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Research report

Effects of kappa opioid receptors on conditioned place aversion and social interaction in males and females

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HIGHLIGHTS

- Low doses of kappa opioid receptor agonist induced place aversion in females but not males.
- Acute effects of kappa agonist on social interaction behavior are similar in males and females.
- Kappa agonist induces activation of p38 map kinase in microglia in the brain.

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ABSTRACT

The effects of kappa opioid receptors (KOR) on motivated behavior are well established based on studies in male rodents, but relatively little is known about the effects of KOR in females. We examined the effects of KOR activation on conditioned place aversion and social interaction in the California mouse (*Peromyscus californicus*). Important differences were observed in long-term (place aversion) and short-term (social interaction) effects. Females but not males treated with a 2.5 mg/kg dose of U50,488 formed a place aversion, whereas males but not females formed a place aversion at the 10 mg/kg dose. In contrast the short term effects of different doses of U50,488 on social interaction behavior were similar in males and females. Acute injection with 10 mg/kg of U50,488 (but not lower doses) reduced social interaction behavior in both males and females. The effects of U50,488 on phosphorylated extracellular signal regulated kinase (pERK) and p38 MAP kinase were cell type and region specific. Higher doses of U50,488 increased the number of pERK neurons in the ventrolateral bed nucleus of the stria terminals in males but not females, a nucleus implicated in male aggressive behavior. In contrast, both males and females treated with U50,488 had more activated p38 cells in the nucleus accumbens shell. Unexpectedly, cells expressing activated p38 co-expressed Iba-1, a widely used microglia marker. In summary we found strong sex differences in the effects of U50,488 on place aversion whereas the acute effects on U50,488 induced similar behavioral effects in males and females.

1. Introduction

Activation of KOR induces an aversive state, producing dysphoric like behaviors [38]. Initial work suggested that females might be more sensitive to KOR activation, as clinical studies showed that the analgesic effects of KOR agonists following dental surgery were stronger in women versus men [27,28]. Further study has demonstrated that sex differences in the analgesic effects of KOR are dependent on the pain modality assessed [33,54,64,46]. Much less is known about whether the behavioral effects of KOR differ in males and females, although recent reports also suggest that sex differences are context-dependent. Injections of the KOR-specific agonist U50,488 had stronger effects on posture and locomotor behavior in male Guinea pigs, but were more effective at blocking cocaine-induced hyperactivity in females [81]. Activation of KOR by U50,488 also was more effective at inhibiting intrancranial self-stimulation (which stimulates brain reward systems) in males compared to females [66]. Suppression of reward-related circuits is thought to contribute to dysphoria. Activation of KOR has been reported to induce dysphoria in humans [60,80] and dysphoric-like states in rodents [42]. In rodents,

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dysphoric-like states frequently lead to the formation of a conditioned place aversion [11,67,20]. The ability of KOR activation to induce place aversion has not been previously reported in females.

Kappa opioid receptors have also been reported to modulate social behaviors, particularly in the context of social conflict. The KOR antagonist nor-binaltorphimine (nor-BNI) reduced submissive behaviors in male C57Bl6 mice exposed to social defeat stress [48] and increased social interaction behavior immediately after exposure to defeat stress [13]. One of the only studies to examine the effects of KOR on social behaviors in females demonstrated that infusions of nor-BNI into the nucleus accumbens (NAc) shell reduced resident-intruder aggression in both male and female prairie voles [65]. These effects of KOR on aggressive and submissive behaviors were induced by relatively short term manipulations of KOR function. Other studies have suggested that certain experiences, such as defeat stress, may induce long term neuroplastic changes in the effects of KOR on social behavior. While U50,488 decreased social approach behavior in C57Bl6 mice that had won aggressive encounters, the same treatment increased social interaction in mice exposed to defeat stress for three weeks [41].

We examined the effects of the selective KOR agonist U50,488 on behavior in female and male California mice (*Peromyscus californicus*). Unlike other rodents, both male and female California mice are aggressive [69], which has allowed for the study of social defeat stress in females [74]. We recently observed that the inhibitory effects of dopamine D1 receptors in the NAc shell on social interaction behavior were stronger in females than males [15]. However, there were no sex differences in D1 receptor mRNA expression. Medium spiny neurons in the NAc that express D1 receptor also express dynorphin [31], the primary endogenous ligand for KOR [19]. Based on these data, we hypothesized that female California mice would be more sensitive than males to KOR stimulation. We tested this hypothesis with a dose–response study examining the effects of U50,488 on conditioned place preferences. Although other specific KOR agonists are available, U50,488 is one of the best characterized KOR ligands in studies of place preference. We also examined the effects of U50,488 on social interaction behavior. Finally, we examined the effects of U50,488 on the activation of extracellular signal regulated kinase (ERK) and p38 MAP kinase, as these pathways have been found to mediate the effects of KOR [4,12,61]. We examined core and shell regions of the NAc as previous reports have shown that the NAc is an important site of KOR action. We also examined the bed nucleus of the stria terminalis, which acts as a link between the mesolimbic dopamine and social behavior circuits [57,58].

2. Methods

2.1. Animals

California mice were bred in the UC Davis laboratory colony. All animals in the study were three month old sexually inexperienced adults. Females were tested at different stages of the estrous cycle as each mouse was tested over the course of one week. Estrous cycle was not continuously monitored because we previously determined that vaginal lavage has strong effects on behavior in California mice [69]. Mice were individually marked with ear punches and housed 2–3 per cage in same sex groups. Animals were housed in clear polypropylene cages with Sani-chips bedding, environ-dri (Shepherd, Milford, NJ), and cotton nestlets. Harlan Tekland 2016 food and water were provided *ad libitum*. Mice were maintained on a 16 h light/8 h dark cycle (lights off 15:00 PST). All experiments were approved by the UC Davis Institutional Animal Care and Use Committee. The mice were maintained in accordance with the recommendations of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

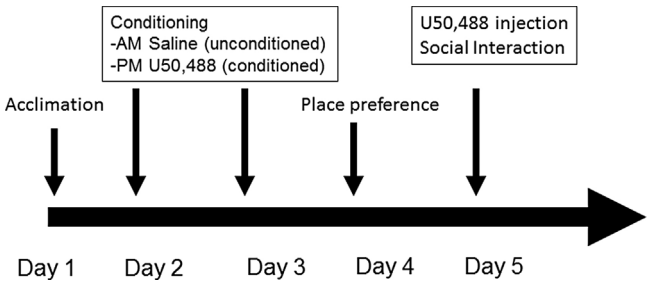

Fig. 1. Timeline for place preference experiments and social interaction testing.

2.2. Conditioned place aversion

The conditioning apparatus consisted of three interconnected standard sized mouse cages (28 × 17.5 × 11 cm). All three of the cages were visually distinct. The center cage (black and white horizontal stripe background) was connected to a left cage (black and white vertical stripe background) and a right cage (black dots on a white background). Each compartment contained no bedding and the apparatus was cleaned with Quatricide (1:64, Quatricide PV in water, Pharmacal Research Labs, Inc) after each conditioning or testing session. Clear polypropylene lids were used to cover the apparatus during experiments to facilitate video recording.

Place preference testing was conducted over four days (Fig. 1). On day 1 each mouse was given free access to the entire arena for a 30 min acclimation test (between 0800 and 1200). Mice were tracked in real time with a visual tracking system (Any-maze, Stoelting). Repeated measures ANOVA showed that on day 1 of testing mice spent significantly more time in the center chamber compared to the side chambers (Table 1, $F_{2,61} = 15.23$, $p < 0.001$). There was also a slight but significant preference for the left chamber compared to the right ($p = 0.03$), but there were no sex or treatment differences (all p's > 0.8). For each mouse, initial place biases were corrected for by assigning drug conditioning to the preferred side chamber [49,63]. Although more mice were conditioned in the left chamber (Table 1), there were no systematic differences between males and females or between the different drug treatments. Importantly, conditioning procedures did not change mean chamber preferences (Table 1). This suggests that the effects of conditioning are based on the aversive or rewarding aspects of U50,488 and not due to nonspecific effects on activity or anxiety-like behavior [77]. Mice were randomly assigned to be conditioned with either 0, 2.5, 5, or 10 mg/kg of the KOR selective agonist U50,488 (Tocris, Ellisville, MO, USA) dissolved in vehicle consisting of sterile phosphate buffered saline (PBS) with 10% Tween 80 (Fisher Scientific, Fair Lawn, NJ, USA).

Conditioning sessions took place on Days 2 and 3 based on previous methods [8]. Each day consisted of one training session in the unconditioned chamber (AM) and one training session in the conditioned chamber (PM). First, between 0800 and 1100 h each mouse was injected i.p. with vehicle and confined to the unconditioned chamber for 30 min. Subjects were confined to the unconditioned chamber by blocking off the entry way to the center chamber with heavy tape. Between 1200 and 1500 h mice

Table 1
Cage preferences before and after conditioning. $^{**}p < 0.01$ vs center, † vs. left side $p < 0.05$.

	Cage time (s)		
	Center cage	Left side	Right side
Pre-test	813 ± 43	544 ± 35**	438 ± 32**,†
Post-test	812 ± 72	580 ± 48**	408 ± 44**,†
Number conditioned	23	46	