Journal Club

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Molecular Underpinnings of Estradiol-Mediated Sexual Dimorphism of Synaptic Plasticity in the Hippocampus of Rodents

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Review of Wang et al.

On a population level, males and females excel at different kinds of memory tasks. In particular, men overall have a greater ability to remember spatial representations, whereas women perform better on tasks requiring semantic memory (Andreano and Cahill, 2009). Initial encoding of different types of memories depends on synaptic plasticity in the hippocampus, and previous work has highlighted sexual dimorphism in these mechanisms. Differences have been found, for example, in the induction of long-term potentiation (LTP) at the Schaffer collateral (SC) synapses, which constitute the main excitatory input from CA3 to CA1 of the hippocampus (Vierk et al., 2012).

The sex hormone estradiol (E2) is likely to participate in the molecular underpinnings of sex differences in memory formation, given its local synthesis and widespread effects in the brain (Woolley, 2007). In fact, the inhibition of local E2 production leads to the loss of hippocampal LTP in female, but not in male, rodents (Vierk et al., 2012; Bender et al., 2017). Nonetheless, when exogenous E2 is applied to hippocampal synapses, LTP is enhanced in both sexes (Kramár et al., 2009). This, in conjunction with the presence of higher levels of hippocampal E2 in males (Ooishi et al., 2012), points toward potential differences in how E2 affects synaptic plasticity in males and females. These might include disparities in the principal estrogen receptor (ER) subtypes involved and alterations in the signaling molecules downstream of these receptors that interact with the LTP pathway.

To investigate these possibilities, Wang et al. (2018) first sought to identify the receptor subtypes that mediate E2 effects on SC-LTP. Application of ERα-selective antagonist during theta burst stimulation (TBS) in acute brain slices permitted initial potentiation but impaired stabilization of SC-LTP in female, but not in male

The normal initiation of SC-LTP in females and the immediate effects of antagonist application on LTP stabilization suggest that the effects rely on local synaptic changes as opposed to ER-driven changes in gene expression at the nucleus. To confirm this, Wang et al. (2018) used hippocampal slices taken from NOER (nuclear-only ER1) and MOER (membrane-only ERα) mutant mice, which lack membrane-associated or nuclear ERα, respectively (Pedram et al., 2013, 2014). As predicted, in slices taken from female NOER mice, LTP failed to stabilize, whereas LTP was unaffected in female MOER mice. In male mice, LTP was not altered by either mutation. Although these results do not exclude contributions of nuclear ERα to longer-term LTP maintenance, they strongly suggest that membrane-associated ERα is necessary for normal SC-LTP stabilization in females. Collectively, these observations provide compelling evidence that the sexually dimorphic effects of E2 on SC-LTP originates from its interactions with the ERα receptor in females, and they suggest that the downstream effects are likely to occur at the synapse rather than in the nucleus.

Next, Wang et al. (2018) investigated the relevant molecular signaling mechanisms involved in this ERα-mediated effect. An involvement of the kinases Src and Erk1/2 is likely, given their role in the immediate component of NMDAR-associated signaling and their link to ERα in neurons (Woolley, 2007). Wang et al. (2018) did, in fact, find that the increased colocalization of phosphorylated Erk1/2 and Src with PSD-95 that normally occurs after TBS was abolished in the presence of a selective ERα antagonist in females, but not in males. Furthermore, changes in the actin cytoskeleton that normally occur downstream of Src and Erk1/2 activation and are required for LTP stabilization were comparable between the sexes. Together, these results suggest that ERK1/2

and Src signaling are the critical targets of ERα action in the context of LTP.

Wang et al. (2018) also found that ERα is expressed at higher levels in female than in male CA1, regardless of estrus stage. More specifically, both the density of postsynaptic ERα and the relative percentage of ERα-positive synapses were greater in females, whereas expression levels of ERβ and GPER1 were similar in males and females. This observation is crucial as it provides a primary neurobiological basis for the ERα dependency of female SC-LTP and its associated down–stream signaling. As a consequence of the lower ERα levels, the effects of E2 on SC-LTP are likely to be smaller in males than in females. This might explain why ERα antagonism affects SC-LTP differentially in the two sexes.

After finding that activation of ERα increases phosphorylation of ERK and Src even in the absence of TBS, Wang et al. (2018) predicted that the effects of E2 on the phosphorylation of these kinases would be additive with regard to LTP. In females, phosphorylation of these kinases is ERα dependent, suggesting that TBS-dependent mechanisms for their activation are weaker in females than in males. If so, the threshold for SC potentiation in females should be higher in the absence of E2 than in its presence. Electrophysiological recordings showed that stimulation with five theta-burst pairs at 2 min intervals was sufficient to elicit SC-LTP in male, but not female, slices. In contrast, the application of four theta-burst triplets resulted in robust LTP in females. When E2 was infused during two-burst stimulation in female slices, LTP levels resembled those seen in male slices. This effect was mediated by both ERα and ERβ, as revealed by the application of the respective receptor antagonists. Therefore, these results support the hypothesis that E2 lowers the threshold for LTP induction threshold in females.

The ability of E2 to lower the threshold for inducing SC-LTP in female mice suggests that memory might be influenced by circulating E2 levels. Wang et al. (2018) tested this hypothesis with an object–location memory task in proestrus and nonestrus female mice, in which circulating levels of E2 are higher and lower, respectively. During a 5 min encoding period, animals explored two objects in an arena. Hippocampus-dependent memory was assessed 24 h later, after the spatial location of one object had been changed. Female mice in proestrus spent significantly more time exploring the object in the novel location than their nonestrus counterparts did, suggesting better encoding and recall of memory when the circulating E2 level was higher. This effect was abolished by peripheral application of ERα- or ERβ-selective antagonists in proestrus animals, consistent with the earlier *in vitro* results.

Arguably, the most perplexing result of the Wang et al. (2018) study is that TBS-induced depolarizing potentials, in the absence of ERα function, are insufficient to activate Erk1/2 and Src signaling and to induce stable SC-LTP in females despite being adequate in males. Wang et al. (2018) propose that this stems from sexually dimorphic cation influx-independent interactions between NMDARs and submembrane proteins. For example, metabotropic signaling of NMDARs regulates Src activity during excitotoxicity (Weilinger et al. 2016), and Ca^{2+} independent co-activation of NMDARs and mGLuR5 has been shown to increase the phosphorylation of ERK (Yang et al. 2004). Why such mechanisms differ between males and females remains unclear. Variations in subunit composition of NMDARs between males and females provide a simple explanation, but this seems unlikely because initial NMDAR activity was comparable between the sexes. Divergence in function could instead occur by virtue of NMDAR modulation. Other work has, in fact, demonstrated that E2-dependent signaling via G-protein-coupled receptor-30 can regulate NMDAR function in the rodent hippocampus (Liu et al., 2012). The receptor partly exerts this effects by coupling to the Erk1/2 signaling cascade and phosphorylating NMDARs, which might alter the downstream coupling of the receptor to other effectors (Dore et al., 2017).

A second explanation for the difference in the E2 dependence of LTP in males and females might be structural and functional differences in cholinergic signaling between the sexes. The cholinergic system directly modulates NMDAR function (Flores-Hernandez et al., 2009), and the expression of sex hormone receptors within cholinergic neurons is known to differ between sexes. In rodents, subsets of male cholinergic neurons express androgen receptors whereas female cholinergic neurons express ERα (Giacobini and Pepeu, 2018). Moreover, the effects of E2 on NMDARs have been shown to be mediated via the cholinergic system in females but not males (Volosin et al., 2006; Hyer et al., 2018). Overall, these findings point toward a cooperative role of E2 signaling and the cholinergic system in the modulation of NMDAR function.

Can alternative mechanisms account for the sexual dimorphism of hippocampal LTP? Another study (Qi et al., 2016) focused on a different hippocampal circuit, the temporoammonic (TA)–CA1 pathway. In brief, they found that male rats express more GluA1-containing AMPARs and GluN1-containing NMDARs on the postsynaptic membrane of TA–CA1 synapses than female rats. Furthermore, male rats displayed larger TA–CA1 LTP and better long-term (>7 d) retention of spatial memory in the Morris water maze. This suggests that differing molecular pathways can underpin the sexual dimorphism of LTP in different circuits. Therefore, cognitive differences between males and females likely result from the complex interplay of multiple sexually dimorphic pathways.

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