NEURAL REGENERATION RESEARCH
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Sexual dimorphism of G protein-coupled receptor signaling in the brain

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G protein-coupled receptors (GPCRs) represent the most substantial family of membrane receptors that are targeted by U.S. Food and Drug Administration-approved drugs. Much of the preclinical research to understand the pharmacology of many membrane receptors including GPCRs is derived from studies in male animal models (Karp and Reavey, 2019). This can be of concern as emerging evidence reveals unexpected sex-dependent differences in GPCR pharmacodynamics. Thus, understanding the sex-specific disparities in GPCR pharmacological fingerprints can aid in developing targeted ligands that precisely correct GPCR signaling modalities in men and women and ultimately, enhance their effectiveness for certain pathophysiological conditions. We will attempt to summarize the current evidence supporting the sex-biased signaling of GPCRs implicated in several brain disorders.

One of the pathological hallmarks of Alzheimer's disease is the deposition of amyloid-β (Aβ) in the brain that is commonly detected in both males and females. Soluble Aβ oligomers disrupt the signaling of the GPCR, metabotropic glutamate receptor 5 (mGluR5), by forming a ternary complex with the receptor in the presence of cellular prion protein (PrP^c) in male but not female brain. Interestingly, this sex-selective Aβ-mGluR5 interaction was not dependent on estrogen, but rather on the ability of mGluR5 to interact with PrP^c, which was only detectable in the male brain. Therefore, the use of pharmacological negative allosteric modulator of mGluR5, CTEP (2-chloro-4-[2-[2,5-dimethyl-1-[4-(trifluoromethoxy)phenyl]imidazol-4-yl] ethynyl]pyridine), was effective in reducing Aβ pathology and cognitive deficits in male APPswe/PS1ΔE9 mice only. These results indicate that, unlike males, mGluR5 does not play a role in Aβ-triggered pathology in females and that sex-specific stratification should be considered when evaluating AD therapeutics in clinical trials (Abd-Elrahman et al., 2020). Furthermore, recent work has established the efficacy of the M1 muscarinic acetylcholine receptor in reducing neuropathology and cognitive impairment in female APPswe/PS1ΔE9 mice but its utility in males remains to be determined (Abd-Elrahman et al., 2022).

Recent work on mGluR5 contribution to Huntington's disease pathophysiology shows that the receptor signals in a sex-biased manner to alter the progression of the disease in zQ175 mice. Unlike males, the mGluR5 negative allosteric modulator CTEP did not improve the grip strength nor reverse the cognitive decline of female zQ175 mice. However, CTEP reduced the number of huntingtin aggregates, improved neuronal survival, and decreased microgliosis in both male and female zQ175 mice. These results suggest that while mGluR5 plays a key role in the pathophysiology of Huntington's disease, sex can alter the efficacy of mGluR5 ligands and must be considered when developing drugs that target this receptor for Huntington's disease patients (Li et al., 2022).

Psychedelics, such as 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), are well known to impact perception, cognition, and sensory processing by acting on the serotonin $5\text{-}HT_{2A}$ receptor. Recent work examined the impact of DOI on head-twitch behavior, which is a behavior in mice believed to mirror human psychedelic experiences. Surprisingly, DOI elicited a more pronounced head-twitch response in female C57BL/6J mice compared to males. Moreover, the pharmacokinetic properties of DOI differed between the sexes, with lower brain and plasma concentrations observed in female C57BL/6J mice compared to males (Jaster et al., 2022). These data indicate that sex can also influence the pharmacokinetic properties of GPCR ligands and should be considered in the drug discovery process.

Interestingly, some other 5-HT GPCR-targeted ligands used for the management of neuropsychiatric conditions, including depression and bipolar have been found to preferentially affect more women than men. One possible explanation for this preferential impact could be attributed to the cyclic changes in estrogen and the elevated basal levels of estrogen in females. Specifically, estrogen is known to interfere with serotonin biosynthesis and affect 5-HT GPCR densities in tissues, and therefore, 5-HT GPCR-targeted drugs are expected to exhibit divergent effects in females. Moreover, females have lower levels of serotonin and a faster rate of serotonin metabolism. Thus, it is possible that the above-discussed sex-specific differences between 5-HT pharmacokinetics and 5-HT GPCRs pharmacodynamics are responsible for the more severe side effects of this class of drugs in females (Jamu and Okamoto, 2022).

Mounting evidence shows that activating GPR30, a membrane-bound estrogen GPCR, has different effects on anxiety in males versus females. Injections of the specific GPR30 agonist G-1 in castrated male mice induced anxiety phenotype, an effect that was not observed in ovariectomized female mice. G-1 administration was also associated with increased nuclear estrogen receptor phosphorylation through extracellular regulated kinase in the ventral hippocampus in male mice only. However, G-1 increased extracellular regulated kinase activation in the dorsal hippocampus in female mice independent of anxiety phenotype (Hart et al., 2014). Another study on the orphan receptor GPR50 showed evidence of an association between GPR50 genetic variants and late-life depression in women but not men. Specifically, women homozygous for the minor allele of *rs2072621* had a 4-fold increased risk of depression and anxiety whereas women heterozygous for *rs13440581* had a 1.6-fold increased risk of depression. This association appeared to be specific to women and those with more severe forms of depression (Ryan et al., 2015).

Cerebral blood flow homeostasis is key for the functional integrity of brain functions and impaired control of blood flow is implicated in many neurodevelopmental, neuropsychiatric, and neurodegenerative diseases. The endothelial lining of cerebral vasculature plays a crucial role in optimizing blood flow to the brain via several mechanisms including endothelium-derived hyperpolarization. This pathway is triggered primarily by the stimulation of endothelial G_q-protein coupled receptors, such as purinergic P2Y receptors, that lead to an increase in Ca^{2+} influx. The rise in intracellular Ca^{2+} activates small- and intermediate-Ca^{2+}-activated K^+ (SKCa/IKCa) channels leading to membrane hyperpolarization that spreads to vascular smooth muscles leading to vasodilation (Behringer and Hakim, 2019). Interestingly, Hakim et al. (2020) show that purinergic receptor function is enhanced in old female mice leading to a more substantial rise in intracellular Ca^{2+} compared to age-matched males. However, upon administration of a positive modulator of SKCA/IKCa, Ca^{2+}-sensitive K^+ channel function was less impaired in old females compared to age-matched males. This evidence of a sex-specific difference in endothelial GPCR and K^+ channels function in cerebral vasculature suggests that females have a greater resilience to maintain optimal cerebral blood flow compared to males in old age and points to key differences between males and females in the GPCR-regulated mechanisms of cerebral blood flow homeostasis (Hakim et al., 2020).

Recent work demonstrates sex differences in the effects of the selective agonist, U50,488H, on mouse kappa opioid receptor phosphorylation (KOR). The phosphorylation of KOR triggers the recruitment of β-arrestins, which leads to desensitization of the receptor as well as initiation of β-arrestin-mediated signaling and therefore modulates physiological function. Interestingly, U50,488H promotes the phosphorylation of KOR at four residues in the C-terminal domain *in vitro*. To understand the functional significance of agonist-induced KOR phosphorylation *in vivo*, a mutant mouse line (K4A) with all four phosphorylation sites mutated was generated. U50,488H promoted KOR phosphorylation in the brains of male and female wildtype mice, but not K4A mice. Moreover, in both genotypes, U50,488H showed similar inhibitory effects on pharmacologically induced scratching. However, repeated pre-treatment with U50,488H resulted in tolerance to its anti-scratching effect in both male and female wildtype mice and female K4A mice, with male K4A mice showing a less pronounced tolerance. In addition, to measure the rewarding and aversive effects of U50,488H, a conditioned place aversion (CPA) test was employed. Results demonstrate that K4A mutations eliminated U50,488H-induced CPA in female mice while having no effect on male mice. Unexpectedly, a higher dose of U50,488H induced CPA in male mice,

but not in females regardless of the genotype. These findings suggest that agonist-induced KOR phosphorylation is strongly associated with U50,488H-induced tolerance and CPA in a sex-dependent manner (Huang et al., 2022). It is also worth noting that Gabel et al. reported that female mice exhibit lower antinociception following the administration of morphine compared to males due to sex differences in the central metabolism of morphine (Gabel et al., 2023). Another promising therapeutic GPCR target of chronic pain is the G-protein-coupled receptor 171 (GPR171) that exhibits close homology to the purinergic GPCR, P2Y. The GPR171 agonist, MS15203, displays sexual dimorphism in alleviating chronic pain in mouse models. In female mice, MS15203 failed to alleviate thermal hypersensitivity and allodynia whereas it was able to reduce the duration of hypersensitivity and improve symptoms of allodynia in male mice (Ram et al., 2021).

In conclusion, we highlight the importance of studying sex as a critical biological variable in GPCR signaling of the brain. Further work, however, is necessary to profile the sex-biased pharmacology of the rest of the GPCR candidates in the brain and provide a better understanding of the underlying biological causes behind these observed sex differences. Plausible causes for sex-selective receptor pharmacodynamics may include variations in receptor isoforms, orthosteric/allosteric binding sites, receptor distribution/expression across tissue, secondary messenger signaling cascades, expression of adaptor proteins, or metabolism of ligands between males and females. It is also important to note that sex-specific differences are not always triggered by variations in sex hormone levels but could also be due to intrinsic differences between sexes (**Figure 1**). A more comprehensive understanding of the underlying mechanisms leading to sex-biased GPCR pharmacology could aid in the development of ligands that are tailored to target GPCRs in each sex and thereby improve efficacy and reduce the occurrence of adverse side effects. We trust that embracing sex-specific differences in GPCR pharmacology will revolutionize the drug-discovery process for many brain diseases in the future.

KSAE is a Michael Smith Health Research BC funded Health-Professional investigator. This work was supported by a New Investigator grant from the Alzheimer's Society of Canada and Alzheimer Disease Research Grant from Djavad Mowafaghian Centre for Brain Health (to KSAE).

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Date of submission: August 9, 2023
Date of decision: September 21, 2023
Date of acceptance: October 11, 2023
Date of web publication: December 11, 2023

https://doi.org/10.4103/1673-5374.389637
How to cite this article: *Aljoudi S, Hamdan H, Abd-Elrahman KS (2024) Sexual dimorphism of G protein-coupled receptor signaling in the brain. Neural Regen Res 19(8):1635-1636.*

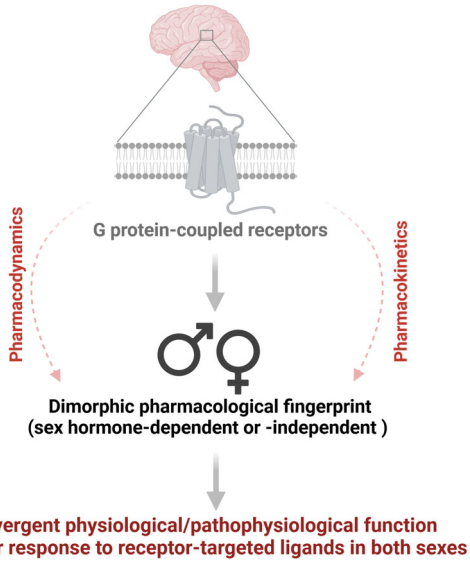

Figure 1 | **Sexual dimorphism in the pharmacological fingerprint of brain G protein-coupled receptors (GPCRs).** The pharmacodynamics and pharmacokinetics properties of GPCRs and their targeted ligands can differ between males and females that could be sex-hormone-dependent or-independent. These differences can lead to divergent physiological/physiological functions in the brain and/or efficacy of GPCR-targeted ligands in some brain disorders between both sexes. Created with BioRender.com.

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C-Editors: Zhao M, Sun Y, Qiu Y; T-Editor: Jia Y