Published in final edited form as:

Neuroendocrinology. 2013 ; 97(4): 363–368. doi:10.1159/000351077.

A Novel Membrane Estrogen Receptor Activated by STX Induces Female Sexual Receptivity through an Interaction with mGluR1a

Amy Christensen and

Department of Neurobiology and Laboratory of Neuroendocrinology of the Brain Research Institute, David Geffen School of Medicine at UCLA, Los Angeles, Calif., USA

Paul Micevych

Department of Neurobiology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095-1763 (USA)

Paul Micevych: pmicevych@mednet.ucla.edu

Abstract

Membrane initiated estradiol signaling has been shown to be vital for multiple physiological processes. Several receptors have been proposed to mediate the actions of estradiol at the membrane. Here, we examined the ability of STX, an agonist of a novel putative membrane estrogen receptor, to activate sexually receptive behavior in the female rat. Infusions of STX into the arcuate nucleus of the hypothalamus resulted in the activation and internalization of μ -opioid receptors in the medial preoptic nucleus, an action that is required for lordosis behavior. Indeed, STX was able to augment sexual receptivity in female rats given a sub-behavioral dose of estradiol. However, if the mGluR1a antagonist, LY367,385, was administered prior to STX, its circuit-activating effects, the internalization of μ -opioid receptors, were lost. This suggests that the receptor stimulated by STX activates rapid membrane-initiated signaling through an interaction with mGluR1a – an effect previously described for estrogen receptor- α at the membrane.

Keywords

STX; Estradiol; Lordosis; Arcuate nucleus; Estrogen receptor

Introduction

Estradiol signaling in the brain is mediated by several estrogen receptors (ERs) on the membrane, as well as by receptors in the nucleus. Both the classical estrogen receptors, ER α and ER β , and putative ERs initiate cell signaling from the membrane. These membrane effects include the phosphorylation of kinases and other proteins, regulation of ion channels, release of intracellular calcium stores, as well as regulation of transcription through the phosphorylation of cAMP response element-binding protein [1–3].

We and others had previously demonstrated that estradiol membrane signaling regulates several aspects of female reproduction [4, 5]. This signaling involves estradiol stimulation of mER α , which transactivates metabotropic glutamate receptor type 1a (mGluR1a), activating a novel protein kinase C, PKC θ [3, 5]. Site-specific application of estradiol in the arcuate nucleus of the hypothalamus (ARH) induces rapid excitation of a circuit responsible for

controlling lordosis behavior, the stereotypic sexually receptive behavior of female rodents. In the ARH, neuropeptide Y (NPY) Y1 receptors activate proopiomelanocortin (POMC) neurons that project to the medial preoptic nucleus (MPN) where they release β -endorphin within minutes of estradiol treatment [6]. The binding of β -endorphin to μ -opioid receptors (MORs) in the MPN produces MOR activation and internalization. This transient activation of MOR is required for full sexual receptivity. Thus, MOR internalization is a useful marker for activation of the lordosis-regulating circuit.

While ER α has been demonstrated to be necessary for the induction of receptivity and MOR activation [7–9], the demonstration that membrane-initiated signaling is involved suggests that one or more putative membrane ERs also have a role in the induction of sexual receptivity. One such putative membrane ER is activated by a tamoxifen analogue, STX [10–12], and has been designated the Gq-mER [13] or STX-binding receptor (STX-R) [14]. While the STX-R has not been cloned, its activation mimics estradiol activation of membrane ER α (mER α) in neurons and astrocytes [10, 15], and it is blocked by the ‘universal’ ER antagonist, ICI182,780. In neurons, STX activates a G α_q signaling cascade that includes the phosphorylation and activation of PKC δ ([10, reviewed in 16]). In astrocytes, STX induces the release of intracellular calcium stores [15]. Significantly, STX activity is retained in ER α /ER β double knockout mice suggesting that this ER is coded by a gene separate from the classic ERs [11].

Previous studies have shown that STX activates POMC neurons of the ARH [10] suggesting that the STX-R may also play a role in activating this part of the circuit regulating sexual receptivity. Tract tracing experiments indicate that ARH POMC neurons have diverse targets, only one of which is the MPN [Hong Wei Dong, unpubl. obs.]. The present experiments were designed to test the hypothesis that membrane ERs activation by STX induces activation of the ARH-MPN circuit, augmenting sexual receptivity. STX was microinjected into the ARH and both MOR internalization in the MPN, a measure of circuit activation, and lordosis behavior, a measure of sexual receptivity were determined.

Membrane ER α signaling has been mimicked with STX and was shown to be sensitive to mGluR1a blockade in cultured astrocytes [15]. Thus, to test whether STX-R signaling required the transactivation of mGluR1a, this glutamate receptor was blocked in the ARH. In rats primed with a sub-behavioral dose of estradiol, site-specific ARH microinfusions of STX activated sexual receptivity. STX induced MOR internalization, which was blocked by LY367,385 suggesting that the STX-R, like mER α may transactivate mGluR1a to initiate signaling.

Materials and Methods

Animals

Male and ovariectomized (ovx; by the supplier) female (200–250 g) Long-Evans rats were purchased (Charles River, Portage, Mich., USA). Upon arrival, rats were housed in a climate-controlled room, 2 per cage in a 12-hour light: 12-hour dark cycle room (lights on at 06:00 h) and provided food and water ad libitum. All experimental procedures were approved by the Chancellor's Animal Research Committee at the University of California, Los Angeles.

Steroid Priming

Animals were allowed to survive 2–3 weeks after ovx prior to steroid treatment. For all experiments, 17 β -estradiol benzoate (EB) was dissolved in safflower oil and injected subcutaneously in a volume of 0.1 ml per rat. Animals received 2 μ g EB or oil every fourth day between 09:00 and 10:00 h to mimic the increase of estradiol during the natural estrous cycle of female rats [17].

Guide Cannulae Implantation Surgery

Bilateral guide cannulae (22 gauge; Plastics One Inc., Roanoke, Va., USA) directed at the arcuate nucleus (coordinates from bregma: anterior –2.8 mm, lateral 0.8 mm, ventral –7.4 mm from dura; tooth bar: –3.3 mm) were implanted using standard stereotaxic procedures while female rats were anesthetized with isoflurane (2–3% in equal parts oxygen and nitrous oxide). Cannulae were secured to the skull with dental acrylic and stainless steel bone screws. Obturators (Plastics One) were placed in the cannulae, which protruded less than 0.5 mm beyond the opening of the guide cannulae. Animals were individually housed after surgery, received oral antibiotics (trimethoprim and sulfamethoxazole, 0.4 mg/ml; Hi-Tech Pharmacal, Amityville, N.Y., USA) in the drinking water and allowed to recover at least 6–7 days before infusion.

Microinjection

The selective ER modulator, STX (from Tom Scanlan at Oregon Health Sciences University) dissolved in DMSO, or the mGluR1 antagonist, (*S*)-(+)- α -amino-4-carboxy-2-methylbenzeneacetic acid (LY367,385; Tocris, Minneapolis, Minn., USA) dissolved in 100 mM NaOH, was infused into the ARH. Microinjections were 1 μ l (50 nmol) per side delivered with an infusion pump (Harvard Apparatus, Holliston, Mass., USA) at a rate of 1.0 μ l/min. This dose was determined to be the smallest effective dose for STX. For LY367,385, a 50-nmol dose was used, which we had demonstrated to be effective in the ARH in previous studies [5]. Microinjection needles (28 gauge) protruded 1 mm or less beyond the opening of the cannula and were allowed to remain in place for 1 min after infusion to allow for diffusion of the drug away from the injector. After microinjection, the obturators were reinserted into the guide cannulae and animals were returned to their home cage.

Behavioral Testing

Testing began 30 h after the third steroid injection. Stimulus males were acclimated to the testing arenas for at least 10 min. Sexual receptivity was measured by placing each female rat in a Plexiglas testing arena with a sexually experienced male. Males were allowed to mount females 10 times and the number of times the female displayed lordosis (lifting of the head, arching of the back, movement of the tail to one side) was recorded. For each female, the sexual receptivity was quantified as a lordosis quotient, i.e. the number of lordosis displays/number of mounts $\times$ 100.

Confirmation of Guide Cannulae Placement

Animals used for immunohistochemical studies were anesthetized and transcardially perfused with chilled 0.9% saline, followed with a fixative, 4% paraformaldehyde dissolved

in 0.2 M Sorenson's phosphate buffer (pH 7.4). Brains were removed and placed in fixative overnight at 4°C and then replaced with 20% sucrose in phosphate buffer for cryoprotection. Brains were blocked, sectioned (20 µm) on a cryostat (Leica CM 1800, Bannockburn, Ill., USA), and collected into chambers filled with PBS. Sections containing the microinjection sites were direct mounted onto Super-Frost/Plus slides (Fisher, Pittsburgh, Pa., USA), stained with thionin, dehydrated, and coverslipped with Krystalon (EMD Chemicals, Gibbstown, N.J., USA). Injection sites were mapped and verified with bright-field illumination for all animals in the study.

Rats with cannulae that were not positioned in the ARH (i.e. located above, lateral to the ARH, or where microinjections had compromised the wall of the third ventricle) were excluded from the study.

Immunohistochemistry

Sections from the MPN were collected as above. Sections were incubated with antibody directed against the µ-opioid receptor (MOR; 1:24,000; Neuromics, Edina, Minn., USA) with 0.5% Triton-X, 1% BSA, and 1% NGS in PBS for two nights at 4°C. Sections were washed before being incubated in biotinylated goat anti-rabbit secondary antibody (Vector Laboratories, Burlingame, Calif., USA) for 1 h at room temperature. The sections were washed in TBS with 0.05% Tween-20 (TNT) before being incubated in streptavidin-horseradish peroxidase (TSA kit; Perkin Elmer, Waltham, Mass., USA) for 30 min at room temperature. The sections were washed again in TNT. Finally, the sections were incubated in fluorescein (TSA kit; Perkin Elmer) for 5 min. The sections were washed in TNT and Tris buffer before being mounted onto slides. The slides were allowed to dry on a slide warmer and coverslipped with Aqua Poly/Mount (PolySciences Inc., Warrington, Pa., USA).

MOR Internalization

To quantify the internalization of MOR, images of the dorsal part of the medial MPN, at the level of maximal MOR staining as previously described [18, 19] were collected using a Zeiss Axioskop 2 equipped with epifluorescent illumination and an Axiocam CCD camera at ×360 magnification using Zeiss Axiovision (version 4.8). For quantification, 4 images were analyzed from each animal. Images were converted to grayscale in Adobe Photoshop (version 12.0.4, San Jose, Calif., USA) and adjusted for brightness and contrast to remove the background staining. To obtain an estimate of relative internalization, optical density was quantified using Image J (version 1.44p) in the pixel inverter function as previously described [5]. Briefly, a circle of about 60 µm was imposed over the MPN and a measurement is taken. The circle was then moved to an area of the picture that does not include the MPN and a background measurement was obtained. The background was subtracted from the MPN measurement to obtain the optical density of MOR staining in the MPN alone. Optical density has been correlated with receptor internalization [5, 6].

Statistics

All data are expressed as the mean ± SEM. MOR internalization data were analyzed by one-way ANOVA with Neuman-Keuls post hoc analysis. Sexual behavior data were analyzed by two-way ANOVA. Differences were considered significant at $p < 0.05$. Statistical analysis

was conducted using GraphPad Prism 5 (version 5.02, GraphPad Software, Inc., La Jolla, Calif., USA) software. The number of animals used in each experiment is specified in the 'Results'.

Results

MOR Internalization

We tested the ability of STX to regulate the activation of the ARH to MPN circuit. Ovariectomized rats were injected with 2 µg EB once every fourth day. On the day animals were to receive the EB injection of the third cycle, some animals were infused with STX rather than EB and perfused 1 h later. These animals showed MOR internalization that was equal to those treated with EB on the third cycle (fig. 1; one-way ANOVA: $p < 0.0001$; $F = 17.78$; $R^2 = 0.8163$). When animals were treated with STX and EB there was no greater internalization. In addition, to test whether estradiol priming was necessary, some females did not receive EB but instead received a single STX microinjection into the ARH and were killed 1 h later. These animals also showed MOR internalization equal to those that had been cycled (fig. 1; STX only; $p > 0.05$ vs. STX + oil and STX + EB).

To test whether STX-R, like ER α on the membrane, interacts with mGluR1a to initiate cell signaling and induce MOR activation/internalization [5], the mGluR1a was blocked prior to treatment with STX. Females infused with the mGluR1 antagonist LY367,385 and then 30 min later with STX had significantly lower levels of MOR internalization compared with animals in which mGluR1 had not been inhibited (fig. 1; LY + STX; $p < 0.05$ vs. STX + oil, STX + EB and STX only; but also $p < 0.05$ vs. LY + EB and LY + oil).

Lordosis

To test whether STX modulated lordosis behavior, females were cycled (injected s.c. once every fourth day) with 2 µg EB or oil. On the third cycle, animals were microinjected with STX or its vehicle, DMSO, and then received an EB injection. Animals were tested for sexual receptivity 30 h later. The subthreshold dose of EB resulted in a modest increase in sexual receptivity compared to oil-treated animals. However, STX was able to strongly increase lordosis behavior in females treated with 2 µg EB (fig. 2; two-way ANOVA, drug, $F(1,15) = 6.366$, $p = 0.0234$; steroid, $F(1,15) = 22.66$, $p = 0.0003$; interaction, $F(1,15) = 5.121$, $p = 0.0389$). As expected, STX was unable to induce lordosis behavior without estradiol priming, since lordosis behavior requires global activation of circuits that involves transcriptional and translational effects of estradiol in addition to membrane signaling. In this experiment, STX was introduced in a site-specific manner to the ARH.

Discussion

The major finding of the present communication is that the STX will augment female sexual receptivity. Like free estradiol and membrane constrained estradiol (E-biotin) [5], infusions of STX into the ARH activated the lordosis regulating circuit as demonstrated by MOR activation/internalization in the MPN. Indeed, infusions of STX into the ARH augmented the ability of a sub-behavioral dose of estradiol to induce sexual behavior, mimicking the estradiol activation of membrane ER α in this nucleus. Significantly, antagonizing mGluR1a,

which abrogates estradiol membrane signaling in the ARH, also strongly attenuated STX-mediated MOR activation/internalization in the MPN. These data are consistent with the emerging idea that there are several mERs involved in the estradiol activation of circuits in the CNS and that signaling through mGluRs is common [20].

While the STX-R has not been structurally characterized or its gene cloned, the present results indicate that it behaves like a mER. STX has no affinity for the classical ERs or for GPR30, another novel estrogen receptor (reviewed in [21]). The pharmacological profile of the STX-R is similar to mER α : STX and the mER α agonist PPT have similar dose-response curves when tested on calcium release in astrocytes [15] and STX action is blocked by the universal ER antagonist, ICI182,780 [11]. In the present study, we report that STX induces MOR internalization and stimulates sexual receptivity, like membrane-constrained estradiol (E-biotin).

The receptor activated by STX has been proposed to be a G α_q coupled membrane ER based on its ability to induce phosphorylation of a novel PKC, PKC δ in guinea pig ARH and uncouple an inwardly rectifying potassium current (GIRK) [10]. These actions have been tied into the estradiol activation of POMC neurons. The present results that LY367,385 inhibition of STX reduced MOR internalization is similar to LY367,385 blockade of STX-induced calcium release in astrocytes [15] and involves mGluR1a in STX signaling within neurons and astrocytes [1, 5, 22]. Such results suggest either a direct STX action on mGluR1a or an interaction between STX-R and the mGluR1a [15]. The former explanation of a direct action is suggested by results that show STX activity in ER α /ER β double knockout mice [11] and in hippocampal neurons derived from males where estradiol has no effect on cAMP response element-binding protein phosphorylation but the STX action remained [Mermelstein, pers. commun.]. However, this hypothesis must be rejected because LY367,385 did not completely block STX-induced MOR internalization, and because of the observation that the specific ER antagonist ICI 182,780 blocked STX action, but did not abrogate mGluR effects [1, 11]. The remaining hypothesis that STX-R interacts or transactivates the mGluR1a is supported by the present results, but cannot be directly tested using co-immunoprecipitation studies, such as have been done for mER α and mGluR1a, until the STX activated protein is characterized [5, 22].

Although STX had estradiol-like actions, MOR internalization and facilitation of lordosis behavior, following infusions into the ARH, the reproductive physiological significance of the STX-R remains opaque. STX retains its activity in both ER α knockout mice and ER α /ER β double knockout mice [11, 15], but female ER α knockout mice are not sexually receptive [23]. One possibility is that STX activates a membrane protein that mediates ER α action, stimulating intracellular cascades but STX cannot activate transcription, which is absolutely critical for inducing lordosis behavior. This activation appears to require nuclear ER α .

Typically when examining the role of a particular drug in a specific site that regulates lordosis behavior, the rat is treated with a systemic subthreshold (i.e. sub-behavioral) dose of estradiol. While such an estradiol dose is insufficient to induce substantial lordosis behavior by itself, it primes the widespread circuits needed to elicit a lordosis response. In the present

experiment, ovx animals were treated with such a sub-threshold dose (2 μ g EB), which induced a very low level of lordosis behavior by itself. When STX was microinjected into the ARH of these rats, lordosis behavior was significantly stimulated. This results indicate that 2 μ g EB is sufficient to activate transcription, but requires a 'trigger'. Over the years several triggers have been identified. The quintessential trigger is progesterone, which augments estradiol to induce full sexual receptivity [19]. Other agents include oxytocin, vasopressin, CCK, GnRH, substance P, galanin, IGF-1, glutamate and dopamine (reviewed in [24]). Interestingly, estradiol acting through membrane ER α , can also augment a previous subthreshold priming dose [4, 5]. What has emerged from a number of studies is that mER signaling accounts for a part of the estradiol regulation of female sexual receptivity (reviewed recently in [20, 25, 26]). Since the activation of MOR is a hallmark of the rapid, membrane action of estradiol, we used MOR knockout mice to assess the estradiol membrane signaling component inducing lordosis behavior [27]. In these mice, MOR knockdown attenuated but did not eliminate lordosis behavior, suggesting that the major component of estradiol action requires nuclear action. The present results indicate that in addition to membrane ER α , STX, which activates a novel ER, is capable of facilitating lordosis. STX without a priming dose of estradiol was incapable of inducing lordosis behavior, which is consistent with the demonstrated need for ER α [23]. Also, these data indicate that activation of MOR in the MPN is correlated with, but by itself is not sufficient to stimulate behavior. While STX alone powerfully induced MOR internalization, it did not induce sexual receptivity.

It is surprising that both mER α and STX-R appear to act through mGluR1a. Membrane ER α and STX activate mGluR1a to initiate a G α_q signaling cascade leading to activation of novel PKCs and elevation of free cytoplasmic calcium [3, 10, 15]. ER α transactivation of mGluR has been demonstrated in hippocampal, striatal and primary sensory neurons, as well as in astrocytes [1, 5, 22, 28, 29]. This is the second demonstration that STX activation of a putative ER requires mGluR1a. In primary cultures of adult astrocytes, STX induced mobilization of intracellular calcium and progesterone synthesis, which was blocked with LY367,385, the mGluR1a antagonist [15]. In those cells, estradiol, the ER α agonist PPT, and STX had a similar dose-response relationship. In fact, STX was actually more efficacious than either estradiol or PPT at inducing progesterone synthesis [15]. It is interesting that estradiol or STX induction of progesterone synthesis does not require transcription, and STX is fully functional. Taken together with the present results, this suggests that STX is capable of estradiol-like membrane signaling that does not require direct transcriptional regulation. In the whole animal, however, regulation of sexual receptivity requires a phase of transcription [30], as does the estradiol induction of hypothalamic progesterone synthesis [31].

In summary, STX, like membrane-constrained estradiol, when microinjected into the ARH, activated ARH to MPN neurotransmission as demonstrated by MOR internalization in the MPN. Like mER α , STX-R transactivation of mGluR1a is needed to facilitate female sexually receptive behavior. These results underscore the importance membrane estradiol cell signaling in the CNS regulation of behavior and of characterizing novel ERs such as STX-R.

Acknowledgments

Supported by DA013185 and T32 HD07228.

References

1. Boulware MI, Weick JP, Becklund BR, Kuo SP, Groth RD, Mermelstein PG. Estradiol activates group I and II metabotropic glutamate receptor signaling, leading to opposing influences on cAMP response element-binding protein. *J Neurosci*. 2005; 25:5066–5078. [PubMed: 15901789]
2. Abraham IM, Han SK, Todman MG, Korach KS, Herbison AE. Estrogen receptor beta mediates rapid estrogen actions on gonadotropin-releasing hormone neurons in vivo. *J Neurosci*. 2003; 23:5771–5777. [PubMed: 12843281]
3. Dewing P, Christensen A, Bondar G, Micevych P. Protein kinase C signaling in the hypothalamic arcuate nucleus regulates sexual receptivity in female rats. *Endocrinology*. 2008; 149:5934–5942. [PubMed: 18653714]
4. Kow LM, Pfaff DW. The membrane actions of estrogens can potentiate their lordosis behavior-facilitating genomic actions. *Proc Natl Acad Sci USA*. 2004; 101:12354–12357. [PubMed: 15302933]
5. Dewing P, Boulware MI, Sinchak K, Christensen A, Mermelstein PG, Micevych P. Membrane estrogen receptor-alpha interactions with metabotropic glutamate receptor 1a modulate female sexual receptivity in rats. *J Neurosci*. 2007; 27:9294–9300. [PubMed: 17728443]
6. Mills RH, Sohn RK, Micevych PE. Estrogen-induced mu-opioid receptor internalization in the medial preoptic nucleus is mediated via neuropeptide Y-Y1 receptor activation in the arcuate nucleus of female rats. *J Neurosci*. 2004; 24:947–955. [PubMed: 14749439]
7. Micevych PE, Rissman EF, Gustafsson JA, Sinchak K. Estrogen receptor-alpha is required for estrogen-induced mu-opioid receptor internalization. *J Neurosci Res*. 2003; 71:802–810. [PubMed: 12605406]
8. Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O. Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc Natl Acad Sci USA*. 1993; 90:11162–11166. [PubMed: 8248223]
9. Rissman EF, Wersinger SR, Taylor JA, Lubahn DB. Estrogen receptor function as revealed by knockout studies: neuroendocrine and behavioral aspects. *Horm Behav*. 1997; 31:232–243. [PubMed: 9213137]
10. Qiu J, Bosch MA, Tobias SC, Grandy DK, Scanlan TS, Ronnekleiv OK, Kelly MJ. Rapid signaling of estrogen in hypothalamic neurons involves a novel G-protein-coupled estrogen receptor that activates protein kinase C. *J Neurosci*. 2003; 23:9529–9540. [PubMed: 14573532]
11. Qiu J, Bosch MA, Tobias SC, Krust A, Graham SM, Murphy SJ, Korach KS, Chambon P, Scanlan TS, Ronnekleiv OK, Kelly MJ. A G-protein-coupled estrogen receptor is involved in hypothalamic control of energy homeostasis. *J Neurosci*. 2006; 26:5649–5655. [PubMed: 16723521]
12. Tobias SC, Qiu J, Kelly MJ, Scanlan TS. Synthesis and biological evaluation of serms with potent nongenomic estrogenic activity. *Chem Med Chem*. 2006; 1:565–571. [PubMed: 16892393]
13. Roepke TA, Qiu J, Bosch MA, Ronnekleiv OK, Kelly MJ. Cross-talk between membrane-initiated and nuclear-initiated oestrogen signalling in the hypothalamus. *J Neuroendocrinol*. 2009; 21:263–270. [PubMed: 19187465]
14. Kenealy BP, Terasawa E. Rapid direct action of estradiol in GNRH neurons: findings and implications. *Front Endocrinol (Lausanne)*. 2011; 2:106. [PubMed: 22654841]
15. Kuo J, Hamid N, Bondar G, Prossnitz ER, Micevych P. Membrane estrogen receptors stimulate intracellular calcium release and progesterone synthesis in hypothalamic astrocytes. *J Neurosci*. 2010; 30:12950–12957. [PubMed: 20881113]
16. Micevych P, Kelly MJ. Membrane estrogen receptor regulation of hypothalamic function. *Neuroendocrinology*. 2012; 96:103–110. [PubMed: 22538318]
17. Micevych PE, Abelson L, Fok H, Ulibarri C, Priest CA. Gonadal steroid control of preprocholecystokinin mRNA expression in the limbic-hypothalamic circuit: comparison of adult with neonatal steroid treatments. *J Neurosci Res*. 1994; 38:386–398. [PubMed: 7932871]

18. Eckersell CB, Popper P, Micevych PE. Estrogen-induced alteration of mu-opioid receptor immunoreactivity in the medial preoptic nucleus and medial amygdala. *J Neurosci.* 1998; 18:3967–3976. [PubMed: 9570823]
19. Sinchak K, Micevych PE. Progesterone blockade of estrogen activation of mu-opioid receptors regulates reproductive behavior. *J Neurosci.* 2001; 21:5723–5729. [PubMed: 11466444]
20. Micevych P, Dominguez R. Membrane estradiol signaling in the brain. *Front Neuroendocrinol.* 2009; 30:315–327. [PubMed: 19416735]
21. Qiu J, Ronnekleiv OK, Kelly MJ. Modulation of hypothalamic neuronal activity through a novel G-protein-coupled estrogen membrane receptor. *Steroids.* 2008; 73:985–991. [PubMed: 18342349]
22. Kuo J, Hariri OR, Bondar G, Ogi J, Micevych P. Membrane estrogen receptor-alpha interacts with metabotropic glutamate receptor type 1a to mobilize intracellular calcium in hypothalamic astrocytes. *Endocrinology.* 2009; 150:1369–1376. [PubMed: 18948402]
23. Ogawa S, Eng V, Taylor J, Lubahn DB, Korach KS, Pfaff DW. Roles of estrogen receptor-alpha gene expression in reproduction-related behaviors in female mice. *Endocrinology.* 1998; 139:5070–5081. [PubMed: 9832446]
24. Micevych, P.; Sinchak, K. The neurochemistry of limbic-hypothalamic circuits regulating sexual receptivity. In: Blaustein, J., editor. *Handbook of Neurochemistry and Molecular Neurobiology.* Vol. 21. Berlin: Springer; 2006.
25. Micevych PE, Dewing P. Membrane-initiated estradiol signaling regulating sexual receptivity. *Front Endocrinol.* 2011; 2:26.
26. Micevych P, Christensen A. Membrane-initiated estradiol actions mediate structural plasticity and reproduction. *Front Neuroendocrinol.* 2012; 33:331–341. [PubMed: 22828999]
27. Sinchak K, Shahedi K, Dewing P, Micevych P. Sexual receptivity is reduced in the female mu-opioid receptor knockout mouse. *Neuroreport.* 2005; 16:1697–1700. [PubMed: 16189480]
28. Chaban V, Li J, McDonald JS, Rapkin A, Micevych P. Estradiol attenuates the adenosine triphosphate-induced increase of intracellular calcium through group II metabotropic glutamate receptors in rat dorsal root ganglion neurons. *J Neurosci Res.* 2011; 89:1707–1710. [PubMed: 21793040]
29. Grove-Strawser D, Boulware MI, Mermelstein PG. Membrane estrogen receptors activate the metabotropic glutamate receptors mGluR5 and mGluR3 to bidirectionally regulate CREB phosphorylation in female rat striatal neurons. *Neuroscience.* 2010; 170:1045–1055. [PubMed: 20709161]
30. Parsons B, Rainbow TC, Pfaff DW, McEwen BS. Hypothalamic protein synthesis essential for the activation of the lordosis reflex in the female rat. *Endocrinology.* 1982; 110:620–624. [PubMed: 7035143]
31. Soma KK, Sinchak K, Lakhter A, Schlinger BA, Micevych PE. Neurosteroids and female reproduction: estrogen increases 3beta-HSD mRNA and activity in rat hypothalamus. *Endocrinology.* 2005; 146:4386–4390. [PubMed: 16020475]

Fig. 1.

An mGluR1 antagonist inhibits STX-induced MOR internalization. Ovx female rats treated with a low systemic dose of estradiol (2 μ g) showed an increase in MOR internalization/activation that is similar to animals in which STX was infused directly into the ARH (STX + oil). In addition, animals needed no priming with EB to be responsive to STX (STX only). Both the estradiol-induced and the STX-induced internalization of MOR were inhibited by the mGluR1a antagonist LY367,385 (LY).

* $p < 0.005$ versus oil ($n = 4-7$ rats).

Fig. 2.

STX-induced lordosis behavior in estradiol-primed rats. Females were injected every fourth day with a sub-behavioral dose of estradiol (2 μ g) or oil control for two cycles. 30 min prior to the injection on the third cycle, animals were infused with STX, which augmented sexual receptivity in estradiol primed rats measured 30 h later. Those rats treated with STX and oil had no increase in sexual receptivity. * $p < 0.05$ compared with DMSO + EB; † $p < 0.05$ compared with DMSO + oil ($n = 4-6$ rats).