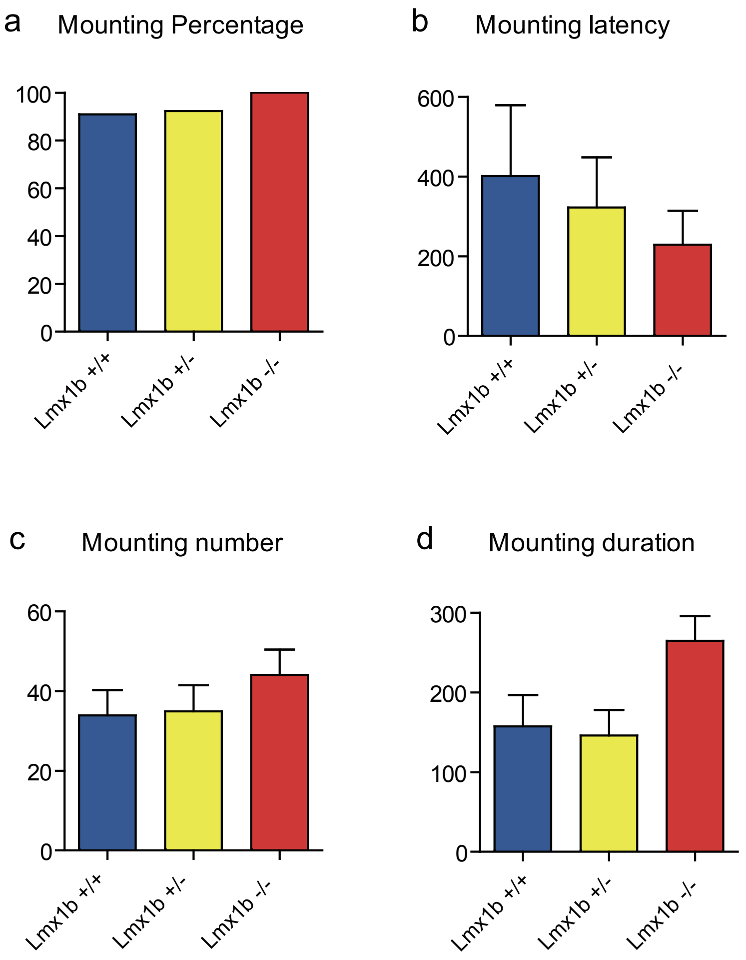
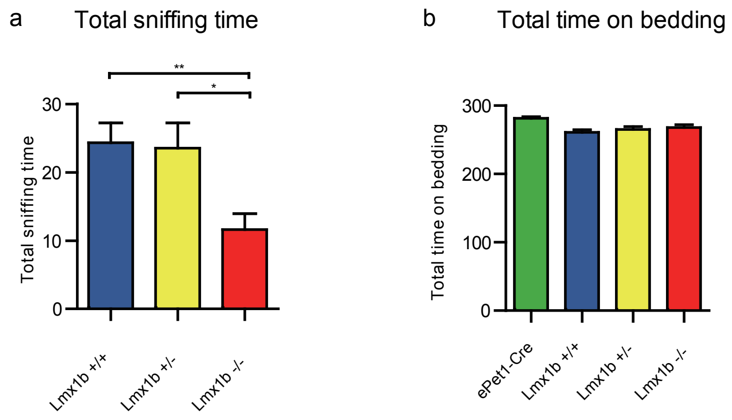
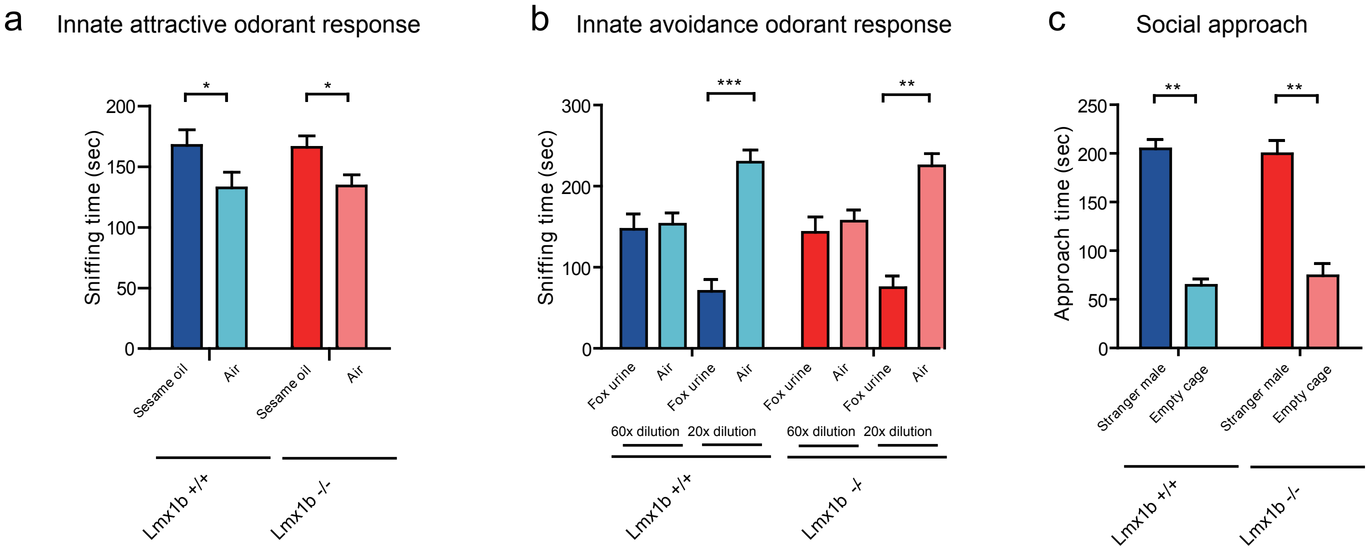
doi:10.1038/nature09822



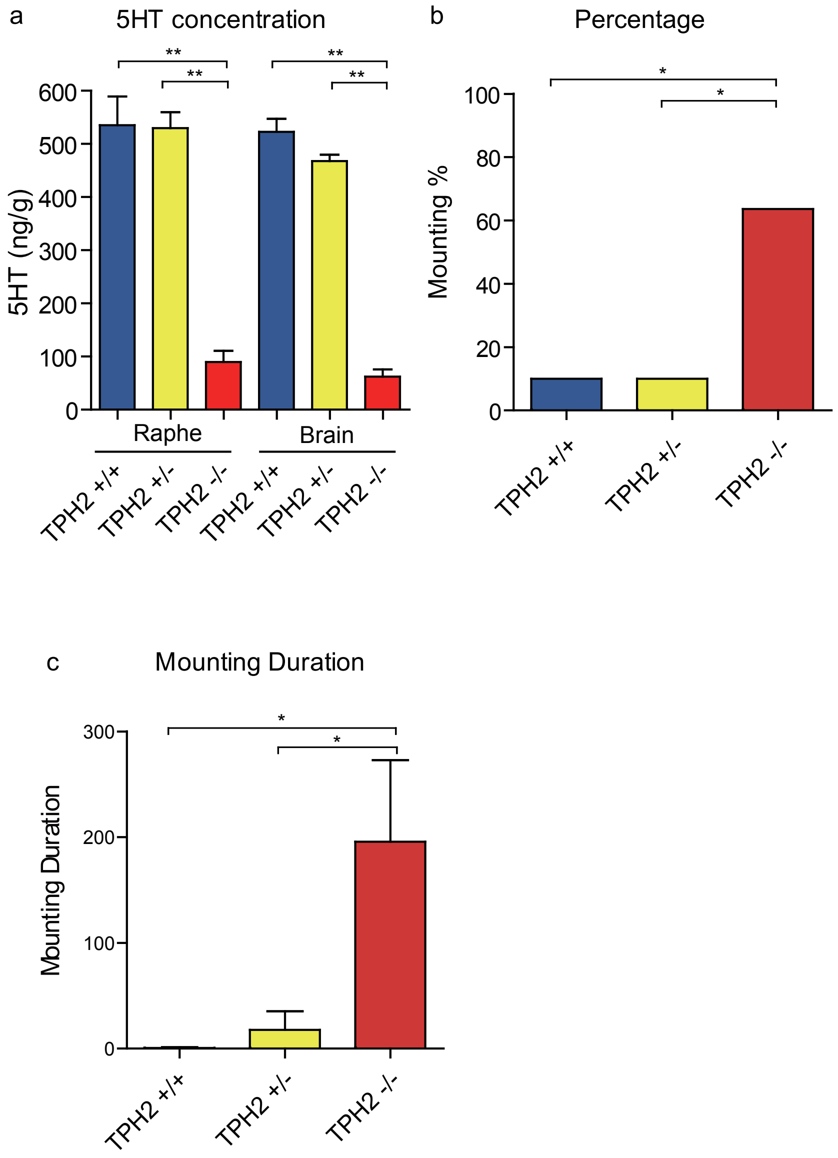
Supplementary Figure 1 | Mounting of wt target female mice by male mice. When a test male was presented with an estrous female, there was no statistically significant difference among *Lmx1b*$^{+/+}$ (n=11) *Lmx1b*$^{+/-}$ (n=13) and *Lmx1b*$^{-/-}$ (n=11) males in mounting percentage (**a,** p>0.5, X^2 test), the latency (**b,** p>0.5, one-way ANOVA), frequency (**c,** p>0.5 one-way ANOVA) or duration (**d,** p>0.5, one-way ANOVA).



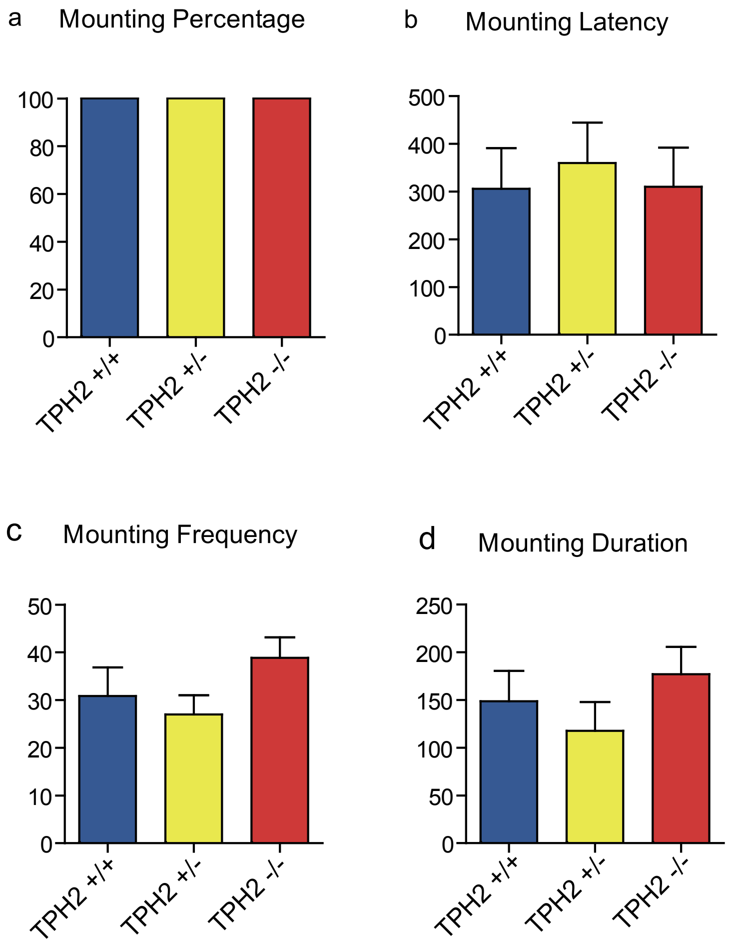
Supplementary Figure 2 | Total sniffing time and total time on bedding. Data from the same experiments as those shown in Fig. 3. In **a,** n=16 for *Lmx1b*$^{+/+}$, n=12 for *Lmx1b*$^{+/-}$, n=16 for *Lmx1b*$^{-/-}$. In **b**, n=9 for *ePet1-Cre*, n=16 for *Lmx1b*$^{+/+}$, n=8 for *Lmx1b*$^{+/-}$ and n=17 for *Lmx1b*$^{-/-}$. **a**, Total sniffing time shows the time (in seconds) of a male mouse spent on sniffing the slide (either the female genital odor side or the male genital odor side) within 3 mins. Total sniffing time was less in *Lmx1b*$^{-/-}$ males than in *Lmx1b*$^{+/+}$ and *Lmx1b*$^{+/-}$ males ($p<0.01$ for *Lmx1b*$^{+/+}$ vs. *Lmx1b*$^{-/-}$, $p<0.05$ for *Lmx1b*$^{+/-}$ vs. *Lmx1b*$^{-/-}$, $p>0.05$ for *Lmx1b*$^{+/+}$ vs. *Lmx1b*$^{+/-}$, one-way ANOVA). **b**, Four groups spent a similar amount of total time on male and female bedding ($p>0.05$ for all pair-wise comparisons, one-way ANOVA).



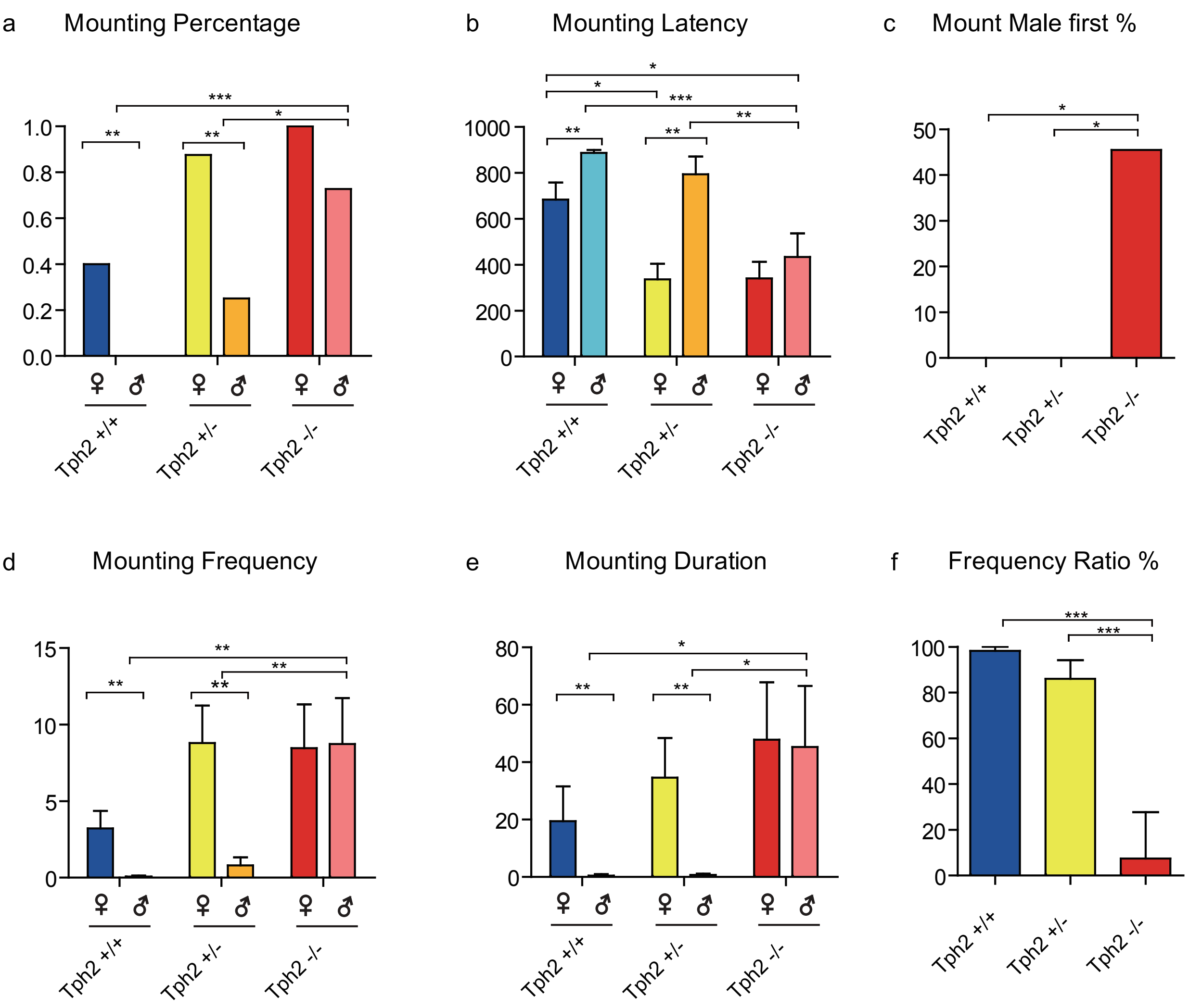
Supplementary Figure 3 | Odor discrimination. a, *Lmx1b*$^{+/+}$ and *Lmx1b*$^{-/-}$ males preferred sesame oil over air ($p<0.05$, t test; n=18 for *Lmx1b*$^{+/+}$, n=14 for *Lmx1b*$^{-/-}$). **b,** *Lmx1b*$^{+/+}$ and *Lmx1b*$^{-/-}$ males were similar in preferring air over fox urine when the concentration of fox urine was high (20X dilution of original urine). *** indicates $p<0.001$ and ** indicates $p<0.01$, t test. n=11 for *Lmx1b*$^{+/+}$, n=10 for *Lmx1b*$^{-/-}$.**c,** Both *Lmx1b*$^{+/+}$ and *Lmx1b*$^{-/-}$ males spent more time in the arm with a caged C57 male than in the empty arm ($p<0.01$, t test; n=13 for *Lmx1b*$^{+/+}$, n=15 for *Lmx1b*$^{-/-}$). No difference was detected between *Lmx1b*$^{+/+}$ and *Lmx1b*$^{-/-}$ males ($p>0.05$ for all other pair-wise comparisons, t test).



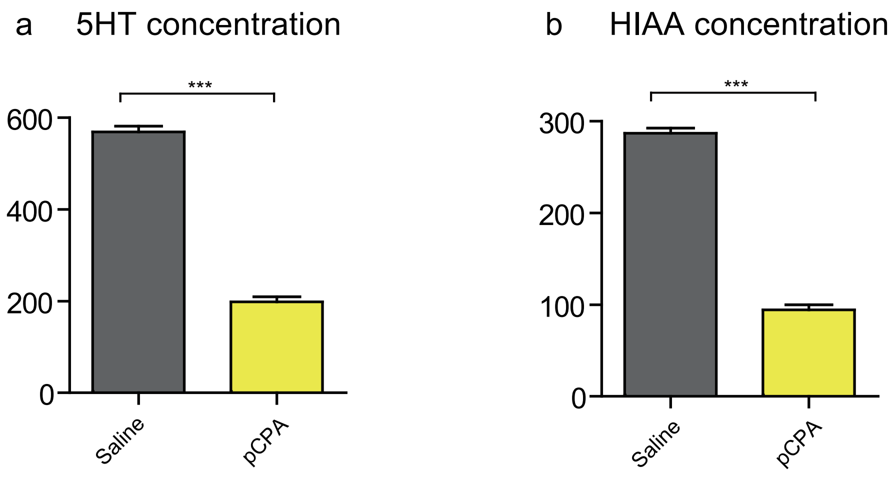
Supplementary Figure 4 | Brain chemistry and behaviors of *Tph2* knockout males. a, Amounts of 5-HT in either the raphe or the whole brain minus the raphe (abbreviated as "brain") in *Tph2*$^{+/+}$ (n=7), *Tph2*$^{+/-}$ (n=9) and *Tph2*$^{-/-}$ (n=7) mice were analyzed by HPLC. 5-HT level was significantly reduced in both the raphe and the brain of *Tph2*$^{-/-}$ mice. **b-c**, Male-male mounting behavior of *Tph2*$^{+/+}$ (n=10), *Tph2*$^{+/-}$ (n=10) and *Tph2*$^{-/-}$ (n=11) mice. Compared with *Tph2*$^{+/+}$ and *Tph2*$^{+/-}$, *Tph2*$^{-/-}$ males showed a higher percentage ($p<0.05$, X^2 test), and longer duration ($p<0.05$ for *Tph2*$^{+/+}$ vs. *Tph2*$^{-/-}$, $p<0.05$ for *Tph2*$^{+/-}$ vs. *Tph2*$^{-/-}$ one-way ANOVA) in mounting males.



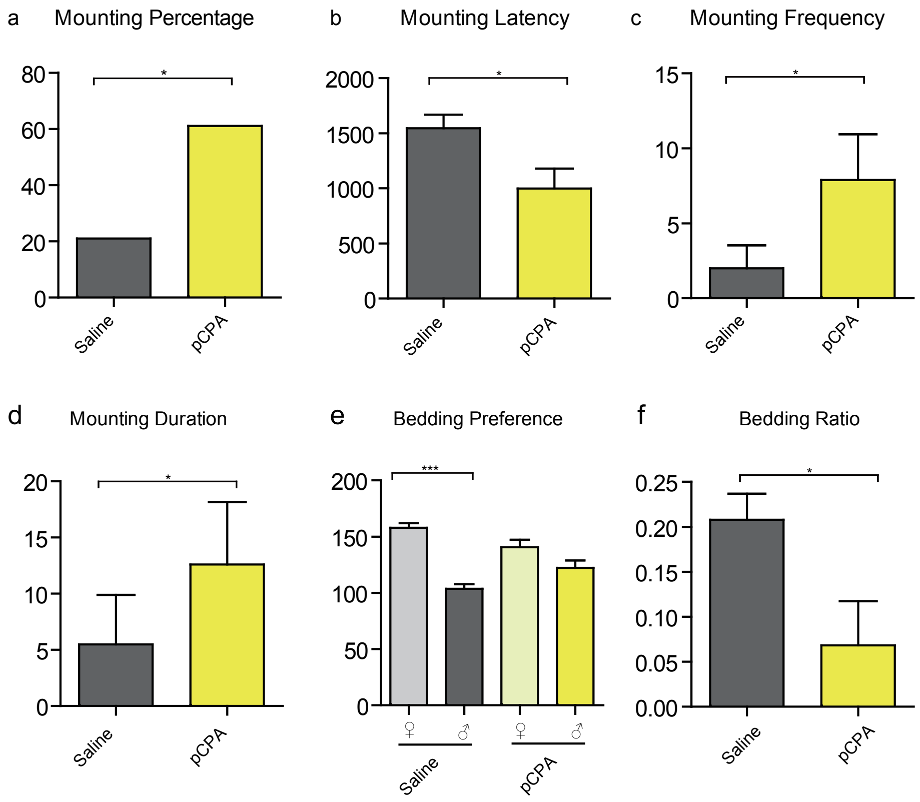
Supplementary Figure 5 | Mounting of female target mice by *Tph2* knockout males. When a test male was presented with an estrous female, there was no statistically difference among *Tph2*$^{+/+}$, *Tph2*$^{+/-}$ and *Tph2*$^{-/-}$ males in mounting percentage (**a,** $p>0.5$, X^2 test), latency (**b,** $p>0.5$, one-way ANOVA), frequency (**c,** $p>0.5$, one-way ANOVA), or duration (**d,** $p>0.5$, one-way ANOVA) of mount. n=13 for *Tph2*$^{+/+}$, n=14 for *Tph2*$^{+/-}$, n=16 for *Tph2*$^{-/-}$.



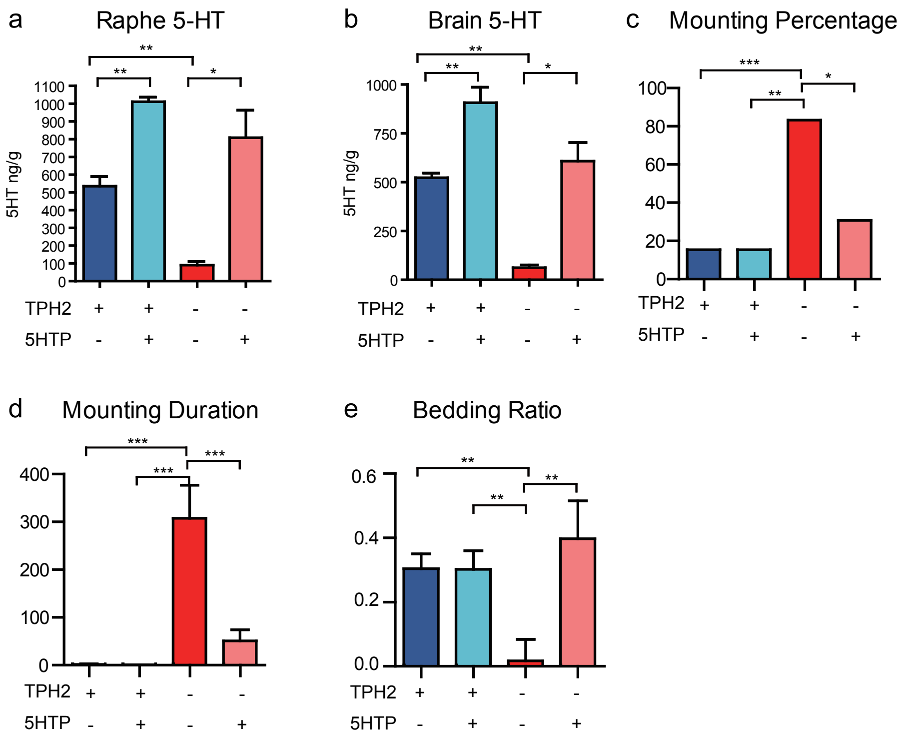
Supplementary Figure 6 | Mounting preference by *Tph2* knockout mice. Each test male was presented with two adult mice, one male and one estrous female, and its mating choice was analyzed for 15 mins. **a**, A higher percentage of *Tph2*$^{+/+}$ mice mounted female than male targets (n=14, $p<0.01$, X^2 test) as did *Tph2*$^{+/-}$ males (n=10, $p<0.01$, X^2 test). A similar percentage of *Tph2*$^{-/-}$ males mounted females and males (n=11, $p>0.05$, X^2 test). More *Tph2*$^{-/-}$ mounted males than *Tph2*$^{+/+}$ mice ($p<0.001$, X^2 test) and *Tph2+/-* mice ($p<0.5$, X^2 test). **b**, *Tph2*$^{+/+}$ males mounted female targets faster than male targets ($p<0.01$, t test), as did *Tph2*$^{+/-}$ males ($p<0.01$, t test). Mounting latencies of *Tph2*$^{-/-}$ males for females and males were not significantly different ($p>0.05$, t test). **c**, More than 40% *Tph2*$^{-/-}$ males, but none of the *Tph2*$^{+/+}$ or *Tph2*$^{+/-}$ males, chose the male as their first mounting targets. ($p<0.05$ for *Tph2*$^{+/+}$ vs. *Tph2*$^{-/-}$; $p<0.05$ for *Tph2*$^{+/-}$ vs. *Tph2*$^{-/-}$, $p>0.05$ for *Tph2*$^{+/+}$ vs. *Tph2*$^{+/-}$, X^2 test). **d**. *Tph2*$^{+/+}$ males mounted females significantly more often than males ($p<0.01$, t test), as did *Tph2*$^{+/-}$ males ($p<0.05$, t test). *Tph2*$^{-/-}$ males mounted females as often as males ($p>0.05$, t test). *Tph2*$^{-/-}$ males mounted males more than *Tph2*$^{+/+}$ ($p<0.01$, one-way ANOVA) and *Tph2*$^{+/-}$ ($p<0.01$, one-way ANOVA). **e**, *Tph2*$^{+/+}$ males spent longer time mounting females than males ($p<0.01$, t test) as did *Tph2*$^{+/-}$ males ($p<0.01$, t test). *Tph2*$^{-/-}$ males did not show difference in duration of mounting males or females ($p>0.05$, t test). *Tph2*$^{-/-}$ males spent more time mounting males than *Tph2*$^{+/+}$ ($p<0.05$, one-way ANOVA) and *Tph2*$^{+/-}$ ($p<0.05$, one-way ANOVA). f. The mounting ratio of *Tph2*$^{-/-}$ was significantly different from *Tph2*$^{+/+}$ and *Tph2*$^{+/-}$ ($p<0.001$, one-way ANOVA).



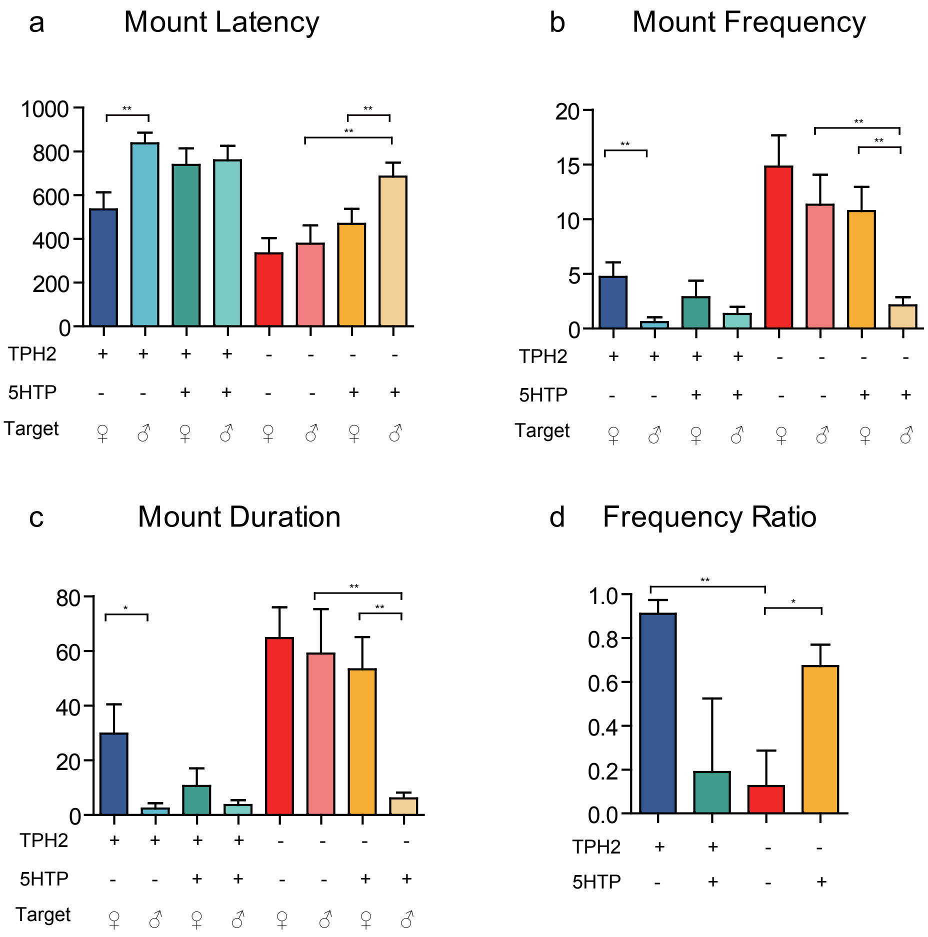
Supplementary Figure 7 | Levels of 5-HT analyzed by HPLC. **a,** 5-HT level was reduced in pCPA treated mice than the control mice in the whole brain ($p<0.001$, t test). **b,** The HIAA level also reduced in pCPA treated mice than the control treated mice in the whole brain ($p<0.001$, t test). n=8 for pCPA treated mice, n=8 for control mice.



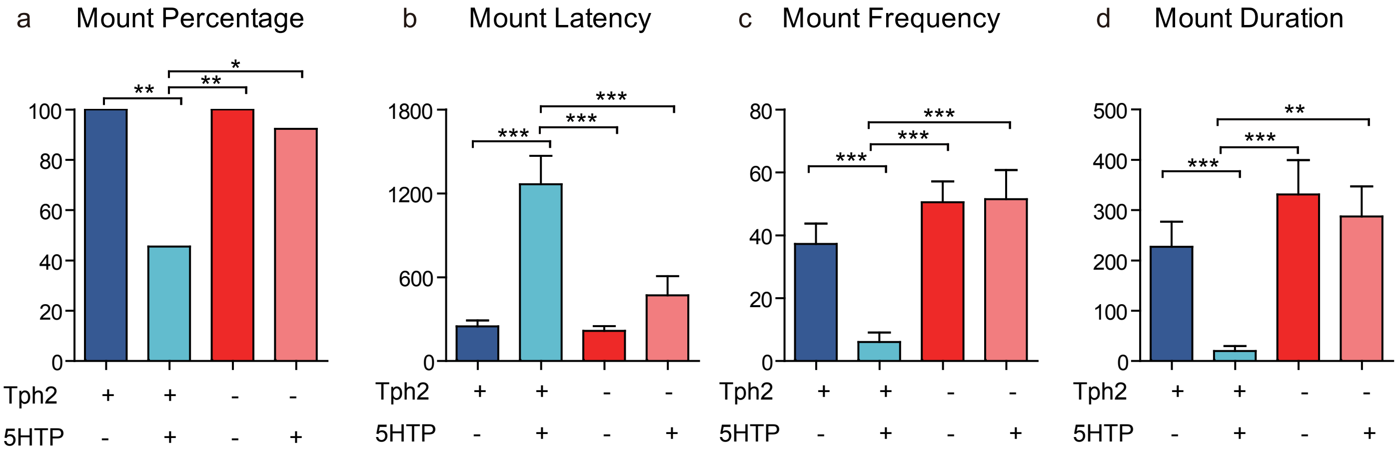
Supplementary Figure 8 | Effect of 5-HT depletion by pCPA on adult behaviors. Male C57 males treated with pCPA or saline in adulthood were tested for sexual behaviors. **a-d** (n=18 for pCPA and n=19 for control males). Mice treated with pCPA showed male-male mounting, with a higher percentage (**a**, $p<0.5$, X^2 test), shorter latency (**b,** $p<0.05$, t test), higher frequency (**c,** $p<0.05$, t test) and longer duration (**d,** $p<0.05$, t test) than control mice. **e,** Computer analysis of bedding preference. The total time spent above both male and female bedding was not different between pCPA treated and control males ($p>0.05$, t test). Control males spent more time above female bedding than male bedding ($p<0.001$, t test). pCPA treated males showed no difference between female and male bedding ($p>0.05$, t test). **f**, The bedding preference ratio of pCPA males were significantly different from the control males ($p<0.05$, t test). n=36 for saline group, n=36 for pCPA group.



Supplementary Figure 9 | 5-HTP rescue of chemical and behavioral deficits in *Tph2* knockout mice. **a-b**, Levels of 5-HT were analyzed in *Tph2*$^{+/+}$ and *Tph2*$^{-/-}$ males 35 min after injection of either 5-HTP (40 mg/kg body weight) or control saline (n=5 for *Tph2*$^{+/+}$ with saline, n=5 for *Tph2*$^{-/-}$ with saline, n=5 for *Tph2*$^{+/+}$ with 5-HTP, and n=6 for *Tph2*$^{-/-}$ with 5-HTP). 5-HTP could significantly rescue the levels of 5-HT. 5-HTP could also increase the levels of 5-HT and in the wt. **c-d**, Male-male mounting in *Tph2*$^{-/-}$ mice was significantly rescued by 5-HTP (results from the same experiments as those in Fig. 6b and c): the mounting percentage was decreased ($p<0.05$) and duration shortened ($p<0.001$). The mounting percentage of *Tph2*$^{-/-}$ mice with 5-HTP injection was not significantly different from those of *Tph2*$^{+/+}$. The mounting duration of *Tph2*$^{-/-}$ mice with 5-HTP injection was not significantly different from those of *Tph2*$^{+/+}$ (with saline or with 5-HTP, $p>0.1$). **e,** Bedding preference was monitored between 35 and 40 min after injection. Although 5-HTP could further increase 5-HT level in *Tph2*$^{+/+}$ mice, it did not cause statistically significant changes in bedding preference of *Tph2*$^{+/+}$ males.



Supplementary Figure 10 | Effect of 5-HTP on mating choice. *Tph2*$^{+/+}$ and *Tph2*$^{-/-}$ males treated with 5-HTP or saline were tested for mating choice with two adult mice, one male and one estrous female. n=15 for *Tph2*$^{+/+}$ and n=16 for *Tph2*$^{-/-}$. 5-HTP significantly rescued the mating choice phenotype of *Tph2*$^{-/-}$ mutant males in mounting latency (**a,** $p<0.01$, t test), frequency (**b,** $p<0.01$, t test), duration (**c,** $p<0.01$, t test),and the frequency ratio (**d,** $p<0.05$, t test).



Supplementary Figure 11 | Effect of 5-HTP on male-female mounting. *Tph2*$^{+/+}$ and *Tph2*$^{-/-}$ males treated with 5-HTP or saline were tested with estrous C57 females. n=11 for *Tph2*$^{+/+}$ and n=13 for *Tph2*$^{-/-}$. 5-HTP did not change male-female mounting of *Tph2*$^{-/-}$ males, but increased the male-female mount latency (**a,** $p<0.001$, t test), decreased the mounting frequency (**b,** $p<0.01$, t test) and duration (**c,** $p<0.01$, t test) of *Tph2*$^{+/+}$.

Supplementary Data Set 1

Statistics for all regular figures. Because of space constraints, statistics and number of animals tested were omitted from the text and the figure legends. They are listed here. In Figure 1 a-d, n=9 for *ePet1-Cre*, n=11 for *Lmx1b*$^{+/+}$, n=13 for *Lmx1b*$^{+/-}$, n=14 for *Lmx1b*$^{-/-}$. a, $p<0.001$ when *Lmx1b*$^{-/-}$ was compared with *Lmx1b*$^{+/+}$, *Lmx1b*$^{+/-}$ or *ePet1-Cre*; $p>0.05$ for other comparisons (X^2 test). b, $p<0.001$ (one-way ANOVA) when *Lmx1b*$^{-/-}$ were compared with *Lmx1b*$^{+/+}$, *Lmx1b*$^{+/-}$ or *ePet1-Cre*. c, $p<0.001$ when *Lmx1b*$^{-/-}$ was compared with *Lmx1b*$^{+/+}$, *Lmx1b*$^{+/-}$ or *ePet1-Cre*; $p>0.05$ for other comparisons (one-way ANOVA). d, $p<0.001$ (one-way ANOVA) when *Lmx1b*$^{-/-}$ were compared with *Lmx1b*$^{+/+}$, *Lmx1b*$^{+/-}$ or *ePet1-Cre*. e-f, n=9 for *ePet1-Cre*, n=14 for *Lmx1b*$^{+/+}$, n=16 for *Lmx1b*$^{+/-}$, n=14 for *Lmx1b*$^{-/-}$. f, USV towards females were similar among males of *ePet1-Cre*, *Lmx1b*$^{-/-}$, *Lmx1b*$^{+/+}$,or *Lmx1b*$^{+/-}$ ($p>0.05$, X^2 test). USV towards male intruders were higher from *Lmx1b*$^{-/-}$ males than from *ePet1-Cre*, *Lmx1b*$^{+/+}$ or *Lmx1b*$^{+/-}$ males ($p<0.05$, X^2 Test). g, USVs towards males were higher from *Lmx1b*$^{-/-}$ males than those from *ePet1-Cre*, *Lmx1b*$^{+/+}$ or *Lmx1b*$^{+/-}$ males ($p<0.05$, one-way ANOVA), whereas there is no statistically significant difference among *ePet1-Cre*, *Lmx1b*$^{+/+}$, *Lmx1b*$^{+/-}$ and *Lmx1b*$^{-/-}$ males in their USVs towards females ($p>0.05$, one-way ANOVA).

In Figure 2, n=9 for *ePet1-Cre*, n=14 for *Lmx1b*$^{+/+}$, n=10 for *Lmx1b*$^{+/-}$ and n=11 for *Lmx1b*$^{-/-}$. a, a higher percentage of *ePet1-Cre*, *Lmx1b*$^{+/+}$ and *Lmx1b*$^{+/-}$ mice mounted female than male targets ($p<0.05$, X^2 test). A similar percentage of *Lmx1b*$^{-/-}$ males mounted females and males ($p>0.05$, X^2 test). b, *ePet1-Cre*, *Lmx1b*$^{+/+}$ and

Lmx1b$^{+/-}$ males mounted female targets faster than male targets ($p<0.05$ for *ePet1-Cre*, $p<0.05$ for *Lmx1b*$^{+/+}$, $p<0.01$ for *Lmx1b*$^{+/-}$, t test). Mounting latencies of *Lmx1b*$^{-/-}$ males for females and males were not significantly different ($p>0.05$, t test). c, $p<0.05$ for *ePet1-Cre* vs. *Lmx1b*$^{-/-}$, $p<0.05$ for *Lmx1b*$^{+/+}$ vs. *Lmx1b*$^{-/-}$; $p<0.05$ for *Lmx1b*$^{+/-}$ vs. *Lmx1b*$^{-/-}$, $p>0.05$ for *Lmx1b*$^{+/+}$ vs. *Lmx1b*$^{+/-}$ (X^2 test). d, *ePet1-Cre* males mounted females significantly more often than males ($p<0.01$, t test), as did *Lmx1b*$^{+/+}$ ($p<0.01$, t test) and *Lmx1b*$^{+/-}$ males ($p<0.05$, t test). *Lmx1b*$^{-/-}$ males mounted females as often as males ($p>0.05$, t test). e, *ePet1-Cre* males spent more time mounting females than males ($p<0.01$, t test), as did *Lmx1b*$^{+/+}$ ($p<0.01$, t test) and *Lmx1b*$^{+/-}$ males ($p<0.05$, t test). *Lmx1b*$^{-/-}$ males did not show differences in mounting males or females ($p>0.05$, t test). f, The mounting frequency ratio of *Lmx1b*$^{-/-}$ was different from those of *ePet1-Cre*, *Lmx1b*$^{+/+}$ and *Lmx1b*$^{+/-}$ ($p<0.01$, one-way ANOVA)

In Figure 3 a-c, n=16 for *Lmx1b*$^{+/+}$, n=12 for *Lmx1b*$^{+/-}$, n=16 for *Lmx1b*$^{-/-}$. In d-f, n=9 for *ePet1-Cre*, n=16 for *Lmx1b*$^{+/+}$, n=8 for *Lmx1b*$^{+/-}$ and n=17 for *Lmx1b*$^{-/-}$. a, *Lmx1b*$^{+/+}$ males spent more time sniffing female than male genital odor ($p<0.001$, t test) as did *Lmx1b*$^{+/-}$ males ($p<0.01$, t test). *Lmx1b*$^{-/-}$ males spent a similar amount of time on female and male genital odor ($p>0.05$, t test). Three groups were not significantly different in male genital odor sniffing time ($p>0.05$, one-way ANOVA) but *Lmx1b*$^{-/-}$ males spent less time in sniffing female genital odor than the other 2 groups ($p<0.01$ for *Lmx1b*$^{+/+}$ vs. *Lmx1b*$^{-/-}$, $p<0.01$ for *Lmx1b*$^{+/-}$ vs. *Lmx1b*$^{-/-}$, one-way ANOVA). b, Sniffing ratio was calculated from (time on female side minus time on male side)/total sniffing time of each mouse. *Lmx1b*$^{-/-}$ males were significantly different from *Lmx1b*$^{+/+}$ and *Lmx1b*$^{-/-}$ males (p<0.05 for *Lmx1b*$^{+/+}$ vs. *Lmx1b*$^{-/-}$, p<0.05 for *Lmx1b*$^{+/-}$ vs. *Lmx1b*$^{-/-}$, p>0.05 for *Lmx1b*$^{+/+}$ vs. *Lmx1b*$^{+/-}$, one-way ANOVA). c, p<0.001 for *Lmx1b*$^{+/+}$ vs. *Lmx1b*$^{-/-}$, p<0.05 for *Lmx1b*$^{+/-}$ vs. *Lmx1b*$^{-/-}$, p>0.05 for *Lmx1b*$^{+/+}$ vs. *Lmx1b*$^{+/-}$ (X^2 test). d, *ePet1-Cre* males spent more time above female bedding than male bedding (p<0.001, t test) as did *Lmx1b*$^{+/+}$ (p<0.001, t test) and *Lmx1b*$^{+/-}$ males (p<0.01，t test). *Lmx1b*$^{-/-}$ males spent a similar amount of time above female and male bedding (p>0.05，t test). Compared with *ePet1-Cre* and *Lmx1b*$^{+/+}$, *Lmx1b*$^{-/-}$ males spent less time above female bedding (p<0.01 for *ePet1-Cre* vs. *Lmx1b*$^{-/-}$, p<0.05 for *Lmx1b*$^{+/+}$ vs. *Lmx1b*$^{-/-}$, one-way ANOVA) but more time above male bedding (p<0.01, one-way ANOVA). e, The bedding time ratio (female-male bedding)/total time on bedding of *Lmx1b*$^{-/-}$ was different from *ePet1-Cre* (p<0.01, one-way ANOVA) and *Lmx1b*$^{+/+}$ (p<0.01, one-way ANOVA). f, Compared with *ePet1-Cre*, *Lmx1b*$^{+/+}$ and *Lmx1b*$^{+/-}$, a significantly higher percentage of *Lmx1b*$^{-/-}$ males spent more time above male bedding (p<0.001 for *ePet1-Cre* vs. *Lmx1b*$^{-/-}$, p<0.001 for *Lmx1b*$^{+/+}$ vs. *Lmx1b*$^{-/-}$, p<0.05 for *Lmx1b*$^{+/-}$ vs. *Lmx1b*$^{-/-}$, X^2 test).

In Figure 4, a, both *Lmx1b*$^{+/+}$ and *Lmx1b*$^{-/-}$ males showed habituation in sniffing time to ovariectomized females presented repeatedly (p<0.001 for 1st vs 4th tests by both *Lmx1b*$^{+/+}$ and *Lmx1b*$^{-/-}$, one-way ANOVA). Dishabituation was observed for both *Lmx1b*$^{+/+}$ and *Lmx1b*$^{-/-}$ when new ovariectomized females were introduced (4th vs 5th p<0.01 for both *Lmx1b*$^{+/+}$ and *Lmx1b*$^{-/-}$, one-way ANOVA). No statistic difference was found between *Lmx1b*$^{+/+}$ and *Lmx1b*$^{-/-}$ males at any point (p>0.05，t test; n=23 for

Lmx1b$^{+/+}$, n=22 for *Lmx1b*$^{-/-}$). b, After 7 training sessions with male and female urine, *Lmx1b*$^{+/+}$ males increased their correct rate from 43.1±1.8% to 84.8±4.0%, while *Lmx1b*$^{-/-}$ males from 37.6±2.7% to 86.4±3.3%. No significant difference was detected between *Lmx1b*$^{+/+}$ and *Lmx1b*$^{-/-}$ males at any point ($p>0.05$, t test; n=14 for *Lmx1b*$^{+/+}$, n=14 for *Lmx1b*$^{-/-}$).

In Figure 5, a-b, Male-male mounting behavior of *Tph2*$^{+/+}$ (n=10), *Tph2*$^{+/-}$ (n=10) and *Tph2*$^{-/-}$ (n=11) mice. Compared with *Tph2*$^{+/+}$ and *Tph2*$^{+/-}$, *Tph2*$^{-/-}$ males showed a shorter latency ($p<0.05$ for both *Tph2*$^{+/+}$ vs. *Tph2*$^{-/-}$ and *Tph2*$^{+/-}$ vs. *Tph2*$^{-/-}$), and higher frequency ($p<0.05$ for both *Tph2*$^{+/+}$ vs. *Tph2*$^{-/-}$ and *Tph2*$^{+/-}$ vs. *Tph2*$^{-/-}$ one-way ANOVA) in mounting males. c, Both *Tph2*$^{+/+}$ (n=22) and *Tph2*$^{+/-}$ (n=17) males significantly preferred female over male bedding ($p<0.01$, t test), whereas *Tph2*$^{-/-}$ (n=20) males did not show preference between male and female bedding ($p>0.05$, t test). d, Both *Tph2*$^{+/+}$ and *Tph2*$^{+/-}$ males significantly preferred female over male genital odor ($p<0.001$ for *Tph2*$^{+/+}$, $p<0.01$ for *Tph2*$^{+/-}$, t test), whereas *Tph2*$^{-/-}$ males did not show preference between male and female genital odor ($p>0.05$, t test).

In Figure 6, a, n=5 for *Tph2*$^{+/+}$ with saline, n=5 for *Tph2*$^{-/-}$ with saline, n=5 for *Tph2*$^{+/+}$ with 5-HTP, and n=6 for *Tph2*$^{-/-}$ with 5-HTP). b-c, n=13 for *Tph2*$^{+/+}$ with saline, n=12 for *Tph2*$^{-/-}$ with saline, n=13 for *Tph2*$^{+/+}$ with 5-HTP, and n=12 for *Tph2*$^{-/-}$ with 5-HTP. Male-male mounting in *Tph2*$^{-/-}$ mice monitored between 20 and 50 min after injection was significantly rescued by 5-HTP: the latency was lengthened ($p<0.01$) and frequency reduced ($p<0.01$). The mounting latency of *Tph2*$^{-/-}$ mice with 5-HTP injection was not significantly different from those of *Tph2*$^{+/+}$ (with saline or with 5-HTP, $p>0.1$). The mounting frequency of *Tph2*$^{-/-}$ mice with 5-HTP injection was not significantly different from those of *Tph2*$^{+/+}$ (with saline or with 5-HTP, $p>0.05$). d, Bedding preference was monitored between 35 and 40 min after injection. 5-HTP could significantly restore the preference of female over male bedding by *Tph2*$^{-/-}$ males.