# Sex Differences in Sensitivity to the Depressive-like Effects of the Kappa Opioid Receptor Agonist U-50488 in Rats

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**Background:** Dynorphin, an endogenous ligand at kappa opioid receptors (KORs), produces depressive-like effects and contributes to addictive behavior in male nonhuman primates and rodents. Although comorbidity of depression and addiction is greater in women than men, the role of KORs in female motivated behavior is unknown.

**Methods:** In adult Sprague-Dawley rats, we used intracranial self-stimulation to measure effects of the KOR agonist (±)-*trans*-U-50488 methanesulfonate salt (U-50488) (.0–10.0 mg/kg) on brain stimulation reward in gonadally intact and castrated males and in females at estrous cycle stages associated with low and high estrogen levels. Pharmacokinetic studies of U-50488 in plasma and brain were conducted. Immunohistochemistry was used to identify sex-dependent expression of U-50488-induced c-Fos in brain.

**Results:** U-50488 dose-dependently increased the frequency of stimulation (threshold) required to maintain intracranial self-stimulation responding in male and female rats, a depressive-like effect. However, females were significantly less sensitive than males to the threshold-increasing effects of U-50488, independent of estrous cycle stage in females or gonadectomy in males. Although initial plasma concentrations of U-50488 were higher in females, there were no sex differences in brain concentrations. Sex differences in U-50488-induced c-Fos activation were observed in corticotropin releasing factor–containing neurons of the paraventricular nucleus of the hypothalamus and primarily in non–corticotropin releasing factor–containing neurons of the bed nucleus of the stria terminalis.

**Conclusions:** These data suggest that the role of KORs in motivated behavior of rats is sex-dependent, which has important ramifications for the study and treatment of mood-related disorders, including depression and drug addiction in people.

**Key Words:** c-Fos, dynorphin, female, stress, intracranial self-stimulation, pharmacokinetics

Women are twice as likely as men to suffer from affective disorders including major depression, anxiety disorders, and posttraumatic stress disorder (1,2), which are often comorbid with drug addiction. Negative emotional states such as stress and depression are more common in women addicts (3–6) and more likely to trigger craving and relapse in women than men (7,8). These findings suggest that brain mechanisms encoding aversive states may differ between the sexes.

Stress and chronic exposure to drugs of abuse promote the synthesis and release of dynorphin, an endogenous kappa opioid receptor (KOR) (9) ligand, that is coincident with the emergence of depressive-like effects (10–14). KOR agonists produce negative affective states in humans and rodents (15–23), whereas KOR blockade attenuates stress- and drug-induced depressive-like states (20,24–27). However, these studies were conducted in males, and little is known about how KORs contribute to affective states in females.

Dynorphin and KORs are found throughout the brain (28,29), and mounting evidence suggests that mood-related effects of KOR activation are due to modulation of neurotransmission within the reciprocally connected mesocorticolimbic, extended amygdala, and hypothalamic systems (30–32). KORs are generally expressed on dopaminergic, gamma-aminobutyric acid (GABA) ergic, and glutamatergic nerve terminals (33–35) where they inhibit transmitter release (17,36,37) via coupling to inhibitory $G_{alpha}$ subunits (38). There is also evidence for postsynaptic KOR expression (34,39), although functional effects are not well understood. Recent studies in guinea pigs demonstrated sex differences in KOR receptor levels and function within neural circuits that regulate motivated behavior (40,41), and sex-specific effects of KOR antagonism on aggressive behavior in prairie voles has been reported (42). Pain studies show that females tend to be less sensitive than males to analgesic properties of KOR agonists (43–47).

Sex differences in behavior can result from activational effects of circulating gonadal hormones or organizational effects during development, and/or sex chromosome effects (48–50). We hypothesized that if depressive-like effects of KOR activation are sex-dependent, then male and female rats would have different sensitivities to the anhedonic effects of the KOR agonist U-50488 as measured with intracranial self-stimulation (ICSS). To identify putative neural substrates for sex differences in motivated behavior, we quantified U-50488-induced c-Fos within mesolimbic, extended amygdala, and hypothalamic systems of male and female rats. On the basis of those results, we initiated studies characterizing the neuronal phenotypes of activated neurons in sexually dimorphic regions.

## Methods and Materials

### Animals

Age-matched, sexually mature female ($n$ = 61) and male ($n$ = 52) Sprague-Dawley rats (Charles River Laboratories, Wilmington,

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Massachusetts) between 75 and 85 days old and weighing 300 to 325 g (female) and 380 to 410 g (male) at the start of experiments were used. Upon arrival at the facility, rats were group housed (4 rats/cage) and segregated by sex. All rats were acclimatized for 1 week in a 12 hour–12-hour light–dark cycle (lights on at 7:00 AM) with free access to food and water. All experiments were conducted during the light phase. Rats were treated according to the guidelines recommended by the Animal Care and Use Committee of McLean Hospital.

To track estrous cycles, vaginal smears were taken at the same time each day for approximately 2 weeks before testing. Males were simultaneously handled. On the morning of test days, vaginal cytology was examined with a light microscope to rapidly determine cycle stage to assign treatments. See Methods and Figure S1 in Supplement 1.

### Intracranial Self-stimulation

Rats ($n = 13$ female; $n = 17$ male) were implanted with stainless steel monopolar electrodes (.25 mm diameter; Plastics One, Roanoke, Virginia) aimed at the medial forebrain bundle at the level of the lateral hypothalamus (2.8 mm posterior to bregma, 1.7 mm lateral to midline, 7.8 mm below dura). Rats were trained using the rate-frequency method, as previously reported (51) (Supplement 1).

For drug testing, three rate-frequency functions (3 × 15 min) were determined for each rat immediately before drug injection. ICSS thresholds and maximum rates for the second and third functions were averaged and served as baseline parameters. Each rat then received an intraperitoneal (IP) injection of drug and four more 15-minute rate-frequency trials. Drug treatment days were separated by 2 or 3 nondrug days during which baseline ICSS thresholds were maintained. Rats were treated with (±)-*trans*-U-50488 methanesulfonate salt (U-50488, Sigma-Aldrich, St. Louis, Missouri) at doses of .0, 2.5, 5.0, and 10 mg/kg dissolved in water, based on weight of the salt. Our goal was to test female rats with each dose of U-50488 during estrous, diestrous, and proestrous, with doses of U-50488 administered in a randomized order. However, most females were tested with only two or three doses of U-50488 at two of the three estrous cycle stages due to loss of head cap, unstable responding during nondrug days (>10% variation from stable baseline thresholds established during training), or inability to capture the rat in a particular estrous stage. Consequently, the number of animals represented in each dose and estrous stage ranges from 7 to 13. To account for repeated testing of females, males were treated twice with each dose of U-50488 in randomized order and effects of the first and second treatments compared (see Figure S4 in Supplement 1). ICSS boxes were cleaned with isopropyl alcohol, and bedding was changed between each animal. Sequentially testing males and females did not affect thresholds.

### Intracranial Self-stimulation: Castrated Males

Adult male rats ($n = 7$) were implanted with stimulating electrodes, trained in ICSS, and treated with U-50488 (.0–10.0 mg/kg, IP) as described earlier. After completion of the U-50488 dose response, baseline ICSS thresholds were reestablished for each rat over a period of 4 to 5 days, and rats were castrated (see Supplement 1). Rats recovered for 4 days and then ICSS was performed 1 hour per day, 5 days a week for 3 weeks as testosterone levels declined and other hormones stabilized. At this point, the U-50488 (.0–10.0 mg/kg, IP) dose response test was repeated.

### Pharmacokinetics

For repeated blood sampling, rats ($n = 11$ females, 6 males) were cannulated through the right external jugular vein and singly housed (see Supplement 1). After 3 days of recovery, vaginal swabs were done on female rats, and male rats were handled. One hour later, rats were treated with U-50488 (10 mg/kg, IP) and blood samples (200 μL/sample) were collected via IV cannula at .083, .25, .5, 1.0, 2.0, 4.0, and 8.0 hour in 1.5-mL Eppendorf tubes stored on wet ice. Twenty-four hours post-U-50488 treatment, rats were decapitated and trunk blood was collected in prechilled 7 mL ethylenediamine tetraacetate–treated blood tubes kept on wet ice. Blood was aliquoted into 1.5-mL tubes and all samples were centrifuged (13,000 *g* at 4°C for 15 minutes). Plasma was removed, aliquoted into 1.5-mL Eppendorf tubes and frozen on dry ice before −80°C storage. For analysis of U-50488 brain levels, separate rats ($n = 14$ females, 9 males) were used. Rats were treated with U-50488 (10 mg/kg, IP) and decapitated 15 minutes, 1 hour, or 24 hours later. Brains were removed and frozen in isopentane kept on dry ice before −80°C storage. U-50488 concentrations in plasma and brain were determined by liquid chromatography tandem mass spectrometry (see Supplement 1).

### Immunohistochemistry

Rats ($n = 19$ females, 18 males) were treated with U-50488 (.0 or 10.0 mg/kg) and perfused 2 hr later for immunohistochemistry (52) (Supplement 1). c-Fos-positive nuclei in brain regions of interest were counted and reported as density (number of c-Fos positive nuclei/area analyzed). To examine possible colocalization of c-Fos and corticotropin releasing factor (CRF) in the paraventricular nucleus (PVN) and bed nucleus of the stria terminalis (BNST), separate rats were treated with U-50488 (.0 or 10.0 mg/kg; $n = 2$ male rats/dose) and perfused 2 hours later. Double label immunohistochemistry was performed using methods described (53) (Supplement 1).

### Electrode Placement Histology

To compare ICSS electrode placements between males and females, a subset of rats was transcardially perfused with 4% paraformaldehyde. After fixation, coronal sections (40 μm) through the lateral hypothalamus were cut. Tissue was stained with cresyl violet and examined under the microscope. Locations of the electrode tips were mapped onto rat brain atlas plates (54).

### Statistics

Dose- and time-dependent effects of U-50488 on ICSS thresholds and maximum rates of responding, as well as time-dependent effects on plasma U-50488 levels measured from repeated blood sampling were analyzed using linear mixed models with sex or cycle stage and dose or time as fixed effects and with a random effect on rat (Supplement 1). Brain concentrations of U-50488 were analyzed with two-way (sex × time) analysis of variance (ANOVA). Quantification of c-Fos expression was analyzed with two-way (sex × treatment) ANOVA for each brain region. Levels of the dependent variable "sex" include male and female, with males subdivided into pre- and postcastration (Figure 3), females into estrous, diestrous, and proestrous (Figure 2) or low and high E (Figures 5–7). Significant effects and interactions were analyzed further with simple main effects tests and Bonferroni multiple comparisons post hoc tests. SPSS Statistics (Version 21; IBM, Armonk, New York) was used for linear mixed model analyses and GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, California) was used for all other statistical analyses.