

## BRIEF COMMUNICATION

# Serotonin Impairs Copulation and Attenuates Ejaculation-Induced Glutamate Activity in the Medial Preoptic Area

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The medial preoptic area (MPOA) is critical for male sexual behavior. Glutamate is released in the MPOA of male rats during copulation, and increasing glutamate levels by reverse dialysis of glutamate uptake inhibitors facilitates mating. Conversely, increased release of serotonin (5-HT) inhibits sexual behavior. In both rats and men, selective serotonin reuptake inhibitors (SSRIs) impair erection, ejaculation, and libido. Here we reverse-dialyzed 5-HT through concentric microdialysis probes in the MPOA of male rats; concurrently we collected 2-min samples for analysis of glutamate and measured sexual behavior. Sexual activity, and especially ejaculation, increased levels of glutamate in the MPOA. However, reverse dialysis of 5-HT into the MPOA impaired ejaculatory ability and attenuated glutamate release. Implications of these results for impairment of sexual behavior that results from administration of SSRIs are discussed.

*Keywords:* serotonin, glutamate, SSRI, libido, ejaculation, preoptic, antidepressants

The medial preoptic area (MPOA) plays an important role in the regulation of male sexual behavior. It receives indirect input from every sensory modality and sends projections to structures critical for the initiation and patterning of mating (Simerly & Swanson, 1986, 1988). Lesions of the MPOA inhibit male sexual behavior in all studied species and, conversely, stimulation facilitates several measures of copulation (reviewed in Hull & Rodriguez-Manzo, 2009). Neural activity in the MPOA increases with sexual activity. Electrophysiological recordings in the MPOA of sexually experienced monkeys showed increased neural activity both when the animals bar-pressed to bring a conspecific female closer and during copulation, whereas activity ceased after ejaculation (Oomura, Aou, Koyama, Fujita, & Yoshimatsu, 1988). Similar results were observed in rats, with continuously increasing activity during mating, until after ejaculation, when activity decreased (Shimura, Yamamoto, & Shimokochi, 1994). Similarly, sexual activity increased Fos-immunoreactivity (ir), a measure of cellular activity, in the MPOA of male rats, hamsters, gerbils, and musk shrews (reviewed in Hull & Rodriguez-Manzo, 2009).

Glutamate release in the MPOA facilitates male sexual behavior (reviewed in Dominguez, 2009). Extracellular glutamate in the MPOA of male rats increased during mating, and reverse dialysis

of glutamate uptake inhibitors enhanced both mating-induced release of MPOA glutamate and male sexual behavior (Dominguez, Gil, & Hull, 2006). Together these data indicate that glutamate mediates mating-induced activity in the MPOA.

On the other hand, increased serotonin (5-HT) generally inhibits sexual behavior (reviewed in Hull, Muschamp, & Sato, 2004). 5-HT might exert this inhibition, in part, by acting in the MPOA. Large doses of 5-HT microinjected directly into the MPOA inhibited mating (Fernandez-Guasti, Escalante, Ahlenius, Hillegaard, & Larsson, 1992; Verma, Chhina, Mohan Kumar, & Singh, 1989). *In vitro* application of 5-HT suppressed glutamate transmission and glutamate-mediated activation in the MPOA of male rats (Malinina, Druzin, & Johansson, 2005). Therefore, we hypothesized that increased 5-HT might also impair mating-induced glutamate release in the MPOA and thus impair sexual behavior.

More than 60% of people who are prescribed selective serotonin reuptake inhibitors (SSRIs) experience impaired sex drive and may also experience impaired ejaculatory ability. The exact mechanisms via which SSRIs impair sexual behavior are not clearly understood. Data presented here suggest that increased 5-HT inhibits glutamatergic activity in the MPOA, resulting in impaired ejaculatory behavior, which is one possible mechanism via which SSRIs inhibit sexual activity.

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## Method

### Subjects

Adult male Long-Evans/Blue Spruce rats (Harlan, Indianapolis, IN) were individually housed in large plastic cages in a climate-controlled room on a 14/10 light/dark cycle (lights off at 11 a.m. and on at 9 p.m.). Stimulus females were ovariectomized under ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4

mg/kg) anesthesia. Behavioral estrus was induced with 10  $\mu$ g estradiol benzoate 48 hr before and 500  $\mu$ g progesterone 4 hr before testing. Receptivity was confirmed shortly before the test by placing the female with a stud male. Experimental males were given sexual experience by copulating to two ejaculations on each of two tests. All procedures were in accordance with the National Institutes of Health Guidelines for the Use of Animals and were approved by the Institutional Animal Care and Use Committee of Florida State University.

### Stereotaxic Surgeries

Sexually experienced male rats ( $n = 18$ ) were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) and received 23 gauge thin-wall stainless steel guide cannulas aimed 2 mm above the MPOA [AP, 2.3 mm; ML, 0.3 mm; DV, 26.2 mm; Pellegrino, Pellegrino, and Cushman (1979)]. Cannulas were secured to the skull and skull screws with dental acrylic. Bacitracin (400 U/g) antibiotic was spread around the wound margins, and an obturator, cut the same length as the guide cannula, was inserted into the guide cannula to prevent entry of foreign matter. Aseptic techniques were used for all surgical procedures.

### Microdialysis and Behavior

Animals were allowed to recover for two weeks after surgery. On the morning of testing, concentric microdialysis probes that had been constructed in the lab were implanted through the guide cannulas into the MPOA. The dialysis membrane (Spectra/Por in vivo microdialysis hollow fibers; Spectrum, Gardena, CA) had an outer diameter of 170  $\mu$ m, an inner diameter of 150  $\mu$ m, an active dialyzing length of 1 mm, and an 18,000 mol wt cutoff. The inflow tubing was encased by a teflon-covered tether. Artificial cerebrospinal fluid (aCSF, in mM: 138 NaCl, 2.7 KCl, 0.5 MgCl<sub>2</sub>, 1.5 KH<sub>2</sub>PO<sub>4</sub>, and 1.2 CaCl<sub>2</sub>, pH 6.8; Dulbecco's solution, Sigma, St. Louis, MO) was perfused at a rate of 2.0  $\mu$ l/min. Dialysate samples were collected every 2 min and were immediately frozen ( $-80^{\circ}\text{C}$ ) for later assay using high performance liquid chromatography with electrochemical detection (HPLC-EC). In vitro recovery through the probe was about 25%. After a 6-hr stabilization period, 3 baseline (BL) samples were collected; the last BL sample was expressed as percent of the mean of the 3 BL samples. Following BL, dialysate for the experimental group was changed to one containing 500  $\mu$ mol/L 5-HT, and 3 additional samples were collected; control animals continued to receive aCSF. Reverse dialysis of 5-HT continued for the experimental group throughout the rest of the experiment. After the initial aCSF/5-HT period, an estrous female in a wire mesh cage was placed over the male's cage, and 3 additional samples were collected (PRE period). The female was then placed into the male's cage, where they could copulate (COP period); during this time, 20 additional samples were collected. If ejaculation occurred, the sample was classified as an ejaculation (EJAC) sample; each sample after an ejaculation and before the next intromission was classified as a postejaculatory interval (PEI) sample. The time required for dialysate to progress from the membrane to the collection vial was  $\sim 30$  sec; therefore, behaviors that occurred during the last 30 sec of a 2-min interval were recorded as occurring during the following interval. After 20

COP, EJAC, and/or PEI samples were collected (i.e., 40 min), the female was removed, if the male was not in a PEI, and 3 more samples were collected (POST). The following behavioral measures were recorded: mount latency, time from introduction of the female to the first mount; intromission latency, time from introduction of the female to the first intromission; ejaculation latency, time from the first intromission to the first ejaculation; postejaculatory interval, time from the ejaculation to the next intromission; mounts and intromissions preceding ejaculation; and ejaculation frequency during the test.

### Data Analysis

Behavioral measures were analyzed with  $t$  tests. Each animal's dialysate samples were categorized as BL, aCSF/5-HT, PRE, COP, EJAC, PEI, or POST and each animal's data were averaged within categories. COP samples included all those collected while the female was with the male, except those during which the male ejaculated or was in PEI. For the glutamate analysis, a two-way analysis of variance (2 groups  $\times$  7 conditions) was run, with comparisons for between-groups measures and within-group repeated measures, followed by Newman-Keuls tests.

### Results

Reverse dialysis of 5-HT into the MPOA impaired mating behavior (see Figure 1). Specifically, animals receiving 5-HT displayed fewer ejaculations,  $t_{(16)} = 3.297, p < .01$ , required more time to reach an ejaculation,  $t_{(16)} = -2.271, p < .05$ , and experienced longer postejaculatory intervals,  $t_{(16)} = -3.787, p < .01$ . No other behavioral measures were affected. Analyses of percent changes from baseline for glutamate levels revealed significant differences attributable to treatment, sample, and interaction (see Figure 2). For treatment, reverse dialysis of 5-HT significantly decreased glutamate levels,  $F_{(1, 330)} = 13.93, p < .001$ ; there was also a significant effect of sample,  $F_{(6, 330)} = 22.02, p < .001$ . In addition, there was an interaction of treatment by sample,  $F_{(6, 330)} = 3.168, p < .01$ . Within-Group Newman-Keuls analyses revealed that glutamate increased in samples collected during copulation ( $p < 0.05$ ) and ejaculation ( $p < 0.05$ ), compared with all other samples, in animals receiving aCSF, while glutamate increased only during ejaculation, compared with all other samples, in animals receiving 5-HT ( $p < 0.05$ ). Between-Groups Newman-Keuls tests revealed that levels of glutamate increased significantly more during ejaculation in animals receiving aCSF alone versus those receiving reverse dialysis of aCSF plus 5-HT ( $p < .01$ ).

### Discussion

Reverse dialysis of 5-HT into the MPOA of male rats impaired ejaculation and glutamate release. Rats receiving reverse dialysis of serotonin displayed fewer ejaculations, required more time to reach an ejaculation, and required more time to reinitiate mating after reaching an ejaculation. In addition, 5-HT decreased glutamate release, especially during copulation and ejaculation. These results support the original hypothesis that increased 5-HT impairs glutamate release in the MPOA and, as a result, impaired ejaculation.

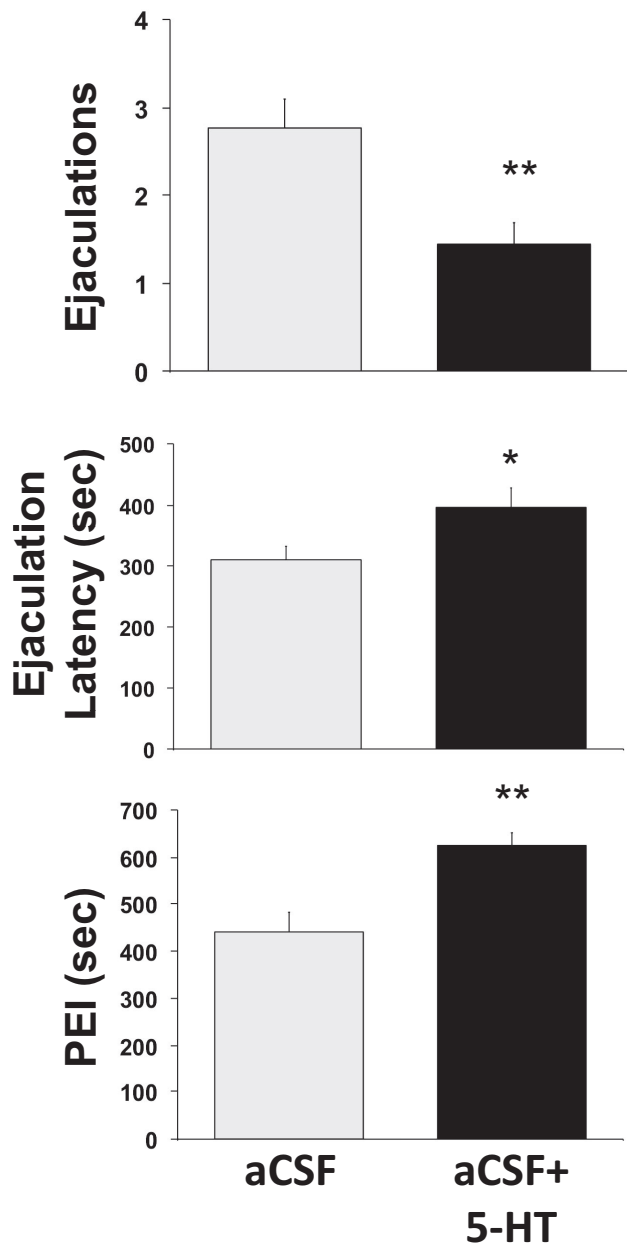


Figure 1. Animals receiving reverse dialysis of artificial cerebrospinal (aCSF) plus serotonin (5-HT) ( $n = 9$ ) displayed fewer ejaculations, required more time to reach an ejaculation, and required more time to reinitiate mating after an ejaculation (PEI), compared to those receiving only aCSF ( $n = 9$ ). Values are expressed as mean  $\pm$  SEM, \*  $p < .05$ , \*\*  $p < .01$ .

With microdialysis it is unclear whether a change in neurotransmitter levels causes, or results from, a behavioral change. In a previous study mating increased glutamate release in the MPOA of male rats, with a 300% increase at the time of ejaculation (Dominguez et al., 2006). In that study reverse dialysis of glutamate uptake inhibitors enhanced both mating-induced increases in extracellular glutamate and male sexual behavior. Furthermore, microinjection of glutamate into the MPOA increased urethrogenital reflexes, a model for orgasm (Marson & McKenna, 1994).

Similar microinjections increased intracavernous pressure in anesthetized rats (Giuliano et al., 1996). Thus, increasing MPOA glutamate has enhanced copulation, urethrogenital reflexes, and erection. In another study, almost all MPOA neurons that expressed mating-induced c-Fos also contained the NR1 subunit of NMDA receptors, and mating increased phosphorylation, and thus activation, of NR1 in the MPOA (Dominguez et al., 2007). This phosphorylation was blocked by the NMDA receptor antagonist MK-801 (Dominguez et al., 2007). In addition, microinjections of MK-801 into the MPOA impaired sexual behavior in both sexually naïve and experienced males and blocked the facilitation otherwise produced by repeated noncopulatory exposures to an estrous female (Dominguez et al., 2007; Vigdorichik, Lagoda, & Hull, 2003). These results provide evidence that mating activates NMDA glutamate receptors in the MPOA and that this activation is important for the expression of male sexual behavior. The microinjection and reverse dialysis studies are important, because they show the direction of the causal relation between MPOA glutamate and behavior. Thus, MPOA glutamate actively facilitates copulation.

Serotonin, on the other hand, is generally inhibitory to male sexual behavior (reviewed in Hull et al., 2004). SSRI antidepressants often result in difficulty achieving ejaculation or orgasm and may also lead to decreased sexual interest (Herman et al., 1990; Hull, Dominguez, & Muschamp, 2007; Montejo-Gonzalez et al., 1997; Rothschild, 2000). Rats, too, have shown decreased sexual motivation and ability to ejaculate following chronic administration of SSRIs (Cantor, Binik, & Pfau, 1999; Frank, Hendricks, & Olson, 2000; Vega Matuszcyk, Larsson, & Eriksson, 1998).

One site for this inhibition may be the MPOA. Microinjection of large doses of 5-HT into the MPOA inhibited mating (Fernandez-Guasti et al., 1992; Verma et al., 1989), likely by acting on 5-HT<sub>1B</sub> receptor subtypes (Fernandez-Guasti et al., 1992). Furthermore, an in

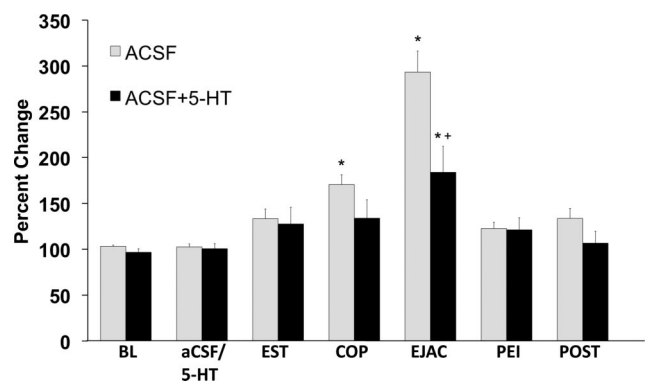


Figure 2. Mating increased levels of extracellular glutamate in the MPOA. Levels were higher in animals receiving dialysis with artificial cerebrospinal fluid (aCSF) ( $n = 9$ ) compared to those receiving aCSF and serotonin (5-HT) ( $n = 9$ ). Samples were collected at 2-min intervals and included: baseline (BL); during initial reverse dialysis of aCSF or aCSF plus 5-HT; in the presence of an inaccessible estrous female (EST); during copulation (COP) without ejaculation; ejaculation (EJAC); postejaculation interval (PEI), and after removal of the female (POST). Glutamate levels increased significantly during COP and EJAC compared to BL (\*  $p < .05$ ) for animals receiving aCSF. Glutamate levels increased only during EJAC for animals receiving 5-HT, and this increase was significantly lower than in those receiving only aCSF (+  $p < .05$ ). Values are expressed as mean  $\pm$  SEM.

vitro study showed that 500  $\mu\text{mol/L}$  5-HT suppressed glutamate transmission and glutamate-mediated activation in the MPOA of male rats, probably by inhibiting both spontaneous and evoked presynaptic glutamate release (Malinina et al., 2005). Therefore, the current results are consistent with previous studies.

Typically, SSRI inhibition of sexual behavior is observed only after chronic treatment, whereas the current data, as well as the two earlier reports noted above (Fernandez-Guasti et al., 1992; Verma et al., 1989), show behavioral inhibition with acute administration of 5-HT directly into the MPOA. The effectiveness of acute direct administration may result in part from its resultant higher concentrations of extracellular 5-HT. However, even acute administration of certain SSRIs can delay ejaculation in men (Kim & Paick, 1999; Pryor et al., 2006; Waldinger, Zwinderman, Schweitzer, & Olivier, 2004) and rats (Looney, C., Thor, K. B., Ricca, D., & Marson, L., 2005).

In summary, reverse dialysis of 5-HT into the MPOA decreased the release of glutamate and inhibited ejaculation. Inhibition of MPOA glutamate release by SSRIs may contribute to their impairment of sexual function in men.

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