), while BMP4 is associated with more of the repressive than the activating mark.

N-ChIP on sperm of F0 males (fathers) conditioned either to Acetophenone (F0-Ace-C57) or Propanol (F0-Prop-C57) reveals no significant differences in the activating (Acetyl H3) (b) or repressive (H3 trimethyl-K27) (c) histone modifications immunoprecipitated in our experiment. (n = 5 epididymis per sample, n = 3 samples/group) (p > 0.05 for both marks). All graphs represent Mean±SEM.