Estrogen, GABA$_{\alpha}$ receptors (GABA$_{\alpha}$R), metabotropic glutamate receptors (mGluRs), and endogenous cannabinoids, such as anandamide, are well known neuromodulators with sex-specific effects on brain development and function. Huang and Woolley link these four signaling pathways by proposing a new feedback loop through which 17β-estradiol triggers an mGluR1-mediated increase in the retrograde release of anandamide, which activates cannabinoid receptors 1 (CB1R) and suppresses GABA$_{\alpha}$-IPSCs. The 17β-estradiol regulation of GABA$_{\alpha}$-IPSCs appears to involve coupling of estrogen receptor $\alpha$ (ER$\alpha$) with mGluR1 and occurs in ovariectomized female, but not in castrated or gonadally intact male rats. The study involves postnatal day (PN) 47 to 57 rats, which are considered as late pubertal or early adults. These findings highlight once again the complex and sex-specific interactions among signaling systems that affect hippocampal activity. The current study was conducted in seizure-naïve rats, with unclear implications for the hippocampus of a rat exposed to seizures.

17β-estradiol is well known for its sex-specific, cell-type, age, and activity-dependent effects (1-4). Indeed, the authors here demonstrate the predilection for 17β-estradiol to reduce GABA$_{\alpha}$R-IPSCs in ovariectomized females but not in gonadally intact or castrated males. The use of nM concentrations of 17β-estradiol was intended to parallel the high local concentrations of the neurosteroid, which range between 5 to 10 nM in the adult male hippocampus (5). It is, however, surprising that the ER$\beta$-preferring agonist diarylpropionitrile, at doses that can activate both ER$\alpha$ and ER$\beta$ (500 nM), was completely inactive (6). ER/mGluR coupling occurs in hypothalamic neurons and glial cells (3), does not require presynaptic glutamate release (1), and may involve ER$\alpha$ or ER$\beta$. The type of mGluR involved (mGluR1/5 or mGluR2/3) determines the downstream effects (3), which can be sex-specific (1). For instance, in early postnatal hippocampal cultures, ER$\alpha$/mGluR1 may trigger phosphorylation of cAMP-responsive element binding protein (pCREB), whereas ER$\alpha$ or $\beta$/mGluR2/3 coupling may reduce pCREB (1).

The authors hypothesize that 17β-estradiol may regulate presynaptic GABA release, although potential effects on postsynaptic GABA$_{\alpha}$Rs are also worth investigating, given the lasting suppression of GABA$_{\alpha}$R-IPSC. However, the search for a presynaptic messenger led to fruitful links to endocannabinoid signaling, since the 17β-estradiol effects seemed to be mediated by retrograde release of anandamide. Although the sex-specific mechanisms are not explored here, these are likely to be tissue-specific, since potentiation of anandamide-induced vasorelaxation by 17β-estradiol occurs in mesenteric arteries of male but not of female rats (7).

CB1R-mediated regulation of GABA$_{\alpha}$R signaling, via 2-arachidonoylglycerol (2-AG) and/or anandamide, has been reported in the hippocampus of rodents of either undetermined sex (8-10) or of young male rats (11). Interestingly, here, only anandamide mediates the 17β-estradiol–induced suppression of GABA$_{\alpha}$R-IPSCs. Inhibition of fatty acid amide hydrolase (FAAH), which catabolizes anandamide, by URB 597 masked the 17β-estradiol effect on GABA$_{\alpha}$R-IPSCs, whereas blockade of 2-AG breakdown had no such effect. This is not necessarily surprising considering that 17β-estradiol may increase anan-
anandamide synthesis or inhibit its catabolizing enzyme, FAAH (12, 13). The inactivity of 17β-estradiol, when the FAAH inhibitor URB 597 is applied, could then be due to the occupancy of its target by URB 597, yet this possibility is not further investigated. In further support, the plasma levels of anandamide correlate nicely with the levels of estrogen during the menstrual cycle in women (13). It is tempting to cite here the clinical studies that find increased frequency of certain types of seizures during menstrual cycle periods with increased estrogen/progesterone serum level ratios (14) and question whether this anandamide-induced decrease in GABAAR signaling might be one of the mechanisms involved in the observed increased seizure frequency. But then, why isn’t the frequency of other focal-onset seizure types increased as well.

Could this 17β-estradiol/anandamide signaling-induced disinhibition of the hippocampus reveal a new mechanism underlying the pro-convulsant actions of high 17β-estradiol levels? In support of this possibility, FAAH(-/-) mice show increased seizure severity in the bicuculline and kainic acid model (17). In contrast, in PN21-24 old rats of undetermined sex, FAAH inhibition had antiseizure effects in the kainic acid model (16). The rest of the existing studies on anandamide effect on seizures have been done in males and are difficult to compare with the authors’ findings. Rather, the existing studies tend to suggest dose and model-specific effects, as well as the involvement of additional signaling pathways (17-20).

It is also unclear how significant this pathway may be in disinhibiting an epileptic hippocampus, where (a) GABA may be depolarizing rather than hyperpolarizing in certain hippocampal regions (21), (b) endocannabinoids may also reduce the excitatory postsynaptic currents (22), and (c) the circuitry of connections and the involved signaling pathways may be undergoing drastic changes from normal controls. Overall, this is an elegant study, which incrementally unravels an exciting sequence of events leading to the sex-specific regulation of GABAAR IPSCs in the hippocampus by 17β-estradiol and endocannabinoids. Further experiments will be needed to clarify the relevance of the current findings not only to epilepsy but also to normal cognitive processes, drug addiction, or other neurological disorders involving the hippocampal structures and endocannabinoid signaling. The relevance of these in vitro observations to an intact, healthy or diseased organism that undergoes continuous changes through time, remains unclear. Deciphering these issues is always a challenge in any research that investigates activity-related functions.

by Aristea S. Galanopoulou, MD PhD

References


American Epilepsy Society

Epilepsy Currents Journal

Disclosure of Potential Conflicts of Interest

Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. Identifying information.
   Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

2. The work under consideration for publication.
   This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”. Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. Relevant financial activities outside the submitted work.
   This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

   Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work’s sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

   For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Other relationships
   Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.
Section #1 Identifying Information

1. Today's Date: 5/21/13

2. First Name Aristea  Last Name Galanopoulou  Degree MD PhD

3. Are you the Main Assigned Author?  Yes  No

   If no, enter your name as co-author:

4. Manuscript/Article Title: Stirring the pot with estrgens

5. Journal Issue you are submitting for:  Vol 13, No 3 (May/June)

Section #2 The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

<table>
<thead>
<tr>
<th>Type</th>
<th>No</th>
<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Grant</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consulting fee or honorarium</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Support for travel to meetings for the study or other purposes</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Fees for participating in review activities such as data monitoring boards, statistical analysis, end point committees, and the like</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Payment for writing or reviewing the manuscript</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Provision of writing assistance, medicines, equipment, or administrative support.</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Other</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This means money that your institution received for your efforts on this study.

** Use this section to provide any needed explanation.
### Section #3 Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the “Add” box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

<table>
<thead>
<tr>
<th>Type of relationship (in alphabetical order)</th>
<th>No</th>
<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Board membership</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consultancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Employment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Expert testimony</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Grants/grants pending</td>
<td></td>
<td></td>
<td>Yes</td>
<td>CURE, NINDS,</td>
<td>Salary support and supplies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Autism Speaks</td>
<td></td>
</tr>
<tr>
<td>6. Payment for lectures including service on speakers bureaus</td>
<td></td>
<td>Yes</td>
<td>Novartis / Sui generis</td>
<td>Honorarium for participating as a speaker in a conference</td>
<td></td>
</tr>
<tr>
<td>7. Payment for manuscript preparation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Patents (planned, pending or issued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Payment for development of educational presentations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Stock/stock options</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Travel/accommodations/meeting expenses unrelated to activities listed.**</td>
<td></td>
<td>Yes</td>
<td>AES, ILAE, CURE, Autism Speaks, Epilepsy Therapy Project, NINDS, University of Davis CA</td>
<td>Travel expenses for participating in conference</td>
<td></td>
</tr>
<tr>
<td>13. Other (err on the side of full disclosure)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This means money that your institution received for your efforts.
** For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

### Section #4 Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?
☒ No other relationships/conditions/circumstances that present a potential conflict of interest.
☐ Yes, the following relationships/conditions/circumstances are present:

Thank you for your assistance.

Epilepsy Currents Editorial Board