1

Epigenetics

Definition
Histone and DNA Modifications
Meaney
Ressler
OPS

Pavlov

2

Epigenetics

Harvy 1651
'partium super-exorientium additamentum', 'the additament of parts budding one out of another'.

Waddington 1942
differentiation of cells from their initial totipotent state in embryonic development

Holliday 1990
the study of the mechanisms of temporal and spatial control of gene activity during the development of complex organisms

Riggs 1996
the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence

Bird 2007
the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states

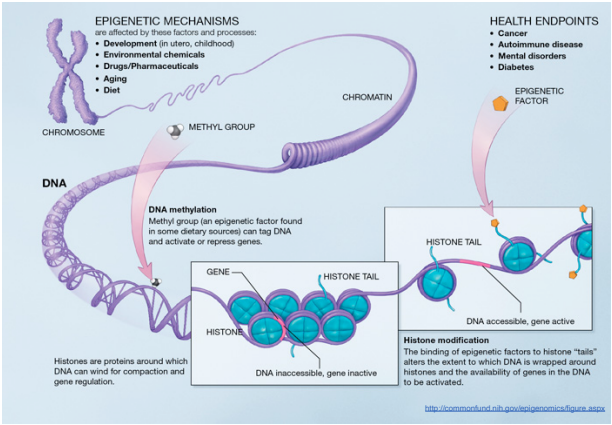
Chromatin Epigenetics, Cold Spring Harbor Mtg 2009
stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence

Histone and DNA Modifications

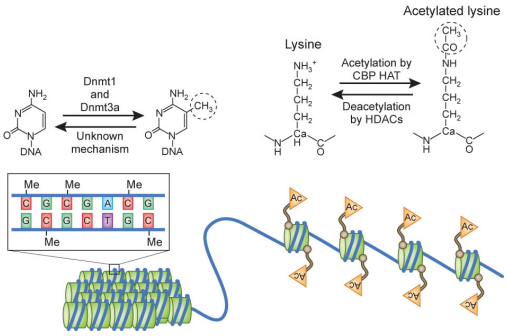
3

Epigenetics: Histone and DNA Modifications

non-coding RNA (microRNAs, sRNAs), Prions



Histone and DNA Modifications



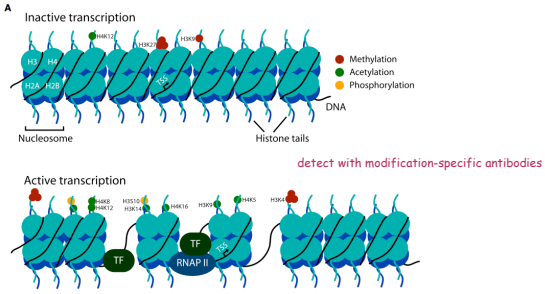
Hypermethylated DNA recruits silencing transcription chromatin remodeling complexes with histone deacetylases (HDACs) and promotes chromatin condensation. Hypomethylated DNA unfolds into a 'beads-on-a-string' structure in which histones are accessible for chromatin remodeling factors such as CREB-binding protein histone acetyltransferase (CBP HAT), the transcriptional coactivator implicated in epigenetic mechanisms controlling memory consolidation3. Ac, acetyl group; Me, methyl group.

Korzus 2010

4

Histone Modifications

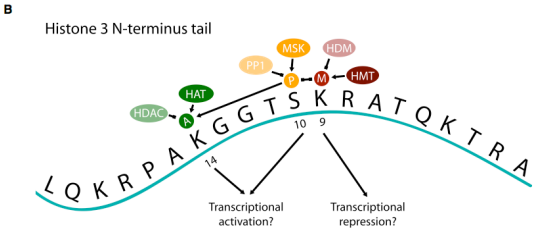
acetylation, phosphorylation, methylation of specific amino acid residues of histone proteins that control access of transcriptional machinery to the DNA



(A) Individual residues on histone tails undergo a number of unique modifications ...surrounding the transcription start site (TSS) for a given gene. These modifications in turn correlate with transcriptional repression (top), in which DNA is tightly condensed on the nucleosome and therefore inaccessible, or transcriptional activation (bottom), in which transcription factors (TF) or RNA polymerase II (RNAP II) can access the underlying DNA to promote gene expression

Day & Sweatt 2011

5

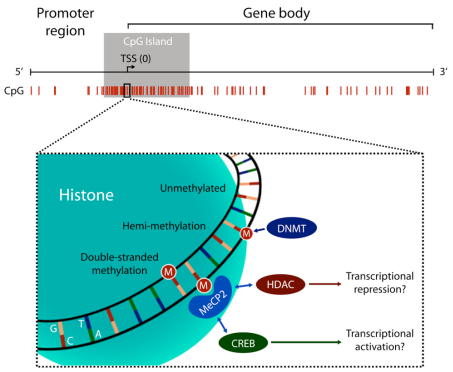


(B) Expanded view of individual modifications on the tail of histone H3. The concept of a histone "code" suggests that individual marks interact with each other to form a combinatorial outcome. In this case, methylation at lysine 9 on H3 (a mark of transcriptional repression) and phosphorylation at serine 10 on H3 repress each other, whereas phosphorylation at serine 10 enhances acetylation on lysine 14 (a mark of transcriptional activation).

Day & Sweatt 2011

6

DNA Methylation of Cytosine bases



A number of plasticity-related genes in the brain possess large CpG islands within the gene promoter region. Each CpG dinucleotide in the DNA sequence can undergo methylation by DNA methyltransferases (DNMTs), resulting in hemimethylation and/or double-stranded DNA methylation. Proteins with methyl-binding domains bind to methylated DNA and associate with other cofactors, such as HDACs or transcription factors like CREB, to alter gene expression. It is presently unclear whether the specific combination of CpG methylation marks constitutes a "code" for unique outcomes or whether the overall or average density of methylation is a larger determinant of transcrip- tional efficacy.

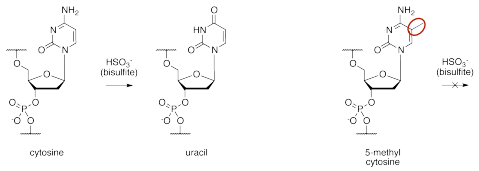
Day & Sweatt 2011

7

Bisulfite Sequencing

The methylation status of a DNA sequence can best be determined using sodium bisulfite. Incubation of the target DNA with sodium bisulfite results in conversion of unmethylated cytosine residues into uracil, leaving the methylated cytosines unchanged. Therefore, bisulfite treatment gives rise to different DNA sequences for methylated and unmethylated DNA.

	Original sequence	After bisulfite treatment
Unmethylated DNA	N-C-G-N-C-G-N-C-G-N	N-U-G-N-U-G-N-U-G-N
Methylated DNA	N-**C**-G-N-**C**-G-N-**C**-G-N	N-**C**-G-N-**C**-G-N-**C**-G-N



Qiagen, EpiTect Bisulfite Handbook 04/2006

http://www.atdbio.com/content/20/Sequencing-forensic-analysis-and-genetic-analysis

8

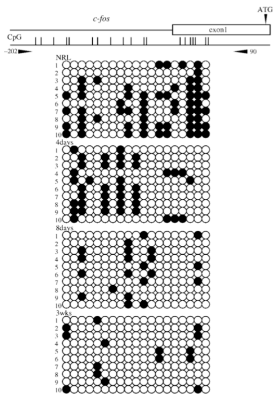


Fig. 4. Methylation analysis of the 5' upstream region of the *c-fos* gene in rat by bisulfite sequencing. The primer pair used for bisulfite sequencing is shown. The transcription start site was defined as +1. Methylated CpG sites are represented by closed circles and unmethylated CpG sites are represented by open circles. NRL, normal liver tissue.

http://alphabiolab.com/page2/page2.html

rat hepatocarcinogenesis, Shimizu 2007

9

Epigenetic programming by maternal behavior
Meaney et al.

Handling Pups -> licking by mom

-> CpG methylation of Glucocorticoid Receptor

-> lower stress responses

-> more licking of grandpups

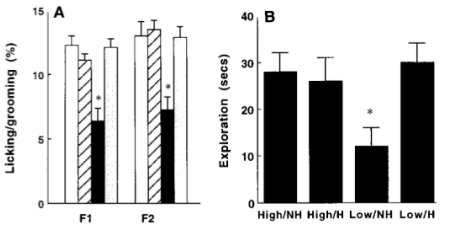
Change in stress behavior mediated by epigenetic modification of DNA

Transmission to next generation by change in maternal behavior (i.e. non-genomically)

10

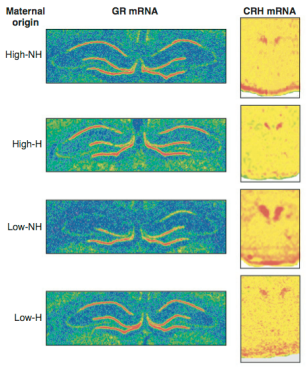
Effects of Maternal Grooming and neonatal handling on behavior of ***grand-pups***

	Not Handled (NH)	Handled (H)
High licking moms (High)	more licking	more licking
Low Licking Moms (Low)	less licking, more stressed	more licking



Francis 1999

11



Francis 1999

12

13

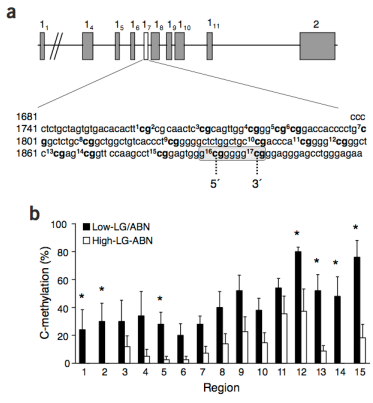


Figure 1 Maternal care alters cytosine methylation of GR promoter. (a) Sequence map of the exon 1γ GR promoter including the 17 CpG dinucleotides (bold) and the NGFI-A binding region16 (encircled). (b,c) Methylation analysis of the 17 CpG dinucleotides of the exon 1γ GR promoter region from adult high- and low-LG- ABN offspring (6–10 clones sequenced/animal; $n = 4$ animals/group; $^*P < 0.01$). (b) Percentage of cytosine residues that were methylated (mean ± s.e.m.) for the first 15 CpG dinucleotides ($^*P < 0.05$).

Weaver 2004

14

Manipulation of Epigenetic Status

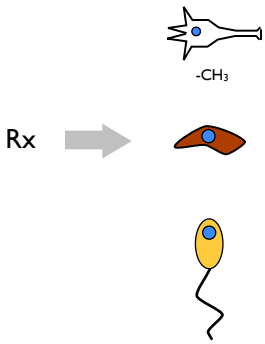
Histone Deactylase (HDAC) inhibitors

Methylation donors

(I'll add a couple more slides here)

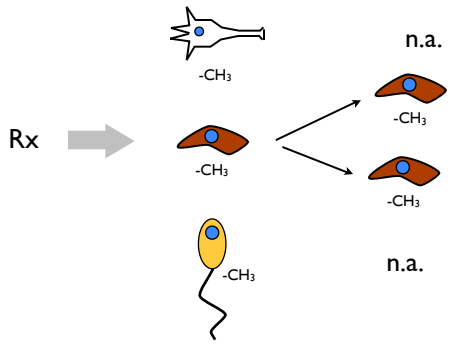
15

Tissue Specific Effects



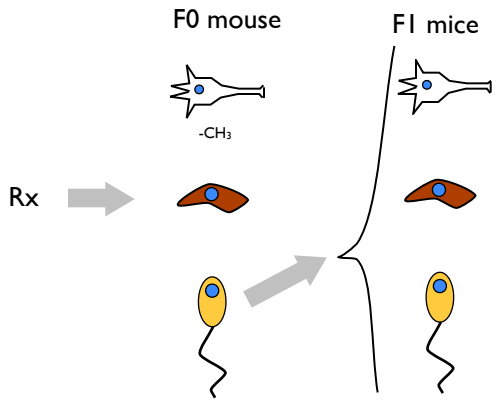
16

Cellular Inheritance



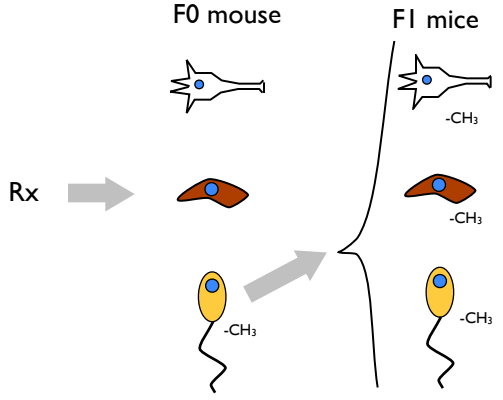
17

Generational Inheritance



18

Generational Inheritance



19

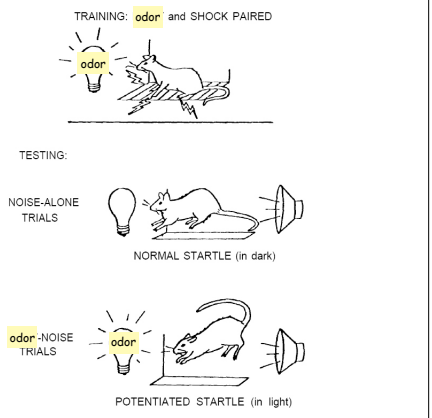
Parental olfactory experience influences behavior and neural structure in subsequent generations

Brian G Dias & Kerry J Ressler

Nat Neurosci. 2014 Jan;17(1):89-96.

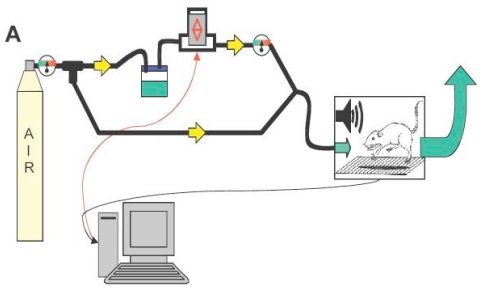
20

Odor Potentiated Startle (OPS)



http://sunburst.usd.edu/~cliff/Courses/Behavioral%20Neuroscience/Fear/

21



SR-Lab Response software controls a solenoid switch (red arrows) which allows compressed air to flow through the odorant jar and into the startle chamber. When closed, clean air flows with no difference in airflow. Backflow is prevented by a series of one-way valves (yellow arrows). The odor is removed via an exhaust hose (green arrow) by outflow fan. Shock is generated by a programmable animal shocker and is delivered through the bars in the cage floor. During behavioral testing, startle is elicited by a 105 dB white noise burst. Activity and startle amplitude are measured by a piezoelectronic device beneath the floor of the cage.

Jones 2009

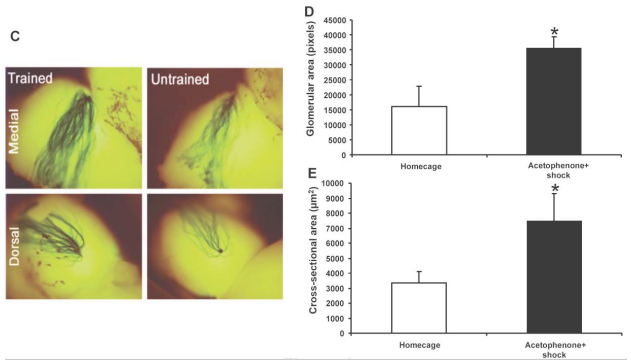
Fear training and testing were conducted using startle response systems (SR-LAB, SDI, San Diego, CA), modified to deliver discrete odor stimuli as previously described(Jones et al., 2005) (Fig 1A). Odorants consisted of 10% acetophenone or 10% propanol (both from Sigma-Aldrich, St. Louis) in propylene glycol. Briefly, mice were habituated to the startle chambers three times (10min per d) prior to training. Mice then received 2 training sessions per week for 3 weeks to ensure strong and stable odor-shock association. Each odor+shock training session consisted of 5 trials of 10-sec odor CS coterminating with a 0.25-sec, 0.4-mA footshock, presented with an average 120-sec intertrial interval (ITI) (range 90 - 150 sec).

The following day, mice were presented with either 10 acetophenone-startle trials or 10 propanol-startle trials randomly intermingled with 10 startle-alone trials and separated by 90-sec ITIs. Each odor-startle trial consisted of a 10-sec odor presentation co-terminated with 50-msec, 105-dB noise burst. For each animal, a fearpotentiated startle score was computed by subtracting the mean of the startle-alone trials from the mean of the odor-startle trials.

Jones 2009

22

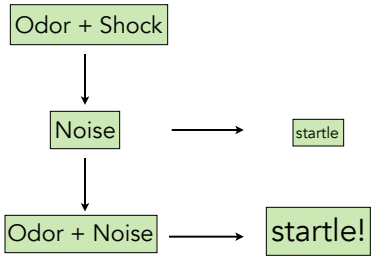
Odor Fear Cond. -> Olfactory Changes



Jones 2009

23

Odor Potentiated Startle (OPS)

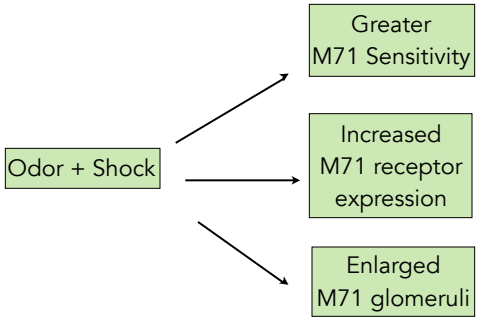


24

Odor Fear Cond. -> Olfactory Changes

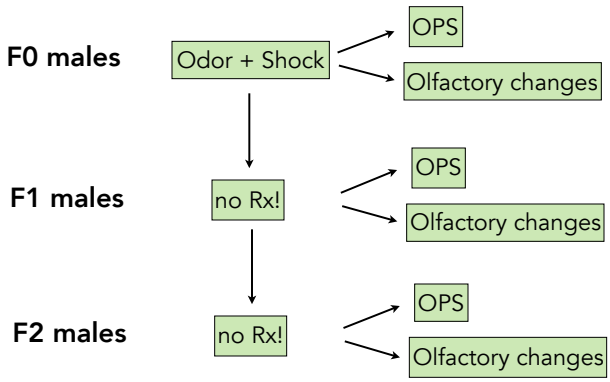
25

Using acetophenone, ligand for M71 receptor
Measure olfactory sensory neurons in M71-lacZ mice



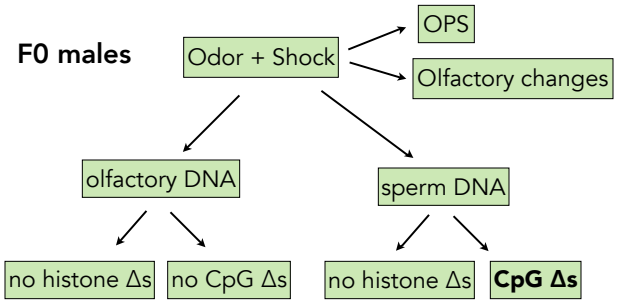
Inheritance of Odor Potentiated Startle & Olfactory Changes

26



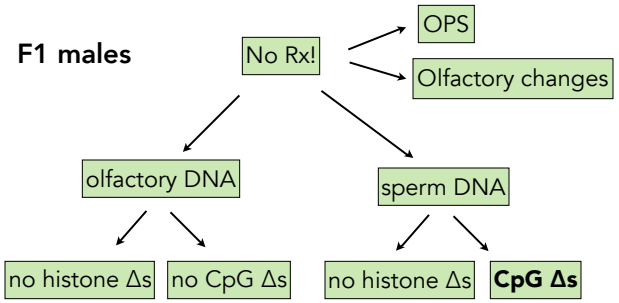
Inheritance of Odor Potentiated Startle & Olfactory Changes

27

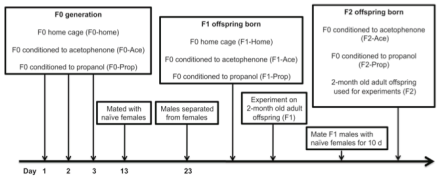


28

Inheritance of Odor Potentiated Startle & Olfactory Changes



29



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

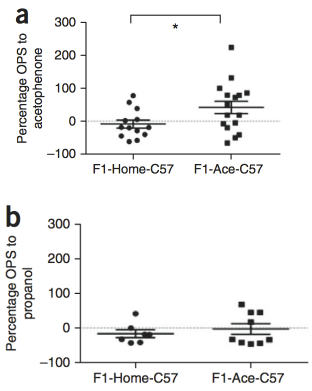
Novel experimental paradigm that uses olfactory fear conditioning to examine the structural and functional changes in the nervous systems of adult mice (F1 and F2) conceived *after* the F0 generation was trained to associate specific odorant presentations with mild foot-shocks. Briefly, F0 adult male 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.

30

Figure 1 Behavioral sensitivity to odor is specific to the paternally conditioned odor. (a,b) Responses of individual C57Bl/6J F1 male offspring conceived after the F0 male was fear conditioned with acetophenone. F1-Ace-C57 mice had an enhanced sensitivity to acetophenone (a), but not to propanol (control odor, b) compared with F1-Home-C57 mice (F1-Ace-C57, n = 16; F1-Home-C57, n = 13; t test, $P = 0.043$, $t_{27} = 2.123$). (c,d) Responses of M71-LacZ F1 male offspring conceived after the F0 male was fear conditioned with acetophenone or propanol. F1-Ace-M71 mice had an enhanced sensitivity to acetophenone (c), but not to propanol (d), compared with F1-Home-M71, and F1-Prop-M71 mice. In contrast, F1-Prop-M71 mice had an enhanced sensitivity to propanol (d), but not acetophenone (c) (F1-Home-M71, n = 11; F1-Ace-M71, n = 13; F1-Prop-M71, n = 9; OPS to acetophenone: ANOVA, $P = 0.003$, $F_{2,30} = 6.874$; F1-Home-M71 versus F1-Ace-M71, $P < 0.05$; F1-Ace-M71 versus F1-Prop-M71, $P < 0.01$; OPS to propanol: ANOVA, $P = 0.020$, $F_{2,26} = 4.541$; F1-Ace-M71 versus F1-Prop-M71, $P < 0.05$). Data are presented as mean ± s.e.m. *$P < 0.05$, **$P < 0.01$.

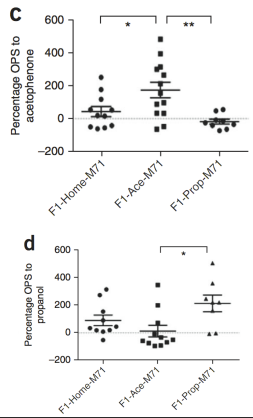
31

Figure 1



32

Figure 1

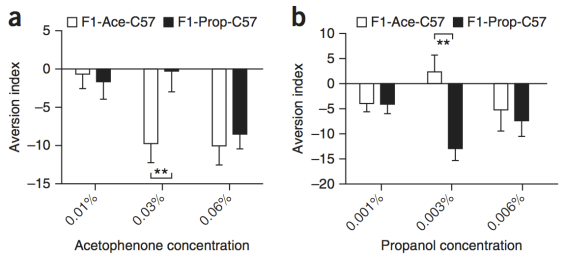


33

Figure 2 Sensitivity of F1 males toward F0-conditioned odor. Association time with either the concentration of odor on the x axis or an empty chamber was recorded. An aversion index was computed by subtracting the amount of time spent in the open chamber from the time spent in the odor chamber. (a) When tested with acetophenone, F1-Ace mice detected acetophenone at a lower concentration (0.03%) than F1-Prop mice, with both groups eventually showing equal aversion at the 0.06% concentration ($P = 0.005$ with Bonferroni correction for multiple comparisons).
(b) When tested with propanol, F1-Prop mice detected propanol at a lower concentration (0.003%) than F1-Ace mice, with both groups eventually showing equal aversion at the 0.006% concentration ($P = 0.0005$ with Bonferroni correction for multiple comparisons) (F1-Ace-C57, n = 16; F1-Prop-C57, n = 16). Data are presented as mean ± s.e.m. (**$P < 0.01$).

34

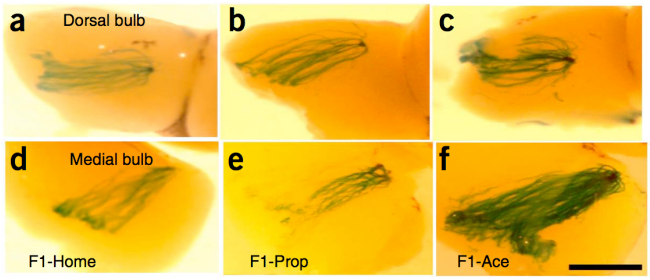
Figure 2



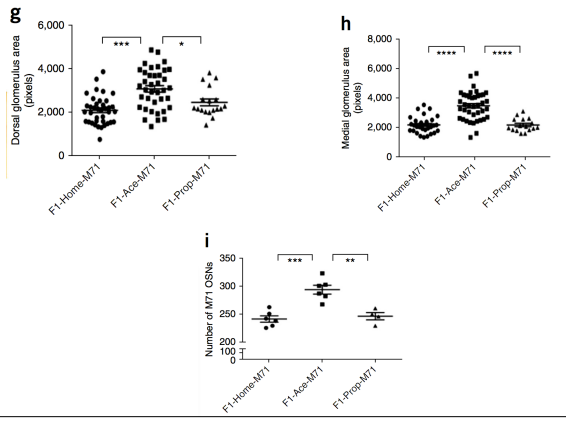
35

Figure 3 Neuroanatomical characteristics of the olfactory system in F1 males after paternal F0 olfactory fear conditioning. (a–f) β-galactosidase staining revealed that offspring of F0 males trained to acetophenone (F1-Ace-M71) had larger dorsal and medial acetophenone-responding glomeruli (M71 glomeruli) in the olfactory bulb compared with F1-Prop-M71 and F1-Home-M71 mice. Scale bar represents 1 mm. (g) Dorsal M71 glomerular area in F1 generation (M71-LacZ: F1-Home, n = 38; F1-Ace, n = 38; F1-Prop, n = 18; ANOVA, $P < 0.0001$, $F_{2,91} = 15.53$; F1-Home-M71 versus F1-Ace-M71, $P < 0.0001$; F1-Ace-M71 versus F1-Prop-M71, $P < 0.05$). (h) Medial M71 glomerular area in F1 generation (M71-LacZ: F1-Home, n = 31; F1-Ace, n = 40; F1-Prop, n = 16; ANOVA, $P < 0.0001$, $F_{2,84} = 31.68$; F1-Home-M71 versus F1-Ace-M71, $P < 0.0001$; F1-Ace-M71 versus F1-Prop-M71, $P < 0.0001$). (i) F1-Ace-M71 mice had a larger number of M71 OSNs in the MOE than F1-Prop-M71 and F1-Home-M71 mice (M71-LacZ: F1-Home, n = 6; F1-Ace, n = 6; F1-Prop, n = 4; ANOVA, $P = 0.0001$, $F_{2,13} = 18.80$; F1-Home-M71 versus F1-Ace-M71, $P < 0.001$; F1-Ace-M71 versus F1-Prop-M71, $P < 0.01$). Data are presented as mean ± s.e.m. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

36



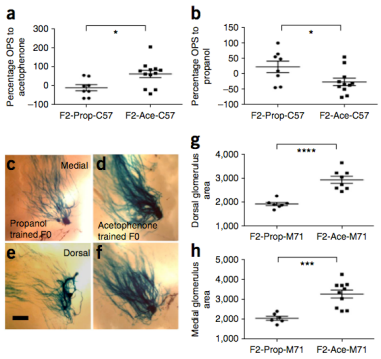
37



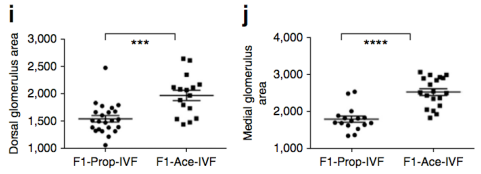
38

Figure 4 Behavioral sensitivity and neuroanatomical changes are inherited in F2 and IVF-derived generations. (a,b) Response of F2-C57Bl/6J
males revealed that F2-Ace-C57 mice had an enhanced sensitivity to acetophenone compared with F2-Prop-C57 mice (a). In contrast, F2-Prop-C57 mice had an enhanced sensitivity to propanol compared
with F2-Ace-C57 mice (b; F2-Prop-C57, n = 8; F2-Ace-C57, n = 12; OPS to acetophenone: t test, P = 0.0158, t_{18} = 2.664; OP to propanol: t test, P = 0.0343, t_{17} = 2.302). (c–f). F2-Ace-M71 mice whose F0 generation male had been conditioned to acetophenone had larger dorsal and medial M71 glomeruli in the olfactory bulb than F2-Prop-M71 mice whose
F0 generation had been conditioned to propanol. Scale bar represents 200 μm. (g) Dorsal M71 glomerular area in F2 generation (M71-LacZ: F2-Prop, n = 7; F2-Ace, n = 8; t test, P < 0.0001, t_{13} = 5.926).
(h) Medial M71 glomerular area in F2 generation (M71-LacZ: F2-Prop,
n = 6; F2-Ace, n = 10; t test, P = 0.0006, t_{14} = 4.44). (i) Dorsal M71 glomerular area in IVF offspring (F1-Prop-IVF, n = 23; F1-Ace-IVF, n = 16; t test, P < 0.001, t_{37} = 4.083). (j) Medial M71 glomerular area in
IVF offspring (F1-Prop-IVF, n = 16; F1-Ace-IVF, n = 19; t test, P < 0.001, t_{33} = 5.880). Data are presented as mean ± s.e.m.*P 0.05,
**P < 0.01, ***P < 0.001, ****P < 0.0001.

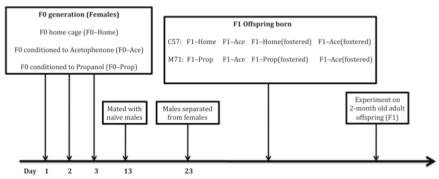
39



40



41



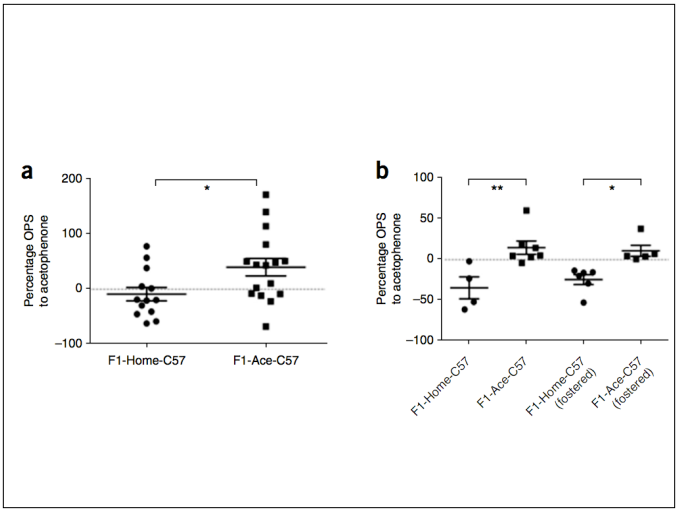
Supplementary Figure 4. Cross-fostering study to determine transmission vs inheritance of observed effects.

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).

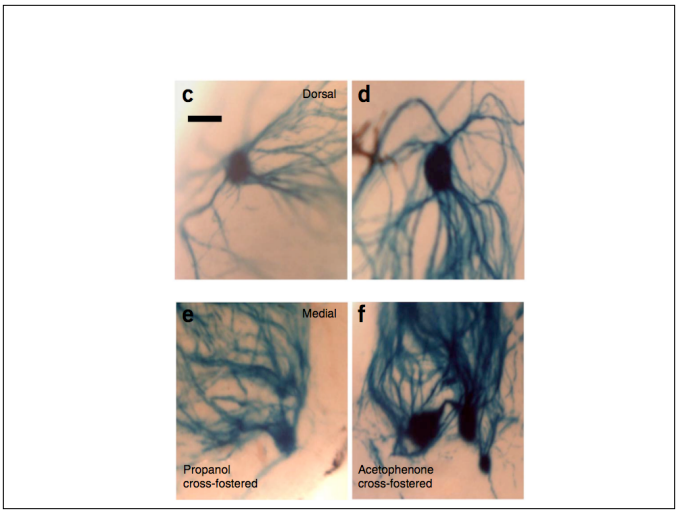
42

Figure 5 Behavioral sensitivity and neuroanatomical changes persist after cross- fostering. (a) F1 offspring of mothers that had been fear conditioned with acetophenone (F1-Ace-C57) showed enhanced sensitivity to acetophenone compared with F1-Home-C57 controls (F1-Home-C57, n = 13; F1-Ace-C57, n = 16; t test, $P = 0.0256$, $t_{27} = 2.362$).
(b) Cross-fostering behavior. F1-Ace-C57 males had higher OPS to acetophenone than F1-Home-C57 males ($P < 0.01$). F1-Ace- C57(fostered) males still had higher OPS to acetophenone than F1-Home-C57(fostered) males ($P < 0.05$) (ANOVA, $P = 0.0011$, $F_{3,18} = 6.874$, planned post hoc comparisons).
(c–f) Cross-fostering neuroanatomy. F1-Ace- M71 males cross-fostered by mothers conditioned to propanol (F1-Ace-M71(fostered)) continued to have larger M71 glomeruli than F1-Prop-M71 males cross-fostered by mothers conditioned to acetophenone (F1-Prop-M71(fostered)). Scale bar represents 100 μm. (g) Dorsal M71 glomerular area in F1 cross-fostered generation (M71-LacZ: F1-Prop, n = 6; F1-Ace, n = 4; F1- Prop(fostered), n = 5; F1-Ace(fostered), n = 3; ANOVA, $P < 0.0001$, $F_{3,14} = 17.52$; F1-Prop versus F1-Ace, $P < 0.001$; F1-Prop(fostered) versus F1-Ace(fostered), $P < 0.01$). (h) Medial M71 glomerular area in F1 cross-fostered generation (M71-LacZ: F1-Prop, n = 4; F1-Ace, n = 3; F1-Prop(fostered), n = 8; F1-Ace(fostered), n = 4; ANOVA, $P < 0.01$, $F_{3,15} = 5.933$; F1-Prop (fostered) versus F1-Ace(fostered), $P < 0.01$). Data are presented as mean ± s.e.m. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

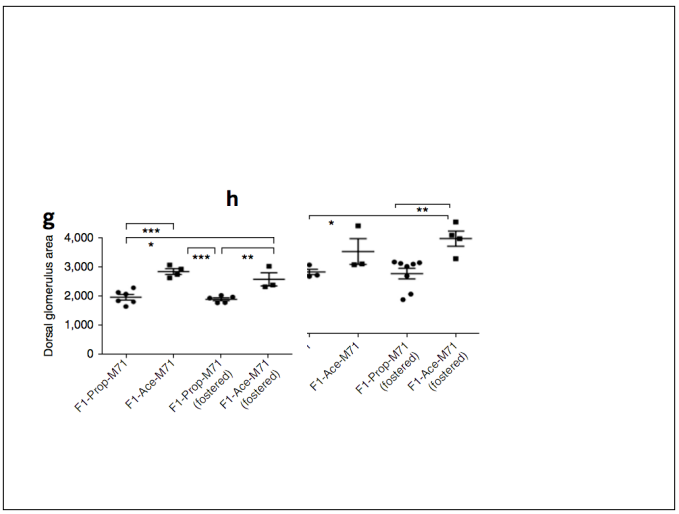
43


a
Percentage OPS to acetophenone
200
100
0
−100
*
F1-Home-C57
F1-Ace-C57
b
Percentage OPS to acetophenone
100
50
0
−50
−100
**
*
F1-Home-C57
F1-Ace-C57
F1-Home-C57 (fostered)
F1-Ace-C57 (fostered)

44


c
Dorsal
d
e
Medial
f
Propanol cross-fostered
Acetophenone cross-fostered

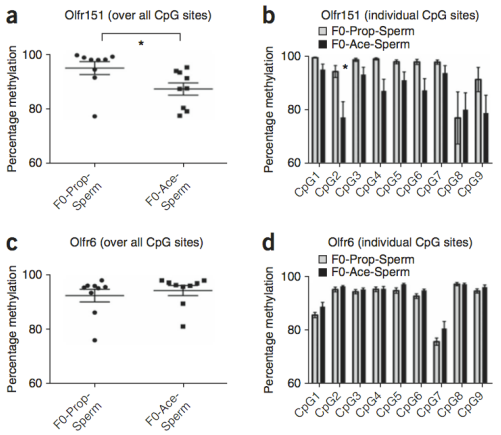
45


g
Dorsal glomerulus area
4,000
3,000
2,000
1,000
0
***
*
***
**
F1-Prop-M71
F1-Ace-M71
F1-Prop-M71 (fostered)
F1-Ace-M71 (fostered)
h
*
**
F1-Ace-M71
F1-Prop-M71 (fostered)
F1-Ace-M71 (fostered)

46

Figure 6 Methylation of odorant receptor genes in sperm DNA from conditioned F0 and odor naive F1 males. (a) Bisulfite sequencing of CpG di-nucleotides in the Olfr151 (M71) gene in F0 sperm revealed that F0-Ace mouse DNA (n = 12) was hypomethylated compared with
that of F0-Prop mice (n = 10) (t test, P = 0.0323, $t_{16} = 2.344$).
(b) A particular CpG di-nucleotide in the Olfr151 (M71) gene in
F0 sperm was hypomethylated in F0-Ace mice (n = 12) compared with F0-Prop mice (n = 10) (P = 0.003, Bonferroni corrected
(c) We found no differences in methylation between F0-Ace (n = 12) and F0-Prop
(n = 10) mice across all of the CpG di-nucleotides queried in the Olfr6 gene in F0 sperm (P > 0.05). (d) Across specific CpG di-
nucleotides in the Olfr6 gene, we found no differences in methylation between F0-Ace (n = 12) and F0-Prop (n = 10) mice
(Bonferroni corrected). (e) Bisulfite sequencing of the Olfr151 (M71) gene in F1 sperm revealed that F1-Ace mouse DNA (n = 4
was hypomethylated compared with that of F1-Prop mice (n = 4) (t test, P = 0.0153, $t_{14} = 2.763$). (f) Bisulfite sequencing of
CpG di-nucleotides in the Olfr151 (M71) gene in F1 sperm revealed that two particular CpG di-nucleotides in the Olfr151 (M71
gene were hypomethylated in F1-Ace mice (n = 4) compared with F1-Prop mice
(n = 4) (P = 0.002, Bonferroni corrected). Data are presented as
mean ± s.e.m. *P < 0.05 after correction.

47



48