## Supplemental data

## Methods

### Determination of PCR cycle number in the ChIP assay

PCR cycle numbers for detection over a log-linear amplification range were carefully determined. To determine the optimal amplification cycle, PCR experiments were performed repeatedly using the same sample and serial cycle number. A cycle that could detect twice the amplification in densitometric quantitative measurements was selected, which allowed comparison of modest differences. In addition, to confirm reproducibility, sample collection was carried out 3 times independently, and experiments were repeated 3 times for each collected sample.

### Open Field Test

The open field consisted of a 100 cm x 100 cm x 45 cm gray polyvinyl chloride box with an open top. The open field arena was partitioned by black lines into 25 equal-sized squares: 16 outer squares and 9 center squares (1). Testing sessions lasted 5 min and were scored by an observer blinded to the drug treatment. The number of squares crossed and the time spent in the center squares were scored. Statistical comparisons between the groups were made using a t-test.

### Determination of the dose of TSA in neonatal intracerebroventricular injection

The effective dose was determined in preliminary trials. There was no effect on male sexual behavior of TSA treatment at 0.05 ng, and there was no additional effect at 5 ng compared with the dose used in the study (0.5 ng).

### Serum Testosterone Level

Serum testosterone was measured by enzyme immunoassay (EIA) (2). Blood was collected from the left ventricle before perfusion, and plasma was stored at -80°C until assayed. Aliquots of plasma were assayed using a Testosterone EIA Kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions. Statistical comparisons between the groups were made using a t-test.

1. McCarthy, M.M., Felzenberg, E., Robbins, A., Pfaff, D.W. & Schwartz-Giblin, S. Infusions of diazepam and allopregnanolone into the midbrain central gray facilitate open-field behavior and sexual receptivity in female rats. 1995 Horm Behav 29: 279-295
2. Sakamoto, H., Mezaki, Y., Shikimi, H., Ukena, K. & Tsutsui, K. Dendritic growth and spine formation in response to estrogen in the developing Purkinje cell. 2003 Endocrinology 144:4466-4477

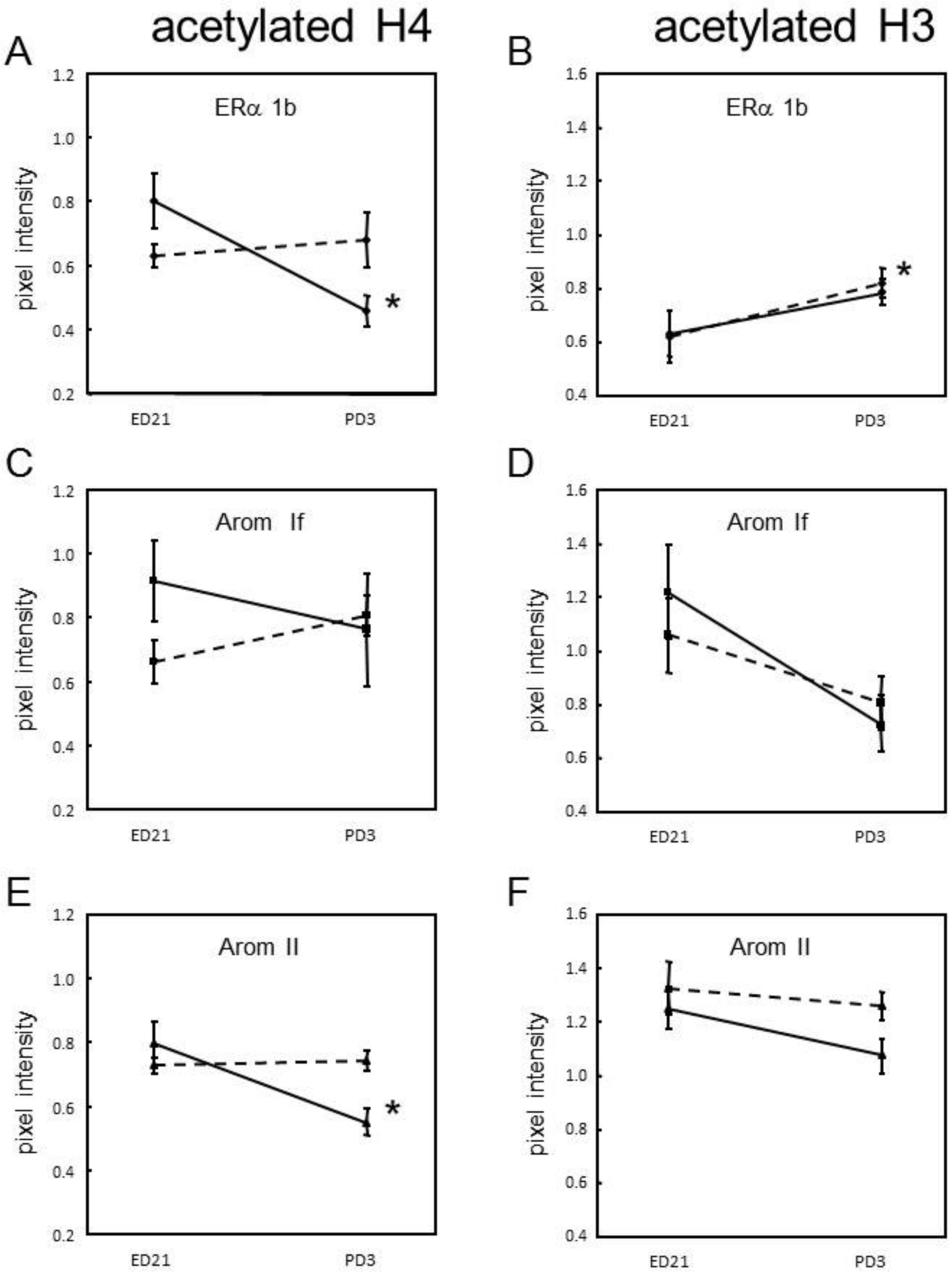
**Supplemental Fig. 1. Comparison of histone acetylation levels at ED21 and PD3 in each sex.** Pixel intensities of gray scale images were measured and ChIP/input ratios were plotted (mean ± SEM) for males (solid lines) and females (dashed lines). (A, C, E) Immunoprecipitated chromatin containing acetylated H4. (b, d, f) Immunoprecipitated chromatin containing acetylated H3. (A, B) ERα 1b promoter. (C, D) Arom lf promoter. (E, F) Arom II promoter. *: $p < 0.05$ for ED21 vs. PD3.

**Supplemental Fig. 2. Latencies of male sexual behavior in rats treated neonatally with TSA.** Latencies of male sexual behavior in TSA-infused (TSA, n=12) and saline-infused (Cont, n=12) rats. Values are means ± SEM for (A) mount latency ($F_{5, 66}$=8.20), (B) intromission latency ($F_{5, 63}$=15.42), and (C) ejaculation latency ($F_{5,}$

$_{50}$=14.70). *: p < 0.05, **: p< 0.01 vs. Cont in each trial.

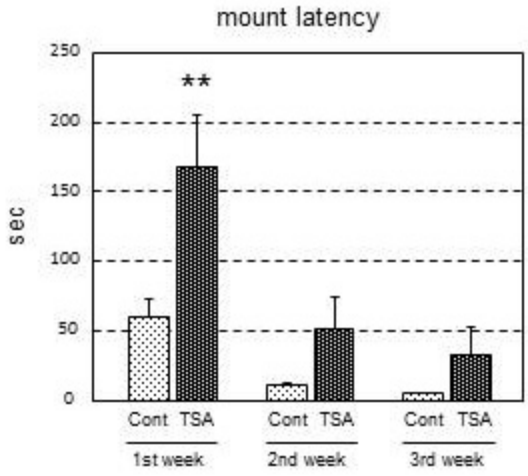
**Supplemental Fig. 3. Lack of an effect of TSA on brain function other than sexual behavior in neonatally TSA-treated male rats.** (A, B) Open field test. After observation of male sexual behavior, rats treated neonatally with TSA (TSA, n=12) or saline (Cont, n=12) were subjected to an open field test to examine locomotor and anxiety-like behavior. Values are means ± SEM of number of squares crossed (A) and time spent in center squares (B). (C-F) To examine the hormonal environment, the serum testosterone level (C), testis weight (D), epididymis weight (E) and body weight (F) were measured. Values are means ± SEM. There were no significant differences between the TSA and Cont groups in all of the analysis (p>0.05) (t-test).

**Supplemental Fig. 4. Latencies of male sexual behavior in rats treated neonatally with HDAC2/4 antisense ODN.** Latencies of male sexual behavior in neonatally HDAC2 antisense ODN-infused (HDAC2, n=9), HDAC4 antisense ODN-infused (HDAC4, n=8), and scrambled ODN-infused (SCRM, n=8) male rats. Values are means ± SEM of (A) mount latency ($F_{8, 63}$=5.76), (B) intromission latency ($F_{8, 63}$=4.42), and (C) ejaculation latency ($F_{8, 59}$=4.37). *: p < 0.05, **: p< 0.01 vs. SCRM in each trial.




Matsuda, supplemental Fig. 1

A

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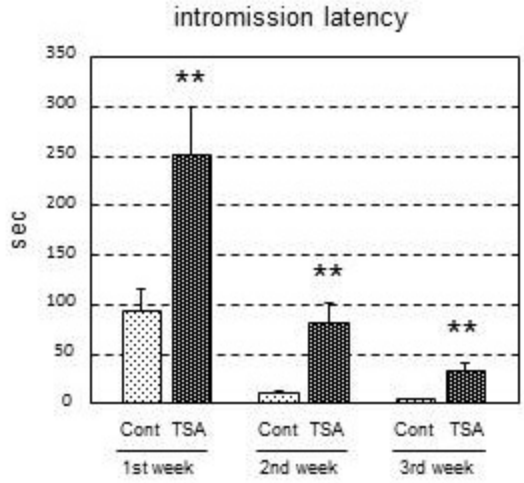


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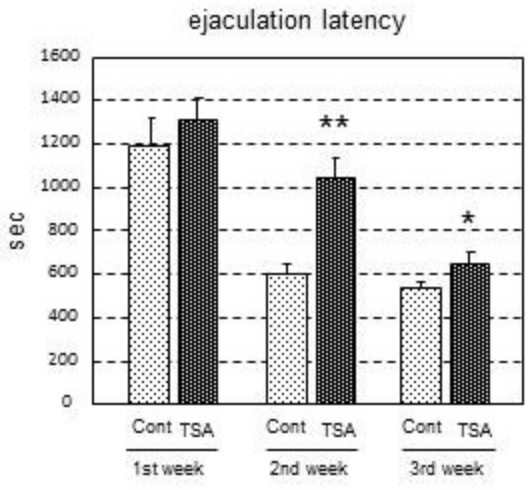
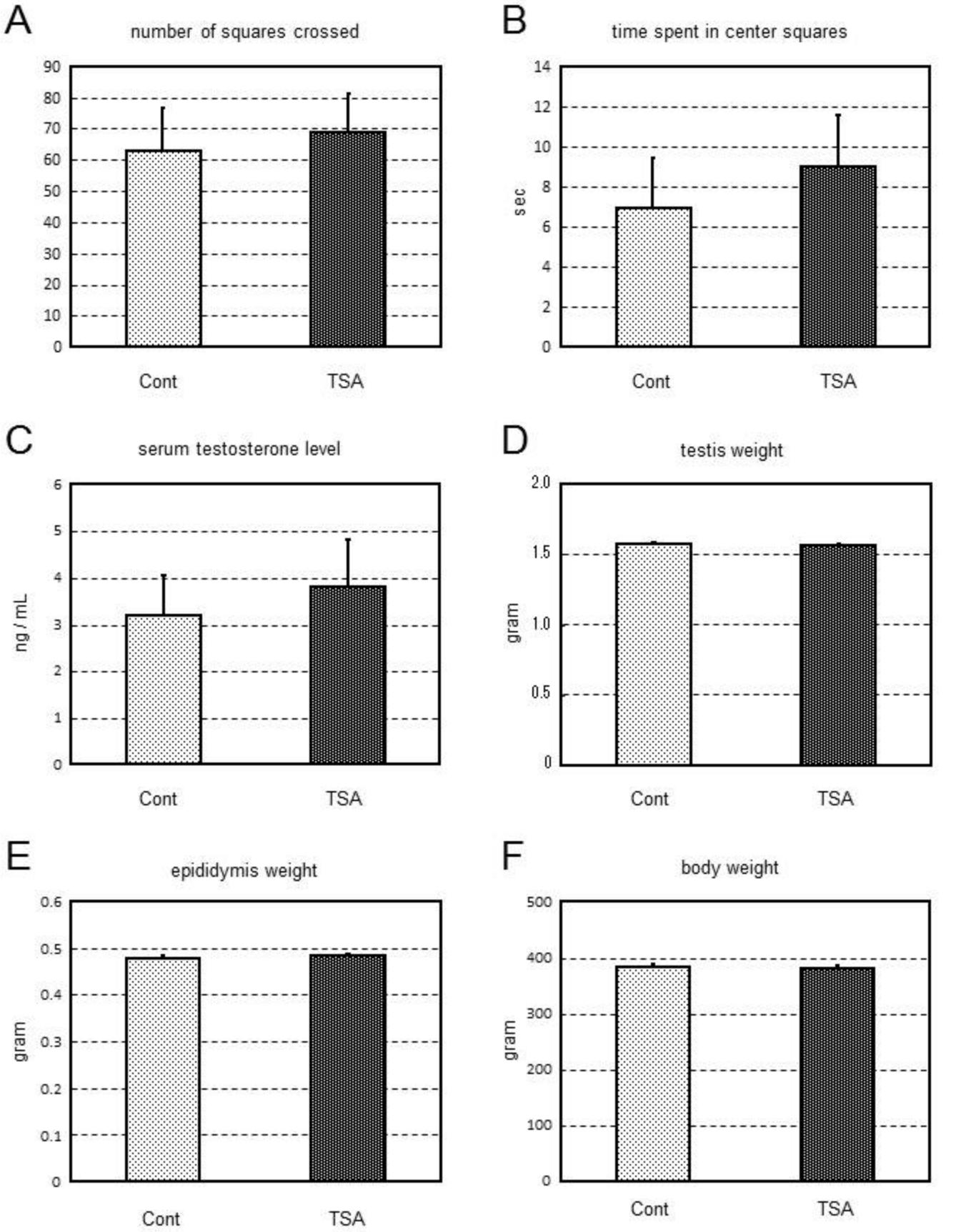
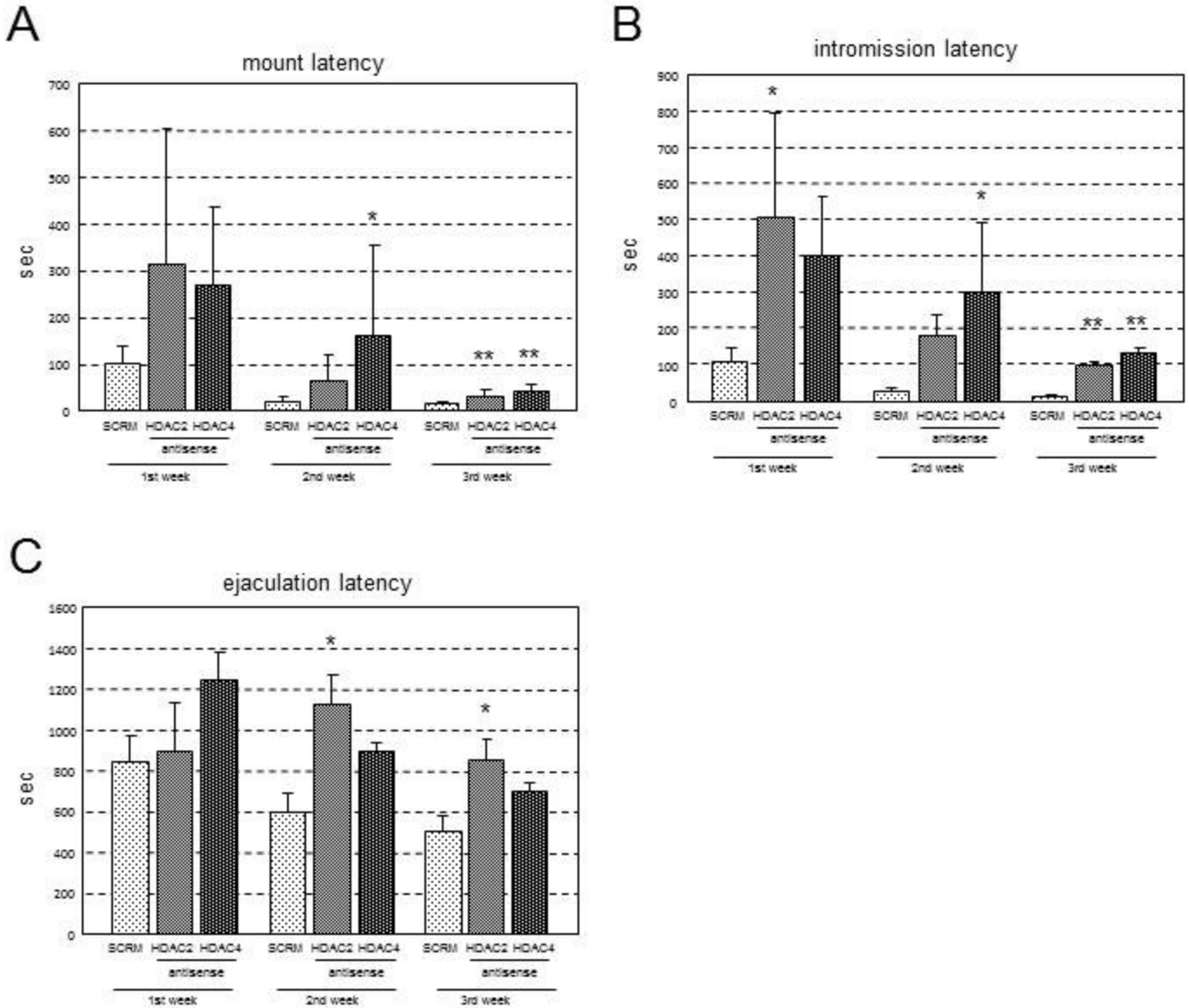



Matsuda, supplemental Fig. 2




Matsuda, supplemental Fig. 3




Matsuda, supplemental Fig. 4