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Endocannabinoid signalling is required for estrogen-dependent modulation of inhibitory transmission

It has long been known that the classic female estrogen, 17β-estradiol (E2), is produced in multiple brain regions including the hypothalamus, hippocampus, as well as sensory areas such as the auditory and visual cortices (McEwen 2002; Woolley 2007; Jeong *et al.* 2011; Tremere *et al.* 2011; Pinaud and Tremere 2012). Importantly, this brain-generated E2, distinct from the gonadal hormone, exerts local effects by acting on estrogen receptors that are also expressed in these diverse brain areas. As such, E2 derived in the brain has been shown to play multiple functional roles ranging from sensory processing, reproductive biology and cognitive processes including those supporting learning and memory formation (McEwen 2002; Woolley 2007; McCarthy 2008; Pinaud and Tremere 2012). Notably, many of E2's effects on neuronal physiology occur rapidly – on a scale of seconds; such rapidity is achieved by E2's actions on the intrinsic and synaptic communication between neurons and via non-genomic mechanisms (Woolley 2007; Pinaud and Tremere 2012). The mechanistic bases of these actions are not well understood and vary across brain regions.

In a recent article published in *Neuron*, Huang and Woolley (2012) shed significant light on the mechanisms through which E2 modulates synaptic transmission in the adult hippocampus, a brain structure heavily implicated in learning and memory (Huang and Woolley 2012). Specifically, the authors revealed a complex basis for E2's suppressive effects on peri-somatic inhibitory neurotransmission for pyramidal cells in the hippocampal CA1 subfield. Surprisingly, the effects of E2 on inhibitory transmission were sex specific, occurring in female, but not male, adult rats despite both being subjected to gonadectomization prior to the experiments.

Huang and Woolley used hippocampal slices where recordings were obtained from CA1 pyramidal cells. Bath application of E2 was used to explore its impact on inhibitory neurotransmission. Interestingly, E2 largely suppressed the amplitude of both unitary and compound inhibitory post-synaptic currents (IPSCs), similar to findings obtained in other preparations (Tremere *et al.* 2009). The authors next demonstrated that such modulatory effects of E2 are mediated by the classic intracellular estrogen receptor α (ERα) given that PPT, a selective ERα agonist, produced effects that were identical to E2 on IPSC amplitude and paired-pulse ratios. No effects were detected for ERβ, as activation of this receptor subtype with a selective agonist (DPN) failed to impact E2-sensitive IPSCs.

One of the most interesting twists of the Huang and Woolley studies was the finding that activation of ERα alone was not sufficient to alter inhibitory transmission. On CA1 neurons, suppression of inhibition requires both the actions of type-1 cannabinoid receptors (CB1Rs) and metabotropic glutamate receptors, in particular mGluR1-containing receptors (Katona *et al.* 1999). Huang and Woolley showed, however, that in the absence of functional CB1R signalling, E2 is incapable of suppressing inhibitory responses, thereby establishing that E2's actions at CA1 neurons depend on cannabinoid signalling through CB1Rs. Specifically, when CB1Rs were blocked, neither E2 nor the ERα agonist affected inhibitory neurotransmission. The reverse scenario was also true: activation of CB1Rs with a selective agonist could largely capture the E2-mediated suppression of IPSCs and paired pulse ratios.

In light of the original discovery that E2's effects were strongly connected to CB1R signalling, a deeper appreciation of this relationship was sought after by the authors. To this end, Huang and Woolley determined how manipulation of either endocannabinoid, 2-arachidonoylglycerol (2-AG) or

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anandamide (AEA), impacts inhibitory transmission. They found that blockade of 2-AG production increased IPSC amplitude in roughly half of the neurons studied; at the remaining neurons it had no effect. Co-application of E2 and the inhibitor of endocannabinoid synthesis revealed that blockade of 2-AG production alone did not interfere with E2-mediated IPSC suppression. The second endocannabinoid tested, AEA, was fully functionally connected to E2's effects on inhibitory transmission. Notably, because no pharmacological tools currently exist to block AEA production, the authors instead blocked its degradation. The overproduction of AEA was a clever means of quantifying AEA's physiological importance within a framework of E2-mediated effects on IPSCs. Increased AEA availability resulted in the suppression of inhibitory transmission. Importantly, no further changes were detected with the addition of E2; therefore, AEA's effects were attributed with fully occluding E2-mediated suppression of inhibition.

The kinetics of the E2-mediated suppression of inhibition also piqued the interest of the authors as to the mechanistic bases of the authors' observations. Specifically, they noted that their results did not resemble the expected depolarization-induced suppression of inhibition (DSI), classically associated with the endocannabinoid system (Wilson *et al.* 2001; Wilson and Nicoll 2001), but rather resembled long-term depression (LTD). While many procedural aspects of the experiments with E2 did not conform to tests for LTD, the authors reasoned that an LTD-like process may be at play and that, by extension, glutamatergic transmission may be involved in E2-mediated IPSC suppression. Notably, ERα can directly activate mGluR1 even in the absence of glutamate. Huang and Woolley showed that selective antagonism of mGluR1-blocked E2-mediated suppression of IPSCs. Intracellular introduction of an inhibitor to G-protein signalling also blocked IPSC suppression induced by E2, demonstrating that the point where mGluR1 signalling was involved, a post-synaptic effect is at play. Importantly, the functional relationship between E2, ERα and mGluR1 signalling were consistent with earlier findings in cultured hippocampal neurons (Boulware *et al.* 2005).

The findings from Huang and Woolley have broad implications for memory and affective behaviours, especially in light of the discovery of this sex-specific mechanism. In particular, disinhibitory mechanisms at CA1 neurons may provide a neural basis for an AND gate (Ang *et al.* 2005). The function of such a gate in CA1 neurons is to create conditions that allow the integration of cortical information at the hippocampal cell soma and to determine the probability and timing of action potential generation. As such, the mechanisms uncovered by Huang and Woolley supporting E2 modulation of female, but not male, hippocampal neuronal function may serve as a gatekeeper in memory retrieval related to sex-specific behaviours like mating or the rearing of offspring.

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