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Effects of defeat stress on behavioral flexibility in males and females: modulation by the mu-opioid receptor

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Abstract

Behavioral flexibility is a component of executive functioning that allows individuals to adapt to changing environmental conditions. Independent lines of research indicate that the mu opioid receptor (MOR) is an important mediator of behavioral flexibility and responses to psychosocial stress. The current study bridges these two lines of research and tests the extent to which social defeat and MOR affect behavioral flexibility and whether sex moderates these effects in California mice (*Peromyscus californicus*). Males and females assigned to social defeat or control conditions were tested in a Barnes maze. In males, defeat impaired behavioral flexibility but not acquisition. Female performance was unaffected by defeat. MOR binding in defeated and control mice in the orbitofrontal cortex (OFC), striatum and hippocampus was examined via autoradiography. Stressed males had reduced MOR binding in the OFC whereas females were unaffected. The MOR antagonist beta-funaltrexamine (1 mg/kg) impaired performance in males naïve to defeat during the reversal phase but had no effect on females. Finally, we examined the effects of the MOR agonist morphine (2.5 and 5 mg/kg) on stressed mice. As expected, morphine improved behavioral flexibility in stressed males. The stress-induced deficits in behavioral flexibility in males are consistent with a proactive coping strategy, including previous observations that stressed male California mice exhibit strong social approach and aggression. Our pharmacological data suggest that a down-regulation of MOR signaling in males may contribute to sex differences in behavioral flexibility following stress. This is discussed in the framework of coping strategies for individuals with mood disorders.

Introduction

Behavioral flexibility is a fundamental process that allows an individual to adapt to changing environmental conditions and can be associated with spatial and/or social strategies that are employed within a given ecological niche (Bond *et al.*, 2007; Tebbich *et al.*, 2010). Coping strategies during times of rapid change can be traced back to an individual's propensity to demonstrate behavioral flexibility under environmental stress (Coppens *et al.*, 2010). Koolhaas *et al.* (1999) have identified two types of coping strategies that are employed as a result of environmental change. Proactive individuals are relatively inflexible and continue a rigid routine despite disturbances, whereas reactive individuals demonstrate more flexibility and respond to environmental changes more readily. Interestingly, behavioral flexibility is frequently impaired in individuals diagnosed with stress-induced mental illnesses such as anxiety or depression (Grant *et al.*, 2001; Dickstein *et al.*, 2010). Animal models show that chronic psychosocial stress can induce deficits in several tests of behavioral flexibility (Liston *et al.*, 2006; Bondi *et al.*, 2008; Danet *et al.*, 2010). Furthermore, prenatal stress induces anatomical differences between males and females in cortical areas associated with behavioral flexibility (Murmu *et al.*, 2006), suggesting that flexibility may be contingent upon both stress and sex.

The mu-opioid receptor (MOR) is abundantly expressed in the OFC and dorsal striatum (Mansour *et al.*, 1995), which are important regions that control performance in tasks assessing behavioral flexibility (Boulougouris *et al.*, 2007; Castane *et al.*, 2010). Psychosocial stress can affect MOR expression and trafficking, often in a sex-dependent manner (Nikulina *et al.*, 1999; Gonzales *et al.*, 2011; Milner *et al.*, 2013). Although the effects of stress on MOR function in the OFC have not been studied, activation of MOR improves behavioral flexibility in males (Olson *et al.*, 1979). Hence we hypothesised that, in males, stress would impair behavioral flexibility (consistent with Liston *et al.*, 2006; Bondi *et al.*, 2008; Danet *et al.*, 2010) and downregulate MOR in brain areas associated with behavioral flexibility. Alternatively, in females we expected that stress would not change flexible performance due to the finding that prenatal stress did not alter female OFC dendritic spine density (Murmu *et al.*, 2006). We used the Barnes maze to examine the effects of defeat stress and MOR activity on behavioral flexibility in male and female California mice

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(*Peromyscus californicus*). Male and female California mice aggressively defend territories under natural conditions, which allows for the study of defeat stress in females (Trainor *et al.*, 2011, 2013). An important gap in this literature is that the vast majority of studies have been conducted on males. Many effects of psychosocial stress are sex-dependent (Goel & Bale, 2009), suggesting that there could be important sex differences in how stress impacts behavioral flexibility. In our previous studies of California mice, we found that the long-term effects of defeat stress are sex-specific and generally consistent with reactive and proactive coping strategies described by Koolhaas *et al.* (1999). In females, the effects of social defeat were more consistent with reactive coping strategies such as social withdrawal (Greenberg *et al.*, 2014) and reduced aggression (Steinman *et al.*, 2015). Behaviors in stressed males were more consistent with proactive coping strategies such as social approach and aggression. In other rodent species and domesticated animals, proactive coping strategies are associated with behavioral inflexibility (Koolhaas *et al.*, 1999). We thus hypothesised that social defeat would impair behavioral flexibility to a greater extent in males.

Materials and methods

Experiment 1: effects of social defeat stress on acquisition and behavioral flexibility

Male and female California mice were randomly assigned to three episodes of social defeat or control handling. Mice assigned to defeat were placed in the cage of an aggressive, same-sex breeder California mouse as previously described (Trainor *et al.*, 2013). This protocol exposes males and females to similar levels of aggression by residents and induces rapid short-term increases in corticosterone in females but not males (Trainor *et al.*, 2013). In contrast, males but not females have elevated levels of baseline corticosterone 2–4 weeks after defeat (Trainor *et al.*, 2011). Mice assigned to control handling were placed in empty cages for 10 min, and then placed back in their home cages with their original cagemates. Four weeks following social defeat or control conditions, mice were tested using the extended Barnes maze protocol (Fig. 1; see 'Barnes maze' methods below).

Experiment 2: MOR autoradiography

In previous studies the effects of social defeat on social interaction behavior at 2 weeks following the last episode of defeat (Trainor *et al.*, 2013) were equivalent to the effects of defeat at 4 weeks after defeat (Trainor *et al.*, 2011). This suggests that long-term changes in brain and behavior detectable at 2 weeks after defeat remain at 4 weeks. Mice were randomly assigned to social defeat or control conditions and then euthanized with isoflurane and decapitated 2 weeks later (Fig. 1). Brains were flash-frozen for autoradiography analysis to assess MOR binding. Sections were cut at 20 μm on a cryostat and mounted on Superfrost Plus Slides (Fisher Scientific, Pittsburgh, PA, USA), and stored at −40 °C. Slides were washed in Tris buffer (in mM: Tris-HCl, 50; NaCl, 120; KCl, 5; pH 7.4). To determine MOR-specific binding, slides were incubated for 1 h at room temperature in a 4.0 nM [^{3}H]DAMGO (PerkinElmer, Waltham, MA, USA) solution containing 400 nM unlabeled U69,593 (Sigma-Aldrich, St Louis, MO, USA), 400 nM unlabeled DPDPE (Tocris Bioscience, Minneapolis, MN, USA), and Tris buffer. To assess nonspecific binding, a separate set of slides were incubated in a 4.0 nM [^{3}H]DAMGO solution and 10 μM naloxone (Tocris Bioscience). All slides were then washed in 4 °C Tris buffer and then ice-cold water. Slides were dried and then exposed to Amersham Hyperfilm MP film for 5 months. Films were developed with Kodak GBX Developer and Fixer. Regions of interest were quantified as previously described (Bales *et al.*, 2007) utilizing a Rodbard curve. Optical densities for MOR binding in the lateral OFC (lOFC), nucleus accumbens (NAc) core and shell, dorsal striatum (caudate–putamen; CPu) and hippocampal regions CA1, CA3 and the dentate gyrus were quantified using ImageJ (NIH, Bethesda, MD, USA).

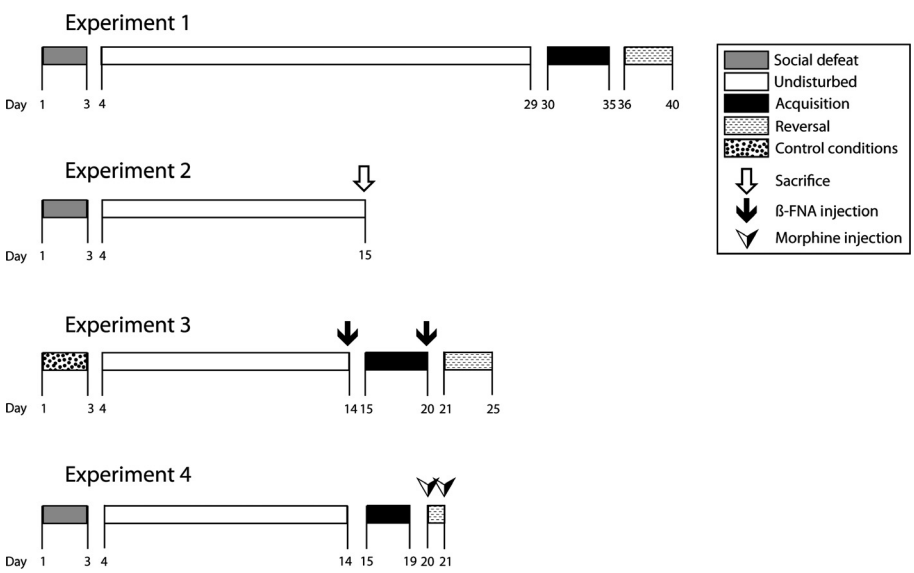

FIG. 1. Behavioral timelines for experiments 1–4 for all animals. Days are indicated with numbers at the bottom of each timeline. Social defeat is marked with gray bars, the periods during which mice were undisturbed following defeat or control conditions are marked with white bars, the acquisition phase of the Barnes maze is marked with black bars, the reversal phase of the Barnes maze is marked by dotted line bars, and control conditions are marked with a bar containing circles. Time of killing for experiment 2 is indicated with a white arrow, β-FNA injections during experiment 3 are marked with black arrows, and morphine injections during experiment 4 are marked with black and white arrows.

Experiment 3: effects of beta-funaltrexamine (β-FNA) on acquisition and behavioral flexibility

Based on the results from experiment 2, we conducted the following behavioral experiments 2 weeks following defeat because changes in MOR density were established following the 2-week time point. Male and female California mice were naïve to defeat and randomly assigned to receive an intraperitoneal (i.p.) injection with either β-FNA (1 mg/kg), an irreversible MOR antagonist (Liu-Chen & Phillips, 1987), or saline vehicle (Meilandt *et al.*, 2004). β-FNA is a long-acting drug that can last up to 18 days, losing affinity after day 9 (Martin *et al.*, 1995). β-FNA was administered once 24 h before acquisition testing, and once 24 h before reversal testing immediately following the last acquisition trial (Fig. 1). The second injection was made to (i) ensure that MOR would be blocked throughout the entire reversal phase and (ii) make the reversal phase consistent with the acquisition phase (one injection prior to day 1). We did not include stressed males and females in this study because previous studies indicated that MOR signaling increases behavioral flexibility. Thus, if β-FNA were administered to stressed males we would expect a null result because stress males demonstrated a deficit in reversal learning in experiment 1.

Experiment 4: effects of morphine on acquisition and behavioral flexibility

All male and female mice experienced social defeat, and were randomly assigned to receive a subcutaneous (s.c.) injection of 2.5 mg/kg morphine, 5.0 mg/kg morphine or saline vehicle (Farahmandfar *et al.*, 2010) 2 weeks following social defeat. As morphine can induce tolerance over repeated long-term use (Stafford *et al.*, 2001), mice in this experiment were run using the condensed Barnes maze protocol, and were injected 30 min before behavioral testing on days 4 and 5 (reversal trials). Thus mice in this study received only two injections (Fig. 1). Similar to experiment 3, we chose not to test the effects of morphine on control males and females. As we predicted that morphine would improve performance in the reversal phase, it is likely that control animals (which did not show a deficit) would show no significant changes following morphine treatment.

Animals

Male and female California mice were bred in a colony at UC Davis and housed two or three per cage in polypropylene cages on either Carefresh (Absorption Corp., Ferndale, WA, USA) or Sanichip (Harlan Teklad, Indianapolis, IN, USA) bedding. Food (2016 Harlan Teklad) and water were provided *ad libitum*. Animals were housed under long-day photoperiods (16 h light : 8 h dark). All procedures were approved by the University of California Davis Institutional Laboratory Animal Care and Use Committee, and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Barnes maze

Experiments 1 and 3 used an extended Barnes maze protocol (Steinman *et al.*, 2011). Testing occurred during the light phase between 08:00 and 13:00 hours Pacific Standard Time (PST). On day 1, each mouse was randomly assigned to a target hole under which was placed an escape container. Each mouse was then placed on the center of the maze and allowed to explore for 5 min. If the mouse did not enter the hole after 5 min, the experimenter guided it to the target hole. Each mouse was tested in one trial per day for a period of five consecutive days (acquisition days 1–5). Twenty-four hours after the last day of acquisition, each mouse was tested in one reversal trial per day for 4 days (days 6–9), which is a measure of behavioral flexibility. During reversal trials, the target hole was switched 180° across the maze platform. AnyMaze (Stoelting, Wood Dale, IL, USA) was used to record path length to reach the target hole, number of incorrect holes entered before reaching the target hole (number of errors), and number of entries into the former target hole during reversal. The experimenter was blind to all treatment groups during Barnes maze testing.

Experiment 4 followed a condensed Barnes maze protocol previously used on *Peromyscus* (Jasarevic *et al.*, 2012). In this protocol, each mouse was tested in two trials per day with a 20 min inter-trial interval (ITI) for three consecutive days (acquisition days 1–3). One day after the last day of acquisition, each mouse was tested in two trials of reversal per day (days 4 and 5, 20 min ITI).

Statistical analyses

Longitudinal mixed-model analyses were used to assess the rate of learning for latency to complete the maze, path length, and number of errors. This model was chosen to analyse the rate of learning because it estimates individual trajectories over time as well as differences in such trajectories across all subjects (Laird & Ware, 1982; Raudenbush & Bryk, 2002). In particular, we estimated intercepts (day 1 or day 6) and slopes (rate of learning), and allowed them to vary across individuals (McArdle & Anderson, 1990). We used repeated-measures (RM) ANOVA to test for main effects of stress and sex, and a sex × stress interaction on path length, number of errors, and number of entries into the former target hole between subjects. Path length was square root- or log-transformed and number of errors was log-transformed for ANOVA due to heterogeneity of variance. MOR receptor binding was analysed using univariate ANOVA for each region of interest and was log-transformed due to heterogeneity of variance.

TABLE 1. Rate of learning: performance across Barnes maze acquisition and reversal phases

	Acquisition		Reversal	
	t-value	*P*-value	*t*-value	*P*-value
Experiment 1				
Latency	−2.29	0.023*	−2.55	0.012*
Path length	−3.71	0.0003**	−2.15	0.033*
Errors	−3.42	0.0008**	−2.60	0.01*
Experiment 2				
Latency	−2.09	0.038*	−0.39	0.70
Path length	−0.65	0.52	0.20	0.84
Errors	−0.39	0.70	1.61	0.11
Experiment 3				
Latency	−3.12	0.0021**	−2.89	0.0046**
Path length	−5.45	<0.0001**	−1.99	0.0485**
Errors	−3.37	0.0009**	−2.64	0.0094**

Table representing the mixed effects model during acquisition and reversal for latency, path length, and number of errors. The table represents *t*- and *P*-values for the estimated slopes (rate of learning) across acquisition (days 1-5) and reversal (days 6-9) for all animals. *$P < 0.05$, **$P < 0.01$.

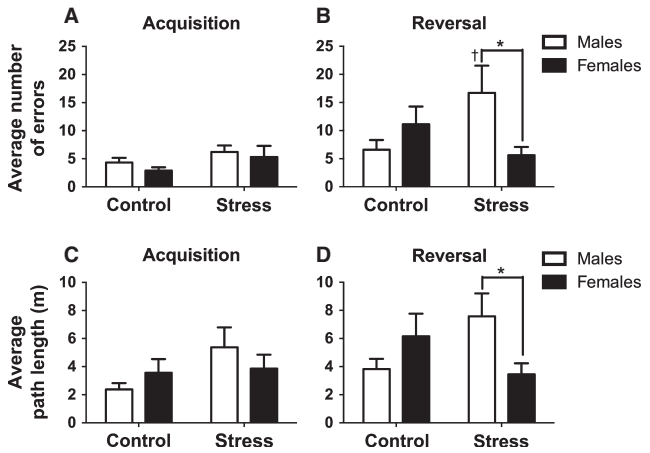

FIG. 2. (A and B) Bar graphs representing the average number of errors before finding the target hole in (A) acquisition and (B) reversal phases for control and socially defeated mice. (C and D) Bar graphs representing the average path length before finding the target hole in (C) acquisition and (D) reversal phases for control and socially defeated mice. White bars represent males and black bars represent females (n = 9–11 mice). *$P < 0.05$, between-sexes comparison; †$P < 0.05$, within-sex comparison.

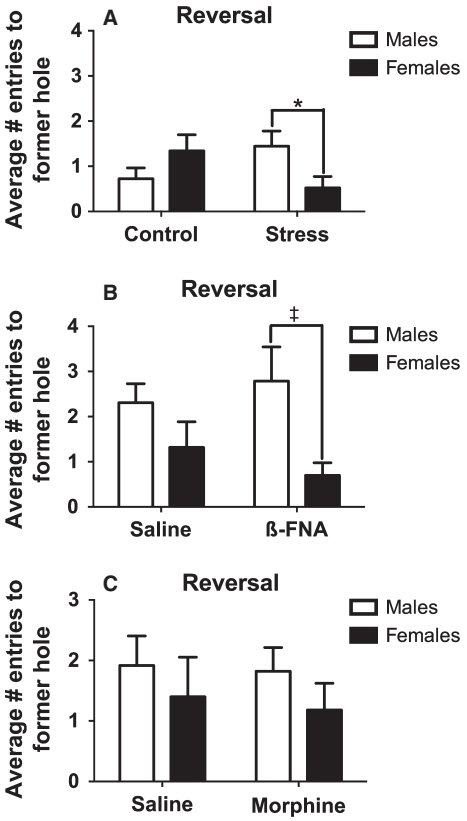

FIG. 3. Bar graphs representing the average number of entries into the former acquisition hole during the reversal phase of the Barnes maze in (A) control and socially defeated mice (n = 9–11 mice), (B) mice receiving saline or ß-FNA (n = 8–10 mice) and (C) mice receiving saline (n = 5–9 mice) or morphine (n = 11–14 mice). White bars represent males and black bars represent females. *$P < 0.05$; ‡$P = 0.05$.

Results

Experiment 1: effects of social defeat on acquisition and behavioral flexibility

Consistent with Jasarevic *et al.* (2012), both males and females improved performance across the acquisition phase with no sex differences in performance (Table 1). Both males and females also improved performance across reversal (Table 1). During reversal, there was a significant sex × stress interaction between subjects for number of errors (Fig. 2B; $F_{1,36} = 5.22$, $P < 0.05$) and path length (Fig. 2D; $F_{1,37} = 6.225$, $P < 0.05$). Stressed males made more errors on average as compared to control males ($F_{1,36} = 4.969$, $P < 0.05$) and stressed females ($F_{1,36} = 5.291$, $P < 0.05$). There was a nonsignificant trend towards a greater average path length to reach the target hole for stressed males as compared to control males ($F_{1,37} = 3.835$, $P = 0.058$). Stressed males demonstrated a significantly longer path length to reach the target hole as compared to stressed females ($F_{1,37} = 4.908$, $P < 0.05$). No treatment or group differences were detected with RM ANOVA in acquisition (Fig. 2A and C; all $P > 0.05$).

To determine whether mice showed perseveration during reversal trials, we analysed the number of entries into the former acquisition hole. There was a significant sex × stress interaction for the number of former target hole entries during reversal (Fig. 3A; $F_{1,37} = 6.45$, $P < 0.05$). Stressed males entered the former target hole more frequently than did stressed females ($F_{1,37} = 4.51$, $P < 0.05$). There was also a nonsignificant trend towards stressed females entering the former target hole less often than control females ($F_{1,37} = 3.95$, $P = 0.05$).

Experiment 2: MOR autoradiography

MOR binding was significantly reduced in the lOFC of stressed males as compared to males experiencing control conditions (Fig. 4; $P < 0.05$), while no differences were detected in females ($P > 0.05$). No differences were observed in the NAc core or shell, CPu or hippocampus (all $P > 0.05$).

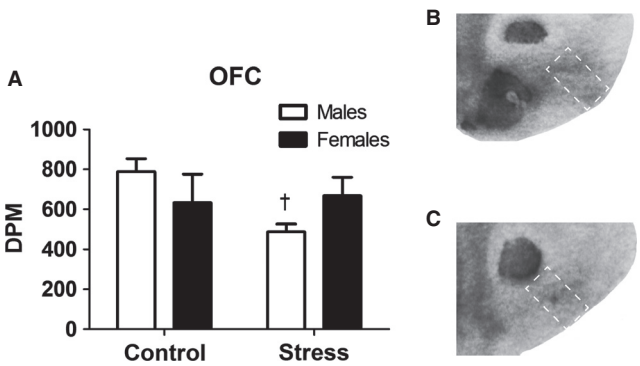

FIG. 4. (A) Bar graph representing the disintegrations per minute (DPM) for MOR binding in the lOFC in socially defeated or control mice. White bars represent males and black bars represent females (n = 4 or 5 mice). (B) A representative image of the lOFC in a control male depicting MOR binding following the autoradiography assay. (C) A representative image of the lOFC in a defeated male depicting MOR binding following the autoradiography assay. White dotted boxes represent the region of interest (lOFC). †$P < 0.05$, within-sex comparison.

Experiment 3: effects of β-FNA on acquisition and behavioral flexibility

All experimental groups improved performance over the course of the acquisition phase, but not reversal (Table 1). During reversal,

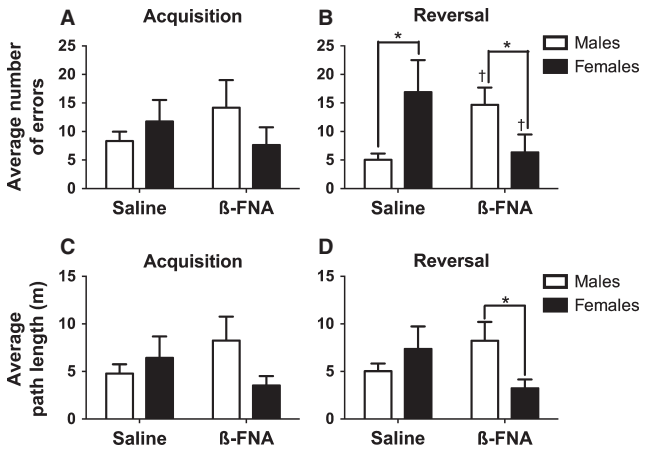

FIG. 5. (A and B) Bar graphs representing the average number of errors before finding the target hole in (A) acquisition and (B) reversal phases for mice receiving saline or β-FNA. (C and D) Bar graphs representing the average path length before finding the target hole in (C) acquisition and (D) reversal phases for mice receiving saline or β-FNA. White bars represent males and black bars represent females (n = 8–10 mice). *$P < 0.05$, between-sexes comparison; †$P < 0.05$, within-sex comparison.

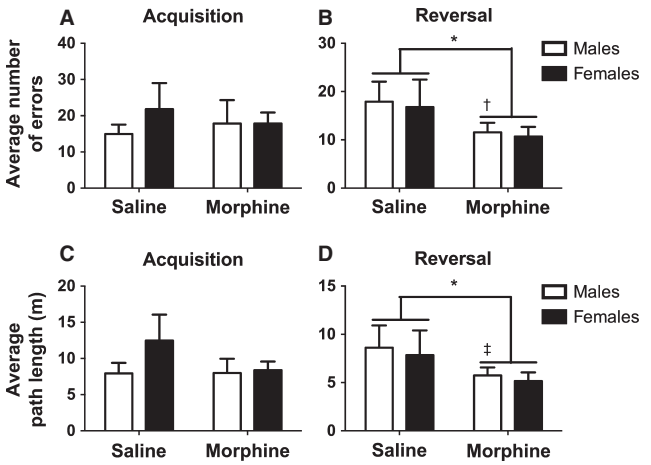

FIG. 6. (A and B) Bar graphs representing the average number of errors before finding the target hole in (A) acquisition and (B) reversal phases for mice assigned to receive (A) or receiving (B) saline or morphine. (C and D) Bar graphs representing the average path length before finding the target hole in (C) acquisition and (D) reversal phases for mice assigned to receive (C) or receiving (D) saline (n = 5–9 mice) or morphine (n = 11–14 mice). White bars represent males and black bars represent females. *$P < 0.05$; †$P < 0.05$, within-sex comparison; ‡$P < 0.1$, within-sex comparison.

TABLE 2. Locomotor behavior

Study	Treatment	Sex	Distance (m)
β-FNA			
Path length day 1	Saline	Male	10.361 ± 2.950
		Female	7.363 ± 3.109
	β-FNA	Male	6.717 ± 3.109
		Female	5.611 ± 3.298
Path length day 6	Saline	Male	7.324 ± 2.098
		Female	7.301 ± 2.211
	β-FNA	Male	6.132 ± 2.211
		Female	4.128 ± 2.346
Morphine			
Path length day 7	Saline	Male	9.086 ± 2.463
		Female	6.516 ± 3.305
	Morphine	Male	9.344 ± 1.975
		Female	6.783 ± 2.228

Locomotor activity for male and female mice receiving β-FNA (days 1 and 6) or morphine (day 7). Distance traveled is reported as mean ± SEM.

RM analyses revealed a significant sex × drug interaction between subjects for number of errors (Fig. 5B; $F_{1,32}$ = 11.70, $P < 0.05$) and path length (Fig. 5D; $F_{1,32}$ = 4.45, $P < 0.05$). Males receiving saline made significantly fewer errors than did males receiving β-FNA ($F_{1,32}$ = 6.99, $P < 0.05$) or females receiving saline ($F_{1,32}$ = 6.54, $P < 0.05$), but did not differ in path length from males receiving β-FNA ($F_{1,32}$ = 1.72, $P > 0.05$). Females receiving β-FNA demonstrated fewer errors ($F_{1,32}$ = 5.24, $P < 0.05$) and a shorter path length to reach the target hole ($F_{1,32}$ = 4.80, $P < 0.05$) as compared to males receiving β-FNA, but showed no differences in path length as compared to females receiving saline ($F_{1,32}$ = 2.78, $P > 0.05$). Females receiving β-FNA also made significantly fewer errors than did females receiving saline ($F_{1,32}$ = 4.87, $P < 0.05$). No treatment or group differences were detected with RM ANOVA in the acquisition phase (Fig 5A and C; all $P > 0.05$).

There was no main effect of sex or drug for the number of former target hole entries, nor was there a significant sex × drug interaction (all $P > 0.05$; Fig 2B). Males receiving β-FNA showed a nonsignificant trend towards entering the former hole more often than females receiving β-FNA ($F_{1,36}$ = 4.06, P = 0.05). There were no effects of β-FNA on locomotor activity (Table 2).

Experiment 4: effects of morphine on acquisition and behavioral flexibility

All experimental groups improved performance over the course of acquisition and reversal phases (Table 1). There were no significant differences between 2.5 and 5.0 mg/kg of morphine, so morphine doses were combined for all of the following analyses. There was a significant main effect of drug treatment in the reversal phase such that mice receiving morphine made significantly fewer errors (Fig. 6B; $F_{1,35}$ = 5.859, $P < 0.05$) and demonstrated significantly shorter path lengths to find the target hole (Fig. 6D; $F_{1,35}$ = 4.562, $P < 0.05$). Unlike previous studies there was no significant sex × treatment interaction. The lack of an interaction was driven by the unexpected poor performance in saline-treated females. We hypothesise that this may be due to handling from injections given 30 min before testing. We have previously observed that handling, such as vaginal lavage, can impact the behavior of female California mice (Silva *et al.*, 2010). Although the predicted interaction was not observed, morphine specifically improved male performance in reversal as predicted ($F_{1,35}$ = 4.946, $P < 0.05$).

No treatment (mice assigned to receive saline versus morphine in reversal) or group differences were detected with RM ANOVA in the acquisition phase (Fig. 6A and C; all $P > 0.05$) or with univariate ANOVA in the number of former hole entries (Fig. 2C; all $P > 0.05$). There were no effects of morphine on locomotor activity (Table 2).

Discussion

We demonstrated that social defeat impaired behavioral flexibility in males exposed to defeat stress, while females were resilient in the face of these deficits. Inhibition of MOR in males, but not females, naive to defeat induced deficits in behavioral flexibility on average,

while activation of MOR ameliorated deficits in behavioral flexibility in mice exposed to defeat. In the MOR antagonist study, mice were treated during both the acquisition and reversal learning stages. However, no effects of β-FNA were observed during the acquisition phase, which further strengthens the conclusion that MOR impacts behavioral flexibility rather than learning per se. We also observed that males but not females exposed to defeat had reduced MOR binding in the lOFC, a brain region that is critical for the expression of flexible behaviors (McAlonan & Brown, 2003; Bissonette *et al.*, 2008). The present studies are consistent with recent data showing that a reversal task is more sensitive to neurological insult in men than in women (Schopp *et al.*, 2001; Niemeier *et al.*, 2007), and that activation of MOR rescues impairments in flexible behaviors (Quednow *et al.*, 2008).

Social organization can have a major impact on sex differences in cognitive function. Previous studies in other species of polygynous rodents have reported that males outperform females in acquiring spatial memories (Gaulin & Fitzgerald, 1986, 1989; Jonasson, 2005). Unlike most species of rodents, California mice are monogamous (Ribble, 1991). Consistent with previous reports in California mice (Jasarevic *et al.*, 2012), we observed no sex differences in the acquisition of spatial memories. Similarly, no sex differences in the acquisition of spatial learning were observed in monogamous pine voles (*Microtus pinetorum*) (Gaulin & Fitzgerald, 1986). Thus studying sex differences in behavioral flexibility in a monogamous species is ideal because the absence of differences during acquisition ensures that any differences during reversal are not an artifact of differences in acquisition learning. Spatial learning and memory are thought to be influenced by sexual selection (Sherry *et al.*, 1992) and have been hypothesised to be more vulnerable to insults that affect steroid hormone signaling pathways (Jasarevic *et al.*, 2011). Behavioral flexibility relies on different neural circuits and neurochemical pathways than does acquisition learning (Ghods-Sharifi *et al.*, 2008; Bissonette & Powell, 2012). Effects of defeat stress on Barnes maze performance were limited to reversal, and primarily to males. This suggests that the signaling pathways mediating behavioral flexibility are more vulnerable to stress in males than in females.

Our results suggest that reduced MOR signaling in males may play an important role in mediating this deficit. Interestingly, the OFC was the only brain region in which MOR binding was affected by defeat stress. A study of healthy male combat veterans showed that combat exposure increased MOR binding in the OFC as compared to a control population (Liberzon *et al.*, 2007). However, veterans diagnosed with post-traumatic stress disorder had significantly lower MOR binding than unaffected veterans. There were no women in this study. In general, sex differences in MOR action are poorly understood. The analgesic effects of MOR have been reported to be stronger in females than in males (Kavaliers & Innes, 1987; Cook *et al.*, 2000), an effect thought to be mediated in part by the periaquductal gray (Bernal *et al.*, 2007). Our results highlight the need for further investigation of sex differences in MOR function in cognitive processes.

The impact of stress on flexible behavior could potentially extend beyond spatial performance. Deficits in executive function, including behavioral flexibility, are commonly observed in stress-induced psychiatric disorders such as depression and post-traumatic stress disorder (Channon, 1996; Kanagaratnam & Asbjornsen, 2007). Few studies have been sufficiently powered to examine sex differences in behavioral flexibility. One study investigating patients with major depressive disorder found that men who ruminated on depressive symptoms demonstrated increased perseverative behaviors, a measure of cognitive inflexibility, as compared to women who ruminated on depressive symptoms (Davis & Nolen-Hoeksema, 2000). Similarly, emerging data suggest that there are sex differences in behavioral flexibility following traumatic brain injury (TBI). Men who experienced TBI performed more poorly on tests of behavioral flexibility than did women who experienced TBI, regardless of the site of injury (Schopp *et al.*, 2001; Niemeier *et al.*, 2007). It is useful to revisit the idea that behavioral flexibility can be associated with different coping strategies following environmental perturbation (Coppens *et al.*, 2010), especially in the context of TBI and major depressive disorder. Individuals who are relatively inflexible in assimilating to environmental change are said to follow a proactive coping strategy (Koolhaas *et al.*, 1999). Interestingly, cognitive behavioral therapy (CBT), which is a clinical approach for treating several forms of mental illnesses (Taylor, 1996; DeRubeis *et al.*, 1999; Butler *et al.*, 2006; Hofmann & Smits, 2008), appears to depend in part on engaging behavioral flexibility. There is some evidence that deficiencies in behavioral flexibility, which could be stress-induced, may impair the effectiveness of CBT (Garety *et al.*, 1997). Our data suggest that future studies need to more closely examine behavioral flexibility in both male and female patients, and consider how sex differences could impact the effects of stress on circuits mediating behavioral flexibility.

Gonadal hormones are a logical mechanism for mediating sex differences in behavior and brain function. In a previous study, we considered the effect of gonadectomy on stress hormones and behavior (Trainor *et al.*, 2013). Castration exaggerated corticosterone responses during defeat, and ovariectomy blunted these responses. Gonadectomy, however, had no effect on long-term social withdrawal responses. In contrast, females raised on corncob bedding (which contains estrogen-like components) had blunted social withdrawal responses, suggesting that the developmental effects of estrogens may be more important for mediating sex differences in behavioral responses to stress. A related possibility is that social housing conditions could impact the effects of defeat on behavioral flexibility. All of our mice were pair-housed, which has been found to blunt the neurobiological effects of defeat stress (Isovich *et al.*, 2001). It is possible that single housing could have sex-specific effects on behavior, and is an interesting avenue for future study. Effects of stress on flexible behaviors could also be modulated by kappa opioid receptor (KOR) signaling, as several forms of stress induce the release of dynorphin and activation of KOR (Bruchas *et al.*, 2007). Although KOR knockout was not found to affect spatial learning in radial mazes or water mazes (Jamot *et al.*, 2003), behavioral flexibility has not been tested.

Our results in California mice show that there are important sex differences in how defeat stress and MOR signaling impact behavioral flexibility. Our results demonstrated impaired male performance during reversal on average following stress. These results are consistent with recent findings linking altered MOR expression with stress-induced psychiatric disorders (Liberzon *et al.*, 2007) and male-biased impairments in flexibility following brain injury (Schopp *et al.*, 2001; Niemeier *et al.*, 2007). These results suggest that clinical studies targeting behavioral flexibility as a therapeutic mechanism to treat psychiatric disorders will need to be sufficiently powered to examine men and women independently. Our conclusions highlight the importance of studying sex differences in biomedical research (Cahill, 2006), specifically focusing on the necessity of including females in models of psychiatric disease due to prominent sex differences in behavior following psychosocial stress.

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Abbreviations

β-FNA, beta-funaltrexamine; CPu, caudate–putamen; lOFC, lateral OFC; MOR, mu-opioid receptor; NAc, nucleus accumbens; OFC, orbitofrontal cortex; RM, repeated-measures.

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