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## **Sexual experience affects reproductive behavior and preoptic androgen receptors in male mice**

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## 2 **Abstract**

3 Reproductive behavior in male rodents is made up of anticipatory and consummatory  
4 elements which are regulated in the brain by sensory systems, reward circuits and hormone  
5 signaling. Gonadal steroids play a key role in the regulation of male sexual behavior *via*  
6 steroid receptors in the hypothalamus and preoptic area. Typical patterns of male  
7 reproductive behavior have been characterized, however these are not fixed but are  
8 modulated by adult experience. We assessed the effects of repeated sexual experience on  
9 male reproductive behavior of C57BL/6 mice; including measures of olfactory investigation  
10 of females, mounting, intromission and ejaculation. The effects of sexual experience on the  
11 number of cells expressing either androgen receptor (AR) or estrogen receptor alpha (ER $\alpha$ ) in  
12 the primary brain nuclei regulating male sexual behavior was also measured. Sexually  
13 experienced male mice engaged in less sniffing of females before initiating sexual behavior  
14 and exhibited shorter latencies to mount and intromit, increased frequency of intromission,  
15 and increased duration of intromission relative to mounting. No changes in numbers of ER $\alpha$ -  
16 positive cells were observed, however sexually experienced males had increased numbers of  
17 AR-positive cells in the medial preoptic area (MPOA); the primary regulatory nucleus for  
18 male sexual behavior. These results indicate that sexual experience results in a qualitative  
19 change in male reproductive behavior in mice that is associated with increased testosterone  
20 sensitivity in the MPOA and that this nucleus may play a key integrative role in mediating the  
21 effects of sexual experience on male behavior.

Keywords: reproduction; sexual behavior; sexual experience; olfaction; testosterone;  
androgen receptor; estrogen receptor; hypothalamus; preoptic area

## 23 **Introduction**

24           Sexual behavior in male rodents consists of species-typical patterns of anticipatory  
25 and consummatory behavior. These involve approach and olfactory investigation of the  
26 female (especially anogenital sniffing), followed by bouts of mounting, intromission and then  
27 ejaculation, with a subsequent refractory period of reduced interest in females. The variations  
28 on this basic behavioral template have been characterized in different species (Hull et al.,  
29 2002) and in strains of the same species (McGill, 1962b), but patterns of male reproductive  
30 behavior also change over the adult life of the individual. One important modifier of mating  
31 behavior is sexual experience, which has long-term effects on both anticipatory and  
32 consummatory behaviors. The detection and investigation of female odor cues is a key trigger  
33 for the initiation of male sexual behavior in rodents and male behavioral responses to female  
34 odors are sensitive to sexual experience. While disruption of either the olfactory or  
35 vomeronasal system has severe effects on male sexual behavior in many rodents (Mandiyan  
36 et al.,2005; Powers and Winans, 1975; Steel and Keverne, 1985), these effects are less  
37 pronounced if subjects are sexually experienced (Meredith, 1986; Pfeiffer and Johnston,  
38 1994). It should be noted, however, that in mice the data are less clear and sexually  
39 experienced males receiving lesions to the main olfactory epithelium have been reported both  
40 to retain normal copulatory performance (Edwards and Burge, 1973) and to suffer total loss  
41 of mating behavior (Keller et al., 2006). While this more recent study suggests that sexual  
42 experience may not protect male mice from the behavioral disruption caused by olfactory  
43 lesions, sexually experienced male mice respond to a wider range of female chemosignals  
44 than virgins (Sipos et al., 1992) and acquire preferences for the odors of receptive females  
45 with sexual experience (Hayashi and Kimura, 1974). As well as affecting pre-copulatory  
46 behaviors, sexual experience also has effects on the motor components of sexual behavior

47 itself. Studies in different rodent species, including rats (Dewsbury, 1969; Larsson, 1959),  
48 guinea pigs (Valenstein et al., 1955) and lemmings (Coopersmith et al., 1986), have shown  
49 that behavioral components of copulation including mounting, intromission and ejaculation  
50 occur with shorter latencies and higher frequencies in sexually experienced males.

51 Male mating behavior is governed by a complex interaction between different systems  
52 in the brain which process sensory inputs, regulate reward and motivation, and integrate  
53 hormonal signals (Hull et al., 2002). Gonadal steroids play a key role in this regulatory  
54 system, as is evident from the suppression of sexual behavior caused by castration, and its  
55 restoration by subsequent testosterone treatment (McGinnis and Dreifuss, 1989). However  
56 even the effects of castration are reduced by sexual experience in some male rodents  
57 (Costantini et al., 2007; Manning and Thompson, 1976). This suggests that while androgens  
58 occupy a primary role in the regulation of sexual behavior, the brain systems that integrate  
59 steroid hormone signals into behavioral output are modified by sexual experience. The  
60 secretion of testosterone itself is also affected by sexual experience. During a sexual  
61 encounter, levels of circulating androgens increase after initial exposure to female cues and  
62 again in response to copulation (Batty, 1978; Gleason et al., 2009). Sexual experience  
63 enhances both these female-elicited reflexive releases of testosterone (Bonilla-Jaime et al.,  
64 2006; Kamel et al., 1975) and increases baseline levels of circulating testosterone (Edinger  
65 and Frye, 2007; Wu and Gore, 2009).

66 Effects of testosterone on sexual behavior are mediated in the brain directly *via* the  
67 androgen receptor (AR) and indirectly (after local aromatization to estradiol) *via* estrogen  
68 receptors, primarily estrogen receptor alpha (ER $\alpha$ ) (Wersinger et al., 1997). The network of  
69 brain areas involved in the regulation of male sexual behavior includes the main olfactory and  
70 vomeronasal systems (Keverne, 2004), the mesocorticolimbic system that governs reward

71 and motivation, (Balfour et al., 2004), and regions in the hypothalamus and preoptic area  
72 (Hull et al., 2002). These are sites of high expression of AR and ER $\alpha$  such as the bed nucleus  
73 of stria terminalis (BNST), medial amygdala (MeAmg), ventromedial hypothalamus (VMH)  
74 and medial preoptic area (MPOA) (Simerly et al., 1990). The MPOA is the critical integrative  
75 nucleus in male sexual behavior (Hull and Dominguez, 2006) and lesions of the MPOA  
76 disrupt mounting, intromission and ejaculation in rats (de Jonge et al., 1989), mice (Bean et  
77 al., 1981) and hamsters (Powers et al., 1987). The importance of testosterone sensitivity in  
78 the MPOA for male sexual behavior is illustrated by studies in which AR antagonists are  
79 injected into the MPOA, resulting in sexual behavior deficits resembling those seen after  
80 MPOA lesions (Harding and McGinnis, 2004; McGinnis et al., 1996). Moreover, conditional  
81 deletion of AR in the brain increases latencies to perform sexual behaviors and reduces  
82 incidences of copulation (Raskin et al, 2009), despite elevated circulating testosterone and  
83 intact MPOA ER $\alpha$  levels in these knockout males. Copulation-induced c-Fos has been shown  
84 to colocalize with AR in the MPOA, BNST and MeAmg of male hamsters (Wood and  
85 Newman, 1993), indicating that testosterone-sensitive neurons are activated in these brain  
86 areas during mating. Sexual experience induces a series of changes across this brain network  
87 that regulates reproductive behavior. After sexual experience, female odor-elicited c-Fos  
88 expression is greater in both the main olfactory and vomeronasal systems and their  
89 downstream androgen-sensitive targets, with particularly strong responses seen in the  
90 MeAmg, BNST and MPOA (Fewell and Meredith, 2002; Hosokawa and Chiba, 2005;  
91 Swaney et al., 2007). Copulation itself increases c-Fos immunoreactivity in the MPOA  
92 (Robertson et al., 1991) and this increase is greater in the brains of sexually experienced male  
93 rats than virgins (Lumley and Hull, 1999).

94           Research into the effects of sexual satiety in rats offers further evidence of neuronal  
95 plasticity in the circuits regulating male sexual behavior and of changes in testosterone  
96 sensitivity. Male rats that are allowed to mate *ad libitum* over a short period of time reach a  
97 state of sexual satiety in which they lose interest in females and do not fully recover sexual  
98 drive for up to 15 days (Phillips-Farfan and Fernandez-Guasti, 2009). This state of sexual  
99 satiety and lack of interest in females is associated with AR density reductions in the nucleus  
100 accumbens (NAc), VMH, lateral septum, MeAmg and especially in the MPOA (Fernandez-  
101 Guasti et al., 2003; Phillips-Farfan and Fernandez-Guasti, 2009). Conversely, ER $\alpha$  is elevated  
102 in the VMH, lateral septum and MPOA of sexually satiated males (Phillips-Farfan et al.,  
103 2007, Fernandez-Guasti, 2010). AR and ER $\alpha$  densities recover to previous levels as libido  
104 returns and this dynamic variation in gonadal steroid sensitivity indicates that receptor  
105 expression in the sexual brain network varies with sexual function in males.

106           In the current study, we explore the long term effects of sexual experience on  
107 reproductive behavior and whether these effects involve changes in regulatory brain areas  
108 that are sensitive to testosterone. Our experimental design involved measurement of the  
109 effects of repeated sexual experience during cohabitation with females on reproductive  
110 behavior in male mice. We predicted that sexual experience would result in changes in  
111 interest in females as well as shorter latencies and higher frequencies of sexual behaviors.  
112 While such effects have been characterized in other rodent species, such as rats and hamsters,  
113 these effects have not been specifically studied in mice despite their increasing use in  
114 behavioral neuroscience research. Given previously reported increases in circulating  
115 testosterone after sexual experience and the dynamic changes in AR and ER $\alpha$  after sexual  
116 activity, we also examined whether behavioral effects of sexual experience are associated  
117 with long-term changes in numbers of AR and ER $\alpha$ -positive neurons in the primary nuclei

118 that regulate sexual behavior in male mice. The MPOA, posterior MeAmg (pMeAmg), and  
119 BNST were selected for investigation based on their involvement in reproductive behavior,  
120 previously reported activity changes after sexual experience, and high levels of expression of  
121 AR and ER $\alpha$ .

## 122 **Materials and Methods**

123 To characterize the effects of sexual experience on reproductive behaviors, age-matched  
124 virgin and sexually experienced C57BL/6 (B6) male mice were given mating tests with  
125 receptive virgin B6 female mice. To investigate effects of sexual experience on AR and ER $\alpha$ ,  
126 age-matched virgin and sexually experienced B6 male mice were sacrificed and their brains  
127 processed for AR and ER $\alpha$  immunohistochemistry. All procedures described were approved  
128 by the Institutional Animal Care and Use Committee of Columbia University.

### 129 *Animals*

130 Adult male and female B6 mice were purchased from Charles River Laboratories (Kingston,  
131 NY) at approximately 2 months of age. Males and females were separately housed in groups  
132 of 4 or 5 at the Department of Psychology at Columbia University for 1 month of habituation  
133 to the animal facility. Mice were housed in 13.5" x 8.1" x 5.5" Plexiglas cages under a 12  
134 hour reversed dark-light cycle (8am: lights off, 8pm: lights on) with wood shaving bedding,  
135 *ad libitum* water and chow. At the start of the experimental phase (see **Figure 1** for summary  
136 of experimental design), male mice were split into two experimental groups: virgin and  
137 sexually experienced males. Each sexually experienced male was housed in a novel clean  
138 cage with two gonadally intact B6 female mice which each received repeated hormonal  
139 priming to induce regular sexual receptivity. This priming involved subcutaneous injections  
140 of 50  $\mu$ g of estradiol benzoate (Fisher Scientific, Pittsburgh PA) dissolved in 50 $\mu$ l of peanut  
141 oil, followed 72 hours later by 400 $\mu$ g progesterone (Fisher Scientific, Pittsburgh PA)



142 dissolved in 50 $\mu$ l of peanut oil. This injection schedule was repeated every 6 days so that  
143 males had repeated access to females that were sexually receptive. The injection schedules  
144 for the two females in each cage were staggered by three days so that males would have the  
145 opportunity to mate with a receptive female every three days. Males were housed under these  
146 conditions for three weeks, and then for a further week the females housed with the sexually  
147 experienced males received a different injection schedule. During this final week females  
148 received no estradiol injections and were given subcutaneous injections of 400 $\mu$ g  
149 progesterone in 50 $\mu$ l peanut oil every two days to prevent estrus (Morin, 1977) and keep  
150 them sexually unreceptive. This ensured that their male cage-mates did not have mating  
151 opportunities during the final week of mixed-sex housing and that any behavioral and brain  
152 effects measured were likely to be a longer-term consequence of sexual experience rather  
153 than recent copulation. Daily observations confirmed that no sexual activity occurred  
154 amongst the sexually-experienced males during this period. Virgin males were re-housed in  
155 new groups of four into a novel clean cage at the start of the experimental phase of the study  
156 (i.e. at the same time that the sexually experienced group males were re-housed with females)  
157 so that they experienced social change, albeit with unfamiliar males rather than females. This  
158 re-housing occurred at the start of the experimental phase and each co-housed male was  
159 included in the analysis. Previous literature has indicated that male-male aggression may  
160 induce changes within the behavioral and neuroendocrine outcomes being examined in the  
161 present study (Fuxjager et al., 2010). To ensure that aggressive encounters within the virgin  
162 male housing condition were not contributing to the long-term effects being assessed in this  
163 experimental design, we conducted daily observations of virgin and sexually-experienced  
164 housed males. We observed minimal male-directed aggression in either condition within the  
165 two hours following re-housing and during the subsequent four week housing period, no  
166 aggression was observed. Following the virgin housing/sexual experience phase, half of the

167 male mice of each experimental group were given mating tests, the other half were sacrificed  
168 for analysis of brain immunohistochemistry.

#### 169 *Tests of male reproductive behavior*

170 The mating behaviors of virgin and sexually experienced B6 males were measured in one-  
171 hour tests with unfamiliar, hormonally-primed, virgin B6 females. These stimulus females  
172 were hormonally primed with subcutaneous injections of 50 µg of estradiol benzoate  
173 dissolved in 50µl of peanut oil 72 hours before testing, followed by 400µg progesterone  
174 dissolved in 50µl of peanut oil 3 hours before testing. Each male was tested during the first 6  
175 hours after lights out in a clean 13.5” x 8.1” x 5.5” Plexiglas cage without food or water. A  
176 male and a stimulus female were placed in the test cage for one hour and behavior was video  
177 recorded from above for later blind scoring. The male reproductive behaviors scored were  
178 anogenital sniffing of the female, mounting, intromission and ejaculation. The measures  
179 scored were latency and frequency of mounting, intromission and ejaculation, duration of  
180 anogenital sniffing before first mount, and the difference between duration of mounting and  
181 intromission (calculated by subtracting mounting duration from intromission duration). If a  
182 subject did not perform a behavior during the hour-long test, a maximum latency value of  
183 3600 seconds was assigned for that behavior.

#### 184 *AR and ERα immunohistochemistry*

185 Virgin and sexually experienced males were euthanized with an overdose of ketamine-  
186 xylazine anesthetic, before being transcardially perfused with 20ml of 0.1M phosphate-  
187 buffered saline (PBS) followed by 20ml of freshly prepared 4% paraformaldehyde in PBS.  
188 Brains were then removed and post-fixed for 4 hours at 4°C in 4% paraformaldehyde in PBS,  
189 before being cryoprotected in 30% sucrose in PBS overnight, then frozen and stored at -80°C.  
190 Brains were sectioned coronally at 40µm on a cryostat at -20°C into two alternate series, and

191 processed for AR and ER $\alpha$  expression. Sections encompassing the areas of interest were  
192 selected and washed twice in PBS, then incubated overnight at 4°C in PBS with 0.3% Triton  
193 x-100 (PBST), 1.5% normal goat serum (Vector Laboratories, Burlingame CA) and either  
194 1:5000 polyclonal anti-AR (rabbit) primary antibody or 1:5000 polyclonal anti-ER $\alpha$  (rabbit)  
195 primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA). After two washes in PBS,  
196 sections were incubated for 15 minutes at room temperature in 4% hydrogen peroxide and  
197 10% methanol in PBST, washed twice in PBS, then incubated for 30 minutes at room  
198 temperature in 1:2000 biotinylated anti-rabbit (goat) secondary antibody (ABC elite kit:  
199 Vector Laboratories) in PBST. After two further washes in PBS, sections were incubated for  
200 30 minutes in avidin-biotin peroxidase solution (ABC elite kit: Vector Laboratories) in  
201 PBST, washed twice in PBS, then stained in Vector SG peroxidase substrate solution (Vector  
202 Laboratories) for 3 minutes. The stained sections were washed twice in PBS before being  
203 mounted on gelatin-coated glass slides, dehydrated and cleared in a series of alcohol and  
204 xylene washes, then coverslipped with DePeX (Fisher Scientific).

#### 205 *Image analysis*

206 For both the AR- and ER $\alpha$ -stained series, the mouse brain atlas of Paxinos and Franklin  
207 (2001) was used to select matched, sequential sections for each animal that encompassed the  
208 BNST (four sections centered around -0.22mm from Bregma), pMeAmg (six sections  
209 centered around -1.46mm from Bregma) and MPOA (six sections centered around 0.02mm  
210 from Bregma). Images of these areas were captured at 20x magnification. The sections were  
211 analyzed using MCID imaging software (Interfocus Imaging, Linton, UK) to normalize  
212 background levels and apply a minimum staining threshold to all images. Stained cells were  
213 counted bilaterally within rectangular boxes overlaid on the areas of interest (BNST:

214 500x350 $\mu$ m; pMeAmg: 500x375 $\mu$ m; MPOA: 600x400 $\mu$ m). In each region the mean number  
215 of stained cells per mm<sup>2</sup> per section was calculated for each animal.

### 216 *Statistical analysis*

217 Latency data from the mating tests were not normally distributed and so were log<sub>10</sub>(y+1)  
218 transformed before analysis. All measures of male reproductive behavior were analyzed using  
219 student's independent two-sample *t*-tests. A Bonferroni correction was applied to account for  
220 multiple comparisons: 8 tests were run, resulting in a corrected alpha value of  $P=0.00625$ . All  
221 immunohistochemical data were normally distributed. Counts of stained AR and ER $\alpha$  cells  
222 were analyzed using Student's independent two-sample *t*-tests, or Welch's *t*-test when the  
223 variances were unequal.

## 224 **Results**

### 225 *Tests of male reproductive behavior*

226 Significant effects of sexual experience were seen across multiple measures of male  
227 reproductive behavior (**Figure 2**). Sexually experienced males had shorter latencies to mount  
228 females than virgin males ( $n=6/\text{group}$ ;  $t_{10}=-5.78$ ,  $P<0.001$ ), and shorter latencies to intromit  
229 than virgins ( $t_{10}=-5.41$ ,  $P<0.001$ ). However there was no significant difference between the  
230 groups in the latency to ejaculate during testing ( $t_{10}=-1.86$ ,  $P=0.093$ ). Comparison of the  
231 frequency of behaviors indicated that intromission frequency was significantly higher among  
232 sexually experienced males than virgins ( $t_{10}=3.95$ ,  $P=0.003$ ), however differences between  
233 sexually experienced and virgin males in frequency of mounting ( $t_{10}=2.19$ ,  $P=0.054$ ) or  
234 frequency of ejaculation ( $t_{10}=1.95$ ,  $P=0.080$ ) did not reach statistical significance. Initial  
235 olfactory investigation of females was markedly reduced in sexually experienced males, who  
236 engaged in significantly less anogenital sniffing of the female before their first mount ( $t_{6.03}=-$   
237 4.51,  $P=0.004$ ). The difference in duration of intromission relative to mounting was also

238 significantly affected by sexual experience, with longer duration of intromission relative to  
239 mounting among sexually experienced males than virgin males ( $t_{10}=4.24$ ,  $P=0.002$ ).

#### 240 *AR and ER $\alpha$ cell counts*

241 Both AR and ER $\alpha$  staining was clearly visible in the regions selected for their importance in  
242 male reproductive behavior. The effects of sexual experience on AR and ER $\alpha$  expression  
243 (n=6 males/group) were not uniform but area and receptor type specific (**Figure 3**). There  
244 was no effect of sexual experience on the number of stained AR cells in either the BNST  
245 ( $t_{10}=1.22$ ,  $P=0.272$ ) or the pMeAmg ( $t_{10}=0.225$ ,  $P=0.827$ ). However, sexually experienced  
246 males had significantly more stained AR cells than virgins in the MPOA ( $t_{10}=2.57$ ,  $P=0.028$ ).  
247 There was no effect of sexual experience on the number of ER $\alpha$  cells counted in any of the  
248 areas of interest, with no significant difference between the sexually experienced and virgin  
249 males in either the BNST ( $t_{10}=-0.516$ ,  $P=0.617$ ), the pMeAmg ( $t_{10}=1.762$ ,  $P=0.108$ ) or the  
250 MPOA ( $t_{10}=0.755$ ,  $P=0.468$ ).

#### 251 **Discussion**

252 Sexual experience resulted in robust changes in reproductive behavior in male mice  
253 and increased numbers of AR-positive neurons in the MPOA, the primary regulatory nucleus  
254 in male reproductive behavior. Mirroring findings in other rodents (Dewsbury, 1969;  
255 Larsson, 1959), sexually experienced male mice had shorter latencies to mount and intromit,  
256 had higher frequencies of intromission, and spent significantly longer engaged in  
257 intromission behavior than mounting. These are interesting results as intromission and  
258 mounting have been shown to have qualitatively different properties from the perspective of  
259 both the mating male and female. Mating-mediated conditioning studies have shown that  
260 while ejaculation is the most effective sexual stimulus for conditioning (Pfaus et al., 2001),  
261 intromission is also intrinsically rewarding and is a sufficiently strong stimulus to produce

262 spatial preferences in B6 male mice (Kudwa et al., 2005). Intromission without ejaculation  
263 results in increased motivation to investigate females, while mounting alone has little effect  
264 (Whalen, 1961), suggesting that intromission is also more rewarding than mounting alone.  
265 Intromission may have been reinforced during previous matings in the sexually experienced  
266 males, leading to longer durations of intromission relative to unrewarding mounting behavior.  
267 Alternatively, sexually experienced males may be more sexually “competent” and so able to  
268 achieve intromission more easily, an idea that is supported by research showing that  
269 inappropriate mounting behavior is reduced in sexually experienced male mice (McGill,  
270 1962a).

271         In addition to the proposed mechanistic explanations for the change in intromissive  
272 behavior, there is also evidence that such a change might have adaptive consequences for  
273 mating males. Intromission, but not mounting, has been shown to result in an increase in NAc  
274 dopamine in mated female hamsters (Kohlert and Meisel, 1999), and extensive mounting  
275 without intromission results in reduced lordosis in female rats (Hardy and Debold, 1971).  
276 This suggests that intromission is a rewarding component of mating for females and that such  
277 stimulation by the male may be important for maintenance of appropriate female behavior  
278 during copulation. Moreover, intromission has also been shown to positively affect female  
279 reproductive physiology. Female rats are both more likely to conceive and less likely to  
280 continue estrous cyclicity after either intromission (Adler, 1969) or analogous artificial  
281 cervical stimulation (Terkel et al., 1990). Copulation with male rats that have high  
282 frequencies of intromission is also more likely to result in pregnancy (Wilson et al., 1965). In  
283 mice, it has been suggested that the pattern of intromission has a direct bearing on the  
284 likelihood of induction of pregnancy or pseudopregnancy (Diamond, 1970). Sexually  
285 experienced males that exhibit higher intromission frequencies and longer total duration of

286 intromission may thus be more likely to successfully impregnate females and so produce  
287 offspring. Sexually experienced male mice have been reported to have higher fecundity than  
288 virgin males (Rastogi et al., 1981), indicating that sexual experience may indeed have such  
289 adaptive consequences for male mice.

290         In contrast to the changes in mounting and intromission, we saw no effect of sexual  
291 experience on either the latency or frequency of ejaculation. This absence of an effect on  
292 ejaculation behavior appears surprising given the fact that both latency to ejaculate and  
293 frequency of ejaculation are changed by sexual experience in rats (Sura et al., 2001).  
294 However the species differences in sexual behavior patterns are important in this regard.  
295 While rats ejaculate repeatedly during a single mating bout, mice lose sexual drive for at least  
296 24 hours after a single ejaculation (McGill, 1962b). None of the B6 mice we tested ejaculated  
297 more than once and some males failed to ejaculate during the hour-long tests despite repeated  
298 mounting and intromission with females. Tests of longer duration, such as the 10 hour tests  
299 employed by Raskin et al (2009), might have improved our data on ejaculation latency and  
300 shown differences between sexually experienced and virgin males. The ejaculation frequency  
301 data for each individual was thus limited to a maximum value of 1, resulting in a narrower  
302 range of values than would be obtained in analogous tests with rats, where at test an  
303 individual is likely to ejaculate repeatedly and variation in ejaculation frequency can be more  
304 easily measured.

305         We also found that sexually experienced males spent significantly less time engaged  
306 in olfactory investigation of stimulus females before initiating sexual behavior. Sexually  
307 experienced males have been shown to spend longer investigating female odor cues  
308 (Hosokawa and Chiba, 2005; Swaney et al., 2007), however these studies involved exposing  
309 males to the urine or volatile odors of females, rather than direct interaction with a stimulus

310 female. Our data suggest that the sexually experienced males required less olfactory and/or  
311 vomeronasal stimulation to initiate sexual behavior. This may be due to more rapid arousal of  
312 neuroendocrine responses to female odors or to more rapid recognition of females as  
313 potential mates.

314 Behavioral changes as a function of sexual experience were associated with increased  
315 numbers of AR-positive cells in the MPOA of sexually experienced males, however we  
316 found no effect of sexual experience on numbers of AR-positive cells in the BNST or  
317 pMeAmg, nor did we see an effect on numbers of ER $\alpha$ -positive cells. This suggests that  
318 sexual experience produces a long-term increase in testosterone sensitivity in the MPOA, the  
319 key nucleus in the regulation of sexual behavior. While both AR and ER $\alpha$  are involved in  
320 reproductive behaviors in male rodents, previous studies indicate that actions of testosterone  
321 *via* AR have a more important role in male sexual behavior. Sexual behavior is restored after  
322 castration by administration of testosterone, but not by estradiol or dihydrotestosterone,  
323 (McGinnis and Dreifuss, 1989). These actions of testosterone after castration are blocked by  
324 AR-antagonists, and while anti-estrogens prevent the testosterone-induced restoration of  
325 social behaviors, they not block the restoration of sexual behavior by testosterone (Vagell and  
326 McGinnis, 1998). The effects of testosterone on sexual behavior appear thus to be primarily  
327 mediated through AR, and our data suggest that the behavioral effects of sexual experience  
328 may be due to some degree to an increase in AR levels within the MPOA. Variation in AR  
329 expression in the male sexual brain has been extensively studied in the context of sexual  
330 satiety, and decreased AR in the MPOA is associated with a lack of sexual drive in sexually  
331 satiated males (Romano-Torres et al., 2007). These studies have shown that AR levels in the  
332 male sexual brain are dynamic and vary with sexual interest. Our data suggest that while



333 recent sexual activity reduces AR in the MPOA, the acquisition of sexual experience  
334 produces a sustained increase in MPOA AR.

335         One caveat to our results is that although we found an increase in MPOA AR in  
336 sexually experienced male mice, a recent study with sexually experienced and virgin male  
337 rats found no difference in AR or ER $\alpha$  levels (Wu and Gore, 2009). This may be due to a  
338 species difference in the mechanisms by which sexual experience affects reproductive  
339 behavior, however it may also be a function of subtle differences in experimental design.  
340 Sexual satiety results in reduced AR in the MPOA, however even a single ejaculation is  
341 sufficient to reduce MPOA AR expression in male rats 24 hours later (Fernandez-Guasti et  
342 al., 2010). Male rat subjects in the Wu and Gore (2009) study were mated every other day for  
343 a month before being sacrificed for immunohistochemistry 24 hours after the final mating  
344 test. It may be that the temporal proximity of ejaculation to the time of AR detection affected  
345 levels of AR, potentially masking any long-term effect of sexual experience on MPOA AR  
346 levels in these male rats.

347         Mating is associated with increases in circulating testosterone, and testosterone has  
348 been shown to upregulate expression of AR (Meek et al., 1997; Wu and Gore, 2010). During  
349 the acquisition of sexual experience, the males in our study mated repeatedly over a three  
350 week period and would thus have experienced repeated increases in levels of testosterone  
351 which may be a mechanism by which levels of AR in the MPOA could be modulated. An  
352 increase in AR could increase the capacity of the MPOA to respond to increased testosterone  
353 signaling after sexual experience, as both basal and mating-associated testosterone levels are  
354 elevated in sexually experienced males (Bonilla-Jaime et al., 2006; Edinger and Frye, 2007).  
355 Increased sensitivity to testosterone in the MPOA also has potential ramifications for mating-  
356 associated dopaminergic activity in the MPOA. Testosterone mediates female-elicited release

357 of dopamine in the MPOA, which is necessary for successful copulation (Hull et al., 2003).  
358 This dopamine release is regulated by nitric oxide synthase, which is itself regulated by  
359 testosterone levels (Du and Hull, 1999; Sato et al., 2005). Increased sensitivity to testosterone  
360 *via* MPOA AR might play a role in nitric oxide synthase-mediated local dopamine release.  
361 Indeed, levels of nitric oxide synthase in the MPOA have been shown to be elevated in  
362 sexually experienced male rats (Dominguez et al., 2006), indicating that this circuit is also  
363 sensitive to sexual experience and potentially pointing to an interaction between gonadal  
364 steroids and dopamine within the MPOA. Elucidating these interactions would provide  
365 insight into the mechanisms of plasticity in the male brain that are recruited during  
366 reproductive experience.

367

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372

373 **References**

- 374 Adler, N.T., 1969. Effects of the male's copulatory behavior on successful pregnancy of the  
375 female rat. *J. Comp. Physiol. Psychol.* 69, 613-22.
- 376 Balfour, M.E., Yu, L., Coolen, L.M., 2004. Sexual behavior and sex-associated  
377 environmental cues activate the mesolimbic system in male rats.  
378 *Neuropsychopharmacology.* 29, 718-30.
- 379 Batty, J., 1978. Acute changes in plasma testosterone levels and their relation to measures of  
380 sexual behaviour in the male house mouse (*Mus musculus*). *Anim. Behav.* 26, 349-  
381 357.
- 382 Bean, N.Y., Nunez, A.A., Conner, R., 1981. Effects of medial preoptic lesions on male  
383 mouse ultrasonic vocalizations and copulatory behavior. *Brain Res. Bull.* 6, 109-12.
- 384 Bonilla-Jaime, H., Vazquez-Palacios, G., Arteaga-Silva, M., Retana-Marquez, S., 2006.  
385 Hormonal responses to different sexually related conditions in male rats. *Horm.*  
386 *Behav.* 49, 376-82.
- 387 Coopersmith, C.B., Huck, U.W., Conant-Huck, L., Banks, E.M., 1986. Effects of pre- and  
388 postpubertal social experience on sexual and social behavior in male and female  
389 brown lemmings (*Lemmus sibiricus*). *J. Comp. Psychol.* 100, 406-412.
- 390 Costantini, R.M., Park, J.H., Beery, A.K., Paul, M.J., Ko, J.J., Zucker, I., 2007. Post-  
391 castration retention of reproductive behavior and olfactory preferences in male  
392 Siberian hamsters: Role of prior experience. *Horm. Behav.* 51, 149-155.
- 393 de Jonge, F.H., Louwerse, A.L., Ooms, M.P., Evers, P., Endert, E., van de Poll, N.E., 1989.  
394 Lesions of the SDN-POA inhibit sexual behavior of male wistar rats. *Brain Res. Bull.*  
395 23, 483-492.

396 Dewsbury, D.A., 1969. Copulatory behaviour of rats (*Rattus norvegicus*) as a function of  
397 prior copulatory experience. *Anim. Behav.* 17, 217-23.

398 Diamond, M., 1970. Intromission pattern and species vaginal code in relation to induction of  
399 pseudopregnancy. *Science.* 169, 995-997.

400 Dominguez, J.M., Brann, J.H., Gil, M., Hull, E.M., 2006. Sexual experience increases nitric  
401 oxide synthase in the medial preoptic area of male rats. *Behav. Neurosci.* 120, 1389-  
402 1394.

403 Du, J. and Hull, E.M., 1999. Effects of testosterone on neuronal nitric oxide synthase and  
404 tyrosine hydroxylase. *Brain Res.* 836, 90-98.

405 Edinger, K.L. and Frye, C.A., 2007. Sexual experience of male rats influences anxiety-like  
406 behavior and androgen levels. *Physiol. Behav.* 92, 443-453.

407 Edwards, D.A. and Burge, K.G., 1973. Olfactory control of the sexual behavior of male and  
408 female mice. *Physiol. Behav.* 11, 867-872.

409 Fernandez-Guasti, A., Arteaga-Lopez, P., Antonio-Cabrera, E., 2010. Copulation modifies  
410 AR and ERα mRNA expression in the male rat brain. *Physiol. Behav.* 101, 738-45.

411 Fernandez-Guasti, A., Swaab, D., Rodriguez-Manzo, G., 2003. Sexual behavior reduces  
412 hypothalamic androgen receptor immunoreactivity. *Psychoneuroendocrinology.* 28,  
413 501-12.

414 Fewell, G.D. and Meredith, M., 2002. Experience facilitates vomeronasal and olfactory  
415 influence on Fos expression in medial preoptic area during pheromone exposure or  
416 mating in male hamsters. *Brain Res.* 941, 91-106.

417 Fuxjager, M. J., Forbes-Lorman, R. M., Coss, D. J., Auger, C. J., Auger, A. P., and Marler, C.  
418 A. (2010) Winning territorial disputes selectively enhances androgen sensitivity in  
419 neural pathways related to motivation and social aggression. *Proc. Natl. Acad. Sci.*  
420 U.S.A. 107, 12393-12398

421 Gleason, E.D., Fuxjager, M.J., Oyegbile, T.O., Marler, C.A., 2009. Testosterone release and  
422 social context: when it occurs and why. *Front. Neuroendocrinol.* 30, 460-9.

423 Harding, S.M. and McGinnis, M.Y., 2004. Androgen receptor blockade in the MPOA or  
424 VMN: effects on male sociosexual behaviors. *Physiol. Behav.* 81, 671-80.

425 Hardy, D.F. and Debold, J.F., 1971. Effects of mounts without intromission upon the  
426 behavior of female rats during the onset of estrogen-induced heat. *Physiol. Behav.* 7,  
427 643-645.

428 Hayashi, S. and Kimura, T., 1974. Sex-attractant emitted by female mice. *Physiol. Behav.* 13,  
429 563-567.

430 Hosokawa, N. and Chiba, A., 2005. Effects of sexual experience on conspecific odor  
431 preference and estrous odor-induced activation of the vomeronasal projection  
432 pathway and the nucleus accumbens in male rats. *Brain Res.* 1066, 101-8.

433 Hull, E.A., Putnam, S.K., Sato, S., 2003. Effects of testosterone metabolites on copulation  
434 and medial preoptic dopamine release in castrated male rats. *Horm. Behav.* 44, 419-  
435 426.

436 Hull, E.M. and Dominguez, J.M., 2006. Getting his act together: roles of glutamate, nitric  
437 oxide, and dopamine in the medial preoptic area. *Brain Res.* 1126, 66-75.

438 Hull, E.M., Meisel, R.L., Sachs, B.D., Male sexual behavior. In: D. W. Pfaff, et al., (Eds.),  
439 Hormones, Brain and Behavior. Academic Press, Amsterdam, 2002, pp. 1-137.

440 Kamel, F., Mock, E.J., Wright, W.W., Frankel, A.I., 1975. Alterations in plasma  
441 concentrations of testosterone, LH, and prolactin associated with mating in the male  
442 rat. *Horm. Behav.* 6, 277-288.

443 Keller, M., Douhard, Q., Baum, M.J., Bakker, J., 2006. Sexual experience does not  
444 compensate for the disruptive effects of zinc sulfate-lesioning of the main olfactory  
445 epithelium on sexual behavior in male mice. *Chem. Senses.* 31, 753-762.

446 Keverne, E.B., 2004. Importance of olfactory and vomeronasal systems for male sexual  
447 function. *Physiol. Behav.* 83, 177-87.

448 Kohlert, J.G. and Meisel, R.L., 1999. Sexual experience sensitizes mating-related nucleus  
449 accumbens dopamine responses of female Syrian hamsters. *Behav. Brain Res.* 99, 45-  
450 52.

451 Kudwa, A.E., Dominguez-Salazar, E., Cabrera, D.M., Sibley, D.R., Rissman, E.F., 2005.  
452 Dopamine D5 receptor modulates male and female sexual behavior in mice.  
453 *Psychopharmacology.* 180, 206-14.

454 Larsson, K., 1959. Experience and maturation in the development of sexual behaviour in  
455 male puberty rat. *Behaviour.* 14, 101-107.

456 Lumley, L.A. and Hull, E.M., 1999. Effects of a D1 antagonist and of sexual experience on  
457 copulation-induced Fos-like immunoreactivity in the medial preoptic nucleus. *Brain*  
458 *Res.* 829, 55-68.

459 Mandiyan, V.S., Coats, J.K., Shah, N.M., 2005. Deficits in sexual and aggressive behaviors  
460 in *Cnga2* mutant mice. *Nat. Neurosci.* 8, 1660-2.

461 Manning, A. and Thompson, M.L., 1976. Postcastration retention of sexual behaviour in the  
462 male BDF1 mouse: the role of experience. *Anim. Behav.* 24, 523-33.

463 McGill, T.E., 1962a. Reduction in "head-mounts" in the sexual behavior of the mouse as a  
464 function of experience. *Psychol. Rep.* 10, 284-284.

465 McGill, T.E., 1962b. Sexual behavior in three inbred strains of mice. *Behaviour.* 19, 341-350.

466 McGinnis, M.Y. and Dreifuss, R.M., 1989. Evidence for a role of testosterone-androgen  
467 receptor interactions in mediating masculine sexual behavior in male rats.  
468 *Endocrinology.* 124, 618-26.

469 McGinnis, M.Y., Williams, G.W., Lumia, A.R., 1996. Inhibition of male sex behavior by  
470 androgen receptor blockade in preoptic area or hypothalamus, but not amygdala or  
471 septum. *Physiol. Behav.* 60, 783-9.

472 Meek, L.R., Romeo, R.D., Novak, C.M., Sisk, C.L., 1997. Actions of Testosterone in  
473 Prepubertal and Postpubertal Male Hamsters: Dissociation of Effects on Reproductive  
474 Behavior and Brain Androgen Receptor Immunoreactivity. *Horm. Behav.* 31, 75-88.

475 Meredith, M., 1986. Vomeronasal organ removal before sexual experience impairs male  
476 hamster mating behavior. *Physiol. Behav.* 36, 737-43.

477 Morin, L.P., 1977. Progesterone: Inhibition of rodent sexual behavior. *Physiol. Behav.* 18,  
478 701-715.

479 Paxinos, G. and Franklin, K.B.J., 2001. *The Mouse Brain in Stereotaxic Coordinates.*  
480 Academic Press, San Diego.

481 Pfau, J.G., Kippin, T.E., Centeno, S., 2001. Conditioning and sexual behavior: a review.  
482 *Horm. Behav.* 40, 291-321.

483 Pfeiffer, C.A. and Johnston, R.E., 1994. Hormonal and behavioral responses of male  
484 hamsters to females and female odors: roles of olfaction, the vomeronasal system, and  
485 sexual experience. *Physiol. Behav.* 55, 129-38.

486 Phillips-Farfan, B.V. and Fernandez-Guasti, A., 2009. Endocrine, neural and  
487 pharmacological aspects of sexual satiety in male rats. *Neurosci. Biobehav. Rev.* 33,  
488 442-55.

489 Phillips-Farfan, B.V., Lemus, A.E., Fernandez-Guasti, A., 2007. Increased estrogen receptor  
490 alpha immunoreactivity in the forebrain of sexually satiated rats. *Horm. Behav.* 51,  
491 328-34.

492 Powers, J.B., Newman, S.W., Bergondy, M.L., 1987. MPOA and BNST lesions in male  
493 Syrian hamsters: differential effects on copulatory and chemoinvestigatory behaviors.  
494 Behav. Brain Res. 23, 181-95.

495 Powers, J.B. and Winans, S.S., 1975. Vomeronasal organ: critical role in mediating sexual  
496 behavior of the male hamster. Science. 187, 961-3.

497 Raskin, K., de Gendt, K., Duittoz, A., Liere, P., Verhoeven, G., Tronche, F., Mhaouty-Kodja,  
498 S., 2009. Conditional inactivation of androgen receptor gene in the nervous system:  
499 effects on male behavioral and neuroendocrine responses. J. Neurosci. 29, 4461-4470.

500 Rastogi, R.K., Milone, M., Chieffi, G., 1981. Impact of socio-sexual conditions on the  
501 epididymis and fertility in the male mouse. J. Reprod. Fertil. 63, 331-4.

502 Robertson, G.S., Pfaus, J.G., Atkinson, L.J., Matsumura, H., Phillips, A.G., Fibiger, H.C.,  
503 1991. Sexual behavior increases c-fos expression in the forebrain of the male rat.  
504 Brain Res. 564, 352-7.

505 Romano-Torres, M., Phillips-Farfan, B.V., Chavira, R., Rodriguez-Manzo, G., Fernandez-  
506 Guasti, A., 2007. Relationship between sexual satiety and brain androgen receptors.  
507 Neuroendocrinology. 85, 16-26.

508 Sato, S., Braham, C.S., Putnam, S.K., Hull, E.M., 2005. Neuronal nitric oxide synthase and  
509 gonadal steroid interaction in the MPOA of male rats: Co-localization and  
510 testosterone-induced restoration of copulation and nNOS-immunoreactivity. Brain  
511 Res. 1043, 205-213.

512 Simerly, R.B., Chang, C., Muramatsu, M., Swanson, L.W., 1990. Distribution of androgen  
513 and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization  
514 study. J. Comp. Neurol. 294, 76-95.

515 Sipos, M.L., Kerchner, M., Nyby, J.G., 1992. An ephemeral sex pheromone in the urine of  
516 female house mice (*Mus domesticus*). Behav. Neural Biol. 58, 138-43.



517 Steel, E. and Keverne, E.B., 1985. Effect of female odour on male hamsters mediated by the  
518 vomeronasal organ. *Physiol. Behav.* 35, 195-200.

519 Sura, A., Overstreet, D.H., Marson, L., 2001. Selectively bred male rat lines differ in naive  
520 and experienced sexual behavior. *Physiol. Behav.* 72, 13-20.

521 Swaney, W.T., Curley, J.P., Champagne, F.A., Keverne, E.B., 2007. Genomic imprinting  
522 mediates sexual experience-dependent olfactory learning in male mice. *Proc. Natl.*  
523 *Acad. Sci. U.S.A.* 104, 6084-9.

524 Terkel, J., Witcher, J.A., Adler, N.T., 1990. Evidence for "memory" of cervical stimulation  
525 for the promotion of pregnancy in rats. *Horm. Behav.* 24, 40-49.

526 Vagell, M.E. and McGinnis, M.Y., 1998. The role of gonadal steroid receptor activation in  
527 the restoration of sociosexual behavior in adult male rats. *Horm. Behav.* 33, 163-179.

528 Valenstein, E.S., Riss, W., Young, W.C., 1955. Experiential and genetic factors in the  
529 organization of sexual behavior in male guinea pigs. *J. Comp. Physiol. Psychol.* 48,  
530 397-403.

531 Wersinger, S.R., Sannen, K., Villalba, C., Lubahn, D.B., Rissman, E.F., De Vries, G.J., 1997.  
532 Masculine sexual behavior is disrupted in male and female mice lacking a functional  
533 estrogen receptor alpha gene. *Horm. Behav.* 32, 176-83.

534 Whalen, R.E., 1961. Effects of mounting without intromission and intromission without  
535 ejaculation on sexual behavior and maze learning. *J. Comp. Physiol. Psychol.* 54, 409-  
536 &.

537 Wilson, J.R., Adler, N., Le Boeuf, B., 1965. The effects of intromission frequency on  
538 successful pregnancy in the female rat. *Proc. Natl. Acad. Sci. U.S.A.* 53, 1392-5.

539 Wood, R.I. and Newman, S.W., 1993. Mating activates androgen receptor-containing neurons  
540 in chemosensory pathways of the male Syrian hamster brain. *Brain Res.* 614, 65-77.

541 Wu, D. and Gore, A.C., 2009. Sexual experience changes sex hormones but not hypothalamic  
542 steroid hormone receptor expression in young and middle-aged male rats. *Horm.*  
543 *Behav.* 56, 299-308.

544 Wu, D. and Gore, A.C., 2010. Changes in androgen receptor, estrogen receptor alpha, and  
545 sexual behavior with aging and testosterone in male rats. *Horm. Behav.* 58, 306-16.

546

547

548 **Figure Captions**

549 Figure 1. Summary of experimental design indicating the treatment of virgin vs. sexually  
550 experienced males

551 Figure 2. Measures of sexual behaviour of virgin (n=6) and sexually experienced (n=6) male  
552 B6 mice in hour long mating tests with sexually receptive B6 female virgins. All values are  
553 means  $\pm$  S.E.M. Asterisks signify  $P < 0.05$ . A) mean latency in minutes of mounting,  
554 intromission and ejaculation; B) mean frequency per minute of mounting, intromission and  
555 ejaculation; C) comparison of mean duration of mounting and intromission; D) mean duration  
556 in seconds of sniffing of the female prior to the male's first mounting attempt.

557 Figure 3. Counts of A) AR- and B) ER $\alpha$ -positive cells in the BNST, MPOA and pMeAmg in  
558 virgin (n=6) and sexually experienced (n=6) male B6 mice. C) Representative  
559 photomicrographs indicating density of AR- and ER $\alpha$ -positive cells in the MPOA of virgin  
560 and sexually-experienced males. All values are means  $\pm$  S.E.M. Asterisks signify  $P < 0.05$ .

**Figure 1**

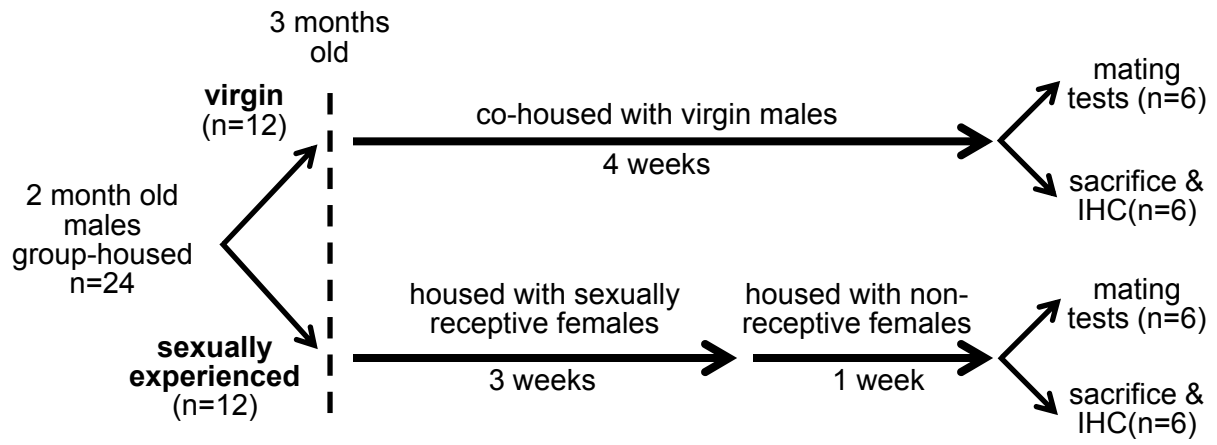
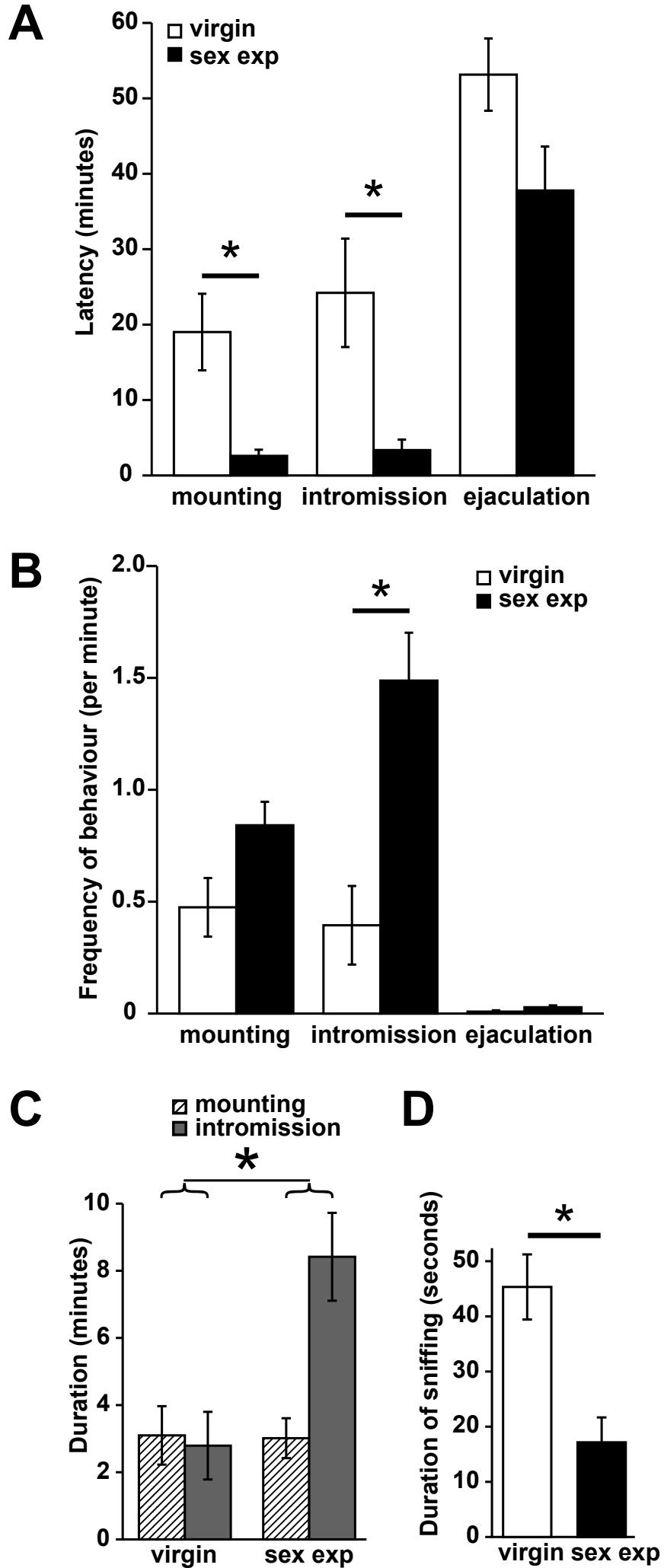


Figure 2



# Figure 3

