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Estradiol Benzoate Potentiates Neuroactive Steroids' Effects on Pain Sensitivity

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FRYE, C. A. AND J. E. DUNCAN. *Estradiol benzoate potentiates neuroactive steroids' effects on pain.* PHARMACOL BIOCHEM BEHAV **53**(1) 27–32, 1996. – Progesterone (P), its metabolites, and other neuroactive steroids alter pain thresholds consistent with their efficacies at modulating γ-aminobutyric acid ($GABA_A$) receptor complexes. We investigated whether estradiol benzoate (EB) potentiates low dosages of neuroactive steroids' effects on pain. Subcutaneous EB (10 μg) or sesame oil vehicle was administered to ovariectomized Long-Evans rats (n = 40) 48 h before intracerebroventricular (ICV) infusion of a neuroactive steroid (0.0, 0.1, 0.3, or 0.5 μg) in cyclodextrin vehicle. Neuroactive steroids (listed from greatest to least efficacious at $GABA_A$ receptor complexes) were THP [5α-pregnan-3α-ol-20-one], THDOC [5α-pregnan-3α, 21-diol-20-one], DHP [5α-pregnan-3,20-dione], P [4-pregnen-3,20-dione], and DHEAS [5-androsten-3β-ol-17-one sulfate]. Pain sensitivity was assessed using the radiant heat tail-flick method before and 20 and 60 min following infusion. Estradiol benzoate interacted with the neuroactive steroids to alter tail-flick latencies. In particular, EB potentiated the antinociceptive effect of THP and DHP by significantly increasing tail-flick latencies above those of non-EB-treated animals. A similar pattern of increased tail-flick latencies occurred in EB-primed animals that received THDOC. Estradiol benzoate less consistently altered the pain threshold of animals administered P, which is less effective at modulating GABAergic activity. Conversely, EB increased the nociceptive effect of the neurosteroid DHEAS, an allosteric antagonist of $GABA_A$ receptor complexes, by significantly decreasing tail-flick latencies of EB- compared to vehicle-primed rats. Thus, EB priming potentiated neuroactive steroids' effects on pain threshold.

Estrogen Progestin Tail-flick latency GABA Membrane Nongenomic Nociception Neurosteroid Neuroactive steroid Steroid mechanism Hormone

FINDINGS from animal and human studies implicate progesterone (P) and its metabolites in pain modulation. Pain thresholds over the estrous cycle (11,16,17,40,41,56,59), luteal functioning (16,22), pregnancy (21,22), and following exogenous P administration (16,41,52) indicate animals are more sensitive to aversive stimuli when P levels are in flux, whereas analgesia occurs when circulating P is stable in high levels. The positive correlation between analgesia and independent measurements of 5α-reduced P metabolites (26,27,49) over endogenous hormonal milieu suggest that P's effects on pain may be mediated by the actions of its metabolites. Systemic (6,18), intrathecal (8,64), and intracerebroventricular (ICV) (18,31) administration of the 5α-reduced P metabolite, 5α-pregnan-3α-ol-20-one (THP), rapidly and consistently promotes analgesia in rats and mice. Women administered P, which increases THP, also report enhanced positive affect and mood (14).

The mechanism by which P and its natural metabolites affect pain threshold is unclear. Whereas steroids can act genomically by entering cells, binding with intracellular receptors to form steroid-receptor complexes, which then alter the genome and subsequently increase RNA and protein synthesis (15), some steroids can also act nongenomically by binding to γ-aminobutyric acid ($GABA_A$) receptor complexes (38) located on neuronal membranes. The latter may represent a putative mechanism for hormonal modulation of pain as THP, the most potent of the endogenous neuroactive steroids at promoting analgesia and the most effective at increasing $GABA_A$ receptor function (38), lacks a high affinity for intracellular progestin receptors (PR) (28).

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Additional evidence that P and/or its metabolites mediate pain via actions at $GABA_A$ receptor complexes is as follows. First, in the absence of steroids, increasing $GABA_A$ receptor function in the ventromedial medulla with diazepam enhances analgesia, whereas antagonizing $GABA_A$ receptor function with bicuculline promotes nociception (42). Second, P and its 3α-hydroxy derivatives, but not 3β metabolites, increase vocalization threshold to tail shock when combined with subanalgesic dosages of $GABA_A$ agonist, muscimol (8). Third, ICV THP's analgesic effects can be attenuated by peripherally administered $GABA_A$ antagonists bicuculline, picrotoxin, and RO-15,788 (31). Finally, ICV implants or infusions (0.5–2.0 μg) of neuroactive steroids produce effects on pain that are only discriminable by the GABAergic efficacy of the neuroactive steroid administered; THP > THDOC >> DHP (5α-pregnan-3,20-dione) > P (4-pregnen-3,20-dione) >> 17 OH-P (17-hydroxyprogesterone) >> PS (5-pregnen-3β-ol-20-one sulfate > DHEAS (5-androsten-3β-ol-17-one sulfate) (18).

Estradiol fluctuates concomitant with P and its metabolites over reproductive cycles and can influence binding of $GABA_A$ receptor agonists (24,35,47,57). To discern potential priming effects of estrogen, the following experiment examined the effects of ICV dosages (0.0–0.5 μg) of neuroactive steroids on pain in the presence and absence of estradiol benzoate priming.

METHOD

Animals and Housing Conditions

We obtained 40 female Long Evans rats, weighing between 175 and 200 g, from Charles River Breeding Laboratories (Wilmington, MA). Animals were housed individually in polycarbonate cages (36 × 18 × 18 cm) in a temperature and humidity-controlled room (22 ± 1°C) with a 12 L : 12 D cycle (lights off at 1000 h).

Surgical Procedures

Ovariectomy and unilateral ICV cannulation were conducted using sodium pentobarbital anesthesia (50 mg/kg or to effect). Ovariectomies were performed via bilateral ventral abdominal incisions. Cannulae were unilaterally implanted into the lateral ventricle at coordinates from bregma AP −1.0, DV −3.6, LAT ±1.6 (50). Following surgery, blank 30-ga stylets were inserted into implanted guide cannulae. Inserts were subsequently removed and cleaned daily with 95% ethanol to prevent clogging of the cannula (19), to reduce handling effects at the time of testing (18), and to avoid stress-induced elevations of 5α-reduced P metabolites (51).

Steroid Administration

Estradiol benzoate (10 μg; Sigma Chemical Co., St. Louis, MO) was suspended in 0.2 cc of sesame oil and administered 48 h before neuroactive steroid infusion. THP, THDOC, DHP, P, and DHEAS were obtained from Sigma and dissolved in 37% cyclodextrin/DH_2O vehicle (Molecusol; Pharmatec, Alachua, FL). The infusion apparatus consisted of a Hamilton syringe (Bonaduz, Switzerland) attached by 20-ga polyethylene tubing to an 8-mm, 30-ga needle. Animals were infused at a rate of 0.1 μl/10 s for 100 s. To prevent displacement of infusate, the infusion needle remained in place for 30 s following administration. Animals were consistently infused with a volume of 1.0 μl; all neuroactive steroids were suspended in cyclodextrin vehicle to concentrations of 0.0, 0.1, 0.3, or 0.5 μg/μl. Thus, the final dosages of all neuroactive steroids were 0.0, 0.1, 0.3, or 0.5 μg.

Behavioral Testing

All animals were tested during the dark phase of the light cycle, beginning at 1100 h. Behavioral testing consisted of gently holding each rat in a towel while smoothing its tail into the tail groove of the tail-flick apparatus (model 33; IITC, Woodland Hills, CA). Prior to neuroactive steroid or vehicle infusion, rats were tested three times on different parts of the tail to obtain baseline measures of 2–4 s. In a small number of instances (3.0% of observations), the median baseline measurement for an animal exceeded the 4-s standard. In these cases, the erroneous value was imputed (54) or randomly and systematically replaced with data from a trial within the same condition. To prevent tissue damage, maximum latency was 10 s throughout the testing period. Pain sensitivity was measured immediately, before, and then 20 and 60 min postneuroactive steroid infusion of 0.0, 0.1, 0.3, or 0.5 μg.

Post-infusion tail-flick latencies (s) were converted to percent maximum possible effect (%MPE) using the following formula (3):

$$\%\text{MPE} = \frac{\text{test latency} - \text{median baseline latency}}{\text{maximum latency (10)} - \text{median baseline latency.}} \times 100$$

A neurologic exam assessing righting response, hindlimb withdrawal, and orientation to a stimulus was used to detect gross neurologic impairment secondary to surgery (39). These responses were present in all animals at every behavioral test session, suggesting the analgesic effects of the neuroactive steroids were not confounded by anxiolytic or anesthetic effects.

Procedure

Ovariectomized/cannulated rats (n = 40) were randomly assigned to receive one of the five neuroactive steroids (n = 8/neuroactive steroid). The eight rats assigned to each neuroactive steroid were tested once per week, over an 8-week period, during which all dosages of the assigned neuroactive steroid were administered with subcutaneous (SC) EB and sesame oil vehicle in a counterbalanced manner. Some cannulae failed to remain patent throughout the entire 8-week testing period (n = 10). For each neuroactive steroid, the total number of animals receiving all eight infusions and subsequent behavioral testing were THP (n = 8), THDOC (n = 5), DHP (n = 7), P (n = 4), and DHEAS (n = 6). Those animals unable to be infused because of occluded cannula continued to be tested. These animals received only counterbalanced EB/sesame oil injections; data from this group were analyzed as an uninfused control group.

Statistical Analysis

Data were analyzed using three-way, repeated-measures analyses of variance (ANOVA) with neuroactive steroid, neuroactive steroid dosage, and EB as variables. Estradiol benzoate administration and dosage were treated as repeated measures, whereas 20 and 60 min time points were analyzed separately so as not to wash out normal changes in the time course of pain behavior (13). These overall analyses were followed by two-way, repeated-measures (EB × neuroactive steroid dosage) ANOVAs for each neuroactive steroid. Where appropriate, these analyses were followed by Duncan new

multiple range posthoc tests comparing EB and non-EB effects at each neuroactive steroid dosage. Only significant differences at $p < 0.05$ are reported.

RESULTS

At 20 min postinfusion, there were main effects of neuroactive steroid [$F(4, 96) = 2.78$, $p < 0.05$] and dosage [$F(3, 96) = 8.11$, $p < 0.0001$], as well as interactions between neuroactive steroid, dosage, and EB [$F(12, 96) = 6.77$, $p < 0.001$]. At 60 min postinfusion, there were main effects of neuroactive steroid [$F(4, 96) = 5.51$, $p < 0.001$], dosage [$F(3, 96) = 9.27$, $p < 0.001$], and EB [$F(1, 96) = 4.01$, $p < 0.05$], as well as an interaction between these variables [$F(12, 96) = 2.35$, $p < 0.01$]. Separate examination of the neuroactive steroids revealed that these interactions resulted from EB priming enhancing each neuroactive steroid's particular antinociceptive (THP, THDOC, and DHP), nociceptive (DHEAS), or inconsistent (P) effect.

As Fig. 1A illustrates, 20 min following THP infusion, there was a main effect of THP dosage [$F(3, 21) = 5.26$, $p < 0.05$] but not EB. Only the 0.1-μg dosage of THP elevated tail-flick latencies over the 0.0-μg control values. At 60 min postinfusion, there were main effects of EB [$F(3, 21) = 5.23$, $p < 0.05$]. Overall, EB-treated animals had longer tail-flick latencies compared with non-EB controls. Within the EB-primed group, animals receiving 0.5 μg THP had significantly lengthened tail-flick latencies compared with 0.0 μg THP.

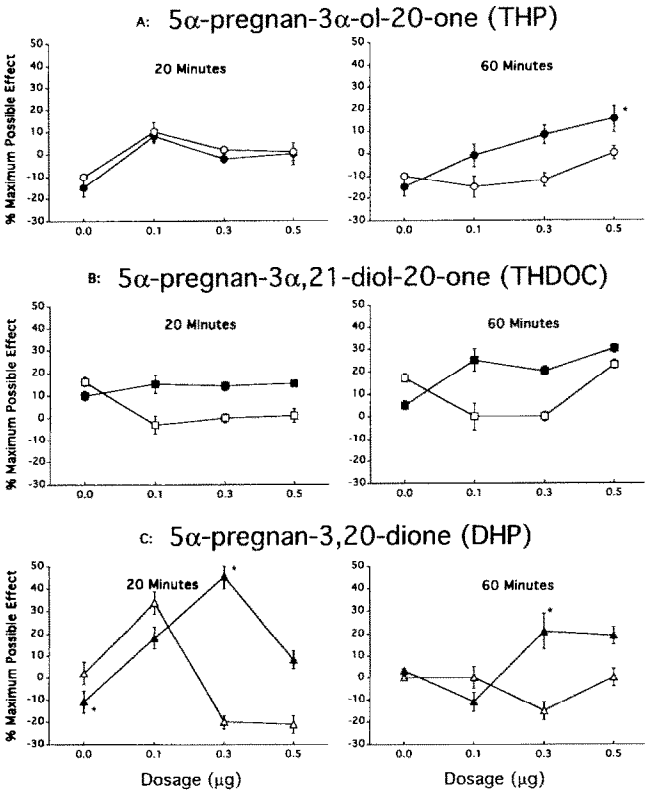

FIG. 1. Mean ± standard error of the mean percent maximum possible effect 20 (left panels) and 60 min (right panels) following infusions of 5α-pregnan-3α-ol-20-one (THP: top panel, A), 5α-pregnan-3α,21diol-20-one (THDOC: middle panel, B) or 5α-pregnan-3,20-dione (DHP: bottom panel, C). *Significant difference between EB and non-EB-primed animals at indicated neuroactive steroid dosage, $p < 0.05$.

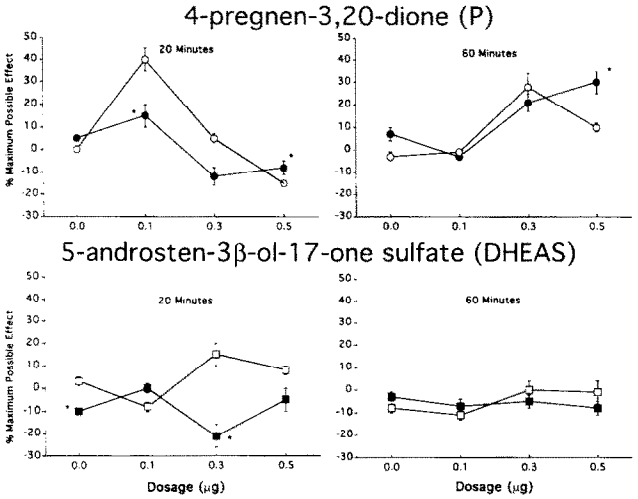

FIG. 2. Mean ± standard error of the mean percent maximum possible effect 20 (left panels) and 60 min (right panels) following infusions of 4-pregnen-3,20-dione (P: top panel, A) or 5-androsten-3β-ol-17-one sulfate (DHEAS: bottom panel, B). *Significant difference between EB and non-EB-primed animals at the same neuroactive steroid dosage, $p < 0.05$.

As Fig. 1B illustrates, EB also potentiated THDOC's effects on the pain threshold. Although not a statistically significant effect, EB-treated animals consistently showed increased pain thresholds compared with non-EB-treated animals 20 and 60 min post-THDOC infusion.

Estradiol benzoate also increased tail-flick latencies 20 and 60 min following DHP infusions (Fig. 1C). At 20 min, there were main effects of EB [$F(1, 18) = 5.98$, $p < 0.05$] and dosage [$F(1, 18) = 13.38$, $p < 0.0001$], in addition to an interaction between EB and DHP dosage [$F(3, 18) = 23.53$, $p < 0.001$]. EB significantly lengthened tail-flick latencies overall, but particularly following 0.3 μg DHP. Similar effects were noted 60 min post-DHP infusion as indicated by a trend for a main effect of EB [$F(1, 18) = 4.40$, $p \leq 0.08$] and an interaction between EB and DHP dosage [$F(3, 18) = 3.04$, $p \leq 0.05$]. Again, EB lengthened tailflick latencies overall; following 0.3 μg DHP, tail-flick latencies were longer compared with non-EB animals receiving 0.3 or 0.0 μg DHP.

It is interesting that EB's effects on pain following P infusion were variable before 60 min. This is consistent with the time course of P implant and infusion effects (18). As Fig. 2A illustrates, there was a main effect of P dosage [$F(3, 30) = 14.87$, $p < 0.0001$] and an interaction between EB and P dosage [$F(3, 30) = 4.40$, $p < 0.01$]. EB potentiated the analgesic efficacy of 0.5 μg P, as indicated by significantly longer tail-flick latencies compared with non-EB animals receiving 0.5 μg P.

Estradiol benzoate potentiated the nociceptive effects of DHEAS, an allosteric antagonist of the $GABA_A$ receptor complex. EB-treated animals had significantly shorter tail-flick latencies compared with non-EB-treated animals 20 min post-DHEAS infusion [$F(1, 15) = 16.12$, $p < 0.01$]. Post hoc tests indicated that EB significantly shortened tail-flick latencies alone and following 0.3 μg DHEAS.

Estradiol benzoate failed to alter nociception independently of neuroactive steroids infusion at either the 20- or 60-min testing points (data not shown).

DISCUSSION

Estradiol benzoate potentiated neuroactive steroids' particular effects on nociception. THP (0.5 μg), DHP (0.3 μg), and P (0.5 μg) failed to induce antinociception in the absence of EB. A similar pattern of results was seen for THDOC-infused animals; tail-flick latencies were longer when animals were EB-primed compared with non-EB-primed ones. Conversely, EB increased the nociceptive effect of the neurosteroid DHEAS (0.3 μg), an allosteric antagonist of the $GABA_A$ receptor (37). These data indicate that EB potentiates a neuroactive steroid's proclivity for affecting pain threshold. Thus, EB and neuroactive steroids may produce orchestrated effects on nociception.

These data confirm both the time course of maximuum effect and the rank ordering of efficacy of the P metabolites or precursors on $GABA_A$ receptor function and their ability to alter pain threshold (THP and THDOC > DHP > P >> DHEAS) (18). The present data reveal that 0.3–0.5 μg is the threshold ICV dose at which neuroactive steroids produce discernable differences in tail-flick latencies that can be potentiated by EB. These findings are consistent with differences in hormonal milieu associated with changes in both estrogen and neuroactive steroids (e.g., gender, gonadectomy, estrous, and menstrual cycles) correlating with changes in seizure susceptibility (1,2,12,25,45,62,63), $GABA_A$-receptor expression, and function (61,62). In this experiment, EB-priming potentiated neuroactive steroids' (anti)nociceptive efficacy, just as withdrawal from a low dosage of SC P (50 μg) only occurs in estrogen-primed animals (20).

Although data strongly suggest that progestins' effects on nociception are mediated via actions at $GABA_A$-receptor complexes, it is unclear how estrogen may potentiate neuroactive steroids' alteration of pain threshold. Evidence suggests that estrogen can alter $GABA_A$-receptor complexes. For example, *in vivo* (24,35) and *in vitro* (36), EB upregulates $GABA_A$ receptors and increases muscimol binding. Autoradiographic studies reveal that estrogen can both increase and decrease the expression of $GABA_A$ receptors depending on the brain site (9,10,47,57). Although there are inconsistencies in the direction of estrogen's effect on $GABA_A$ receptors, in the absence of P, estrogen alone alters $GABA_A$-receptor complexes.

When P is administered to estrogen-primed animals, GABAergic activity is also changed, and in some cases, estrogen and P's effects can be opposite (57) or additive (35). A recent report suggests that P may be metabolized and subsequently interact at neuronal membranes, whereas estrogen's effects may be limited to areas of the brain where $GABA_A$-receptor complexes and intracellular estrogen receptors are colocalized (10). That estrogen alone can affect $GABA_A$-receptor complexes but EB alone did not alter pain thresholds in this study suggests that EB's present effects may be mediated via a GABAergic mechanism that depends on intracellular estrogen receptors (ER). That administration of EB 48 h before behavioral testing enhanced neuroactive steroids' modulation of pain also supports this notion, as estrogen-mediated effects via intracellular receptors are associated with much longer latencies than membrane-mediated effects of estrogen.

Estrogen may not produce its effects directly via GABA-receptor complexes or intracellular estrogen receptors/$GABA_A$-receptor complex interactions, but rather through priming effects on intracellular progestin (PR) and/or androgen receptors (AR). It has long been recognized that estradiol can increase the expression of intracellular PR's and AR's (4,7,53). *In vitro* evidence indicates that the $GABA_A$ receptor complex-mediated effects of some neuroactive steroids, particularly neurosteroids, may require intracellular events. Neurosteroids are synthesized by glial cells, and an estrogen-inducible PR has been demonstrated in cultured oligodendrocites of female rats (29). As well, both estrogen and P induce dramatic morphologic changes in astrocytes and oligodendrocites (30). Furthermore, after influencing GABA-mediated chloride currents, both THP and THDOC can activate intracellular PRs without binding to them (55). These data and others (23) which show that $GABA_A$-receptor complex channel functioning may be modified by agents that increase protein phosphorylation suggest that "crosstalk" between membrane GABA-receptor complexes and intracellular events may occur (34). Considering the long time course of estrogen's effects, the present data may reflect interactions between intracellular (ER, PR, or AR) and $GABA_A$-receptor complexes.

Estrogen's effects may not occur through intracellular or $GABA_A$-receptor complex sites, but rather via other membrane loci. Estrogen can alter pain sensitivity via the opiate system [see (48) for review] by modifying morphine-induced analgesia (5,46), reducing sensitivity to opioid antagonists (43), and stimulating the release of β-endorphin (60). Furthermore, 17α-estradiol and its metabolites show selective affinity for opiate receptors (33), supporting the notion that estradiol may have a direct impact on the opioid system. In humans, estrogens reduce the incidence of cluster headache (32) and increase endogenous opioid activity (58). Although the opiate system is another possible site of EB actions, one cannot discount the possibility that EB may effect another cell surface receptor (44).

In conclusion, EB potentiates neuroactive steroids' modulation of pain, although the mechanism by which EB does so remains unclear. Future studies will elucidate the mechanism and CNS sites for these alterations in pain threshold.

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REFERENCES

1. Backstrom, T. Epileptic seizures in women related to plasma estrogen and progesterone during the menstrual cycle. Acta Neurol. Scand. 54:321–347; 1976.
2. Backstrom, T.; Zetterlund, B.; Blom, S.; Romano, M. Effects of intravenous progesterone infusions on the epileptic discharge frequency in women with partial epilepsy. Acta Neurol. Scand. 69:240–248; 1984.
3. Berge, O. G.; Garcia-Cabrera, I.; Hole, K. Response latencies in the tail-flick test depend on tail skin temperature. Neurosci. Lett. 86:284–288; 1988.
4. Blaustein, J. D. Estrogen receptors in neurons: New subcellular locations and functional implications. Endocr. J. 2:249–258; 1994.
5. Bodnar, R. J.; Romero, M.; Kramer, E. Organismic variables

and pain inhibition: Roles of gender and aging. Brain Res. Bull. 21:947-953; 1988.
6. Bukusoglu, C.; Thalhammer, J. G.; Krieger, N. R. Analgesia with anesthetic steroids and ethanol. Anesth. Analg. 77:27-31; 1993.
7. Burgess, L. H.; Handa, R. J. Hormonal regulation of androgen receptor mRNA in the brain and anterior pituitary gland of the male rat. Mol. Brain Res. 19:31-38; 1993.
8. Caba, M.; Gonzalez-Mariscal, G.; Beyer, C. Perispinal progestins enhance the antinociceptive effects of muscimol in the rat. Pharmacol. Biochem. Behav. 47:177-182; 1994.
9. Canonaco, M.; Carelli, A.; Maggi, A. Steroid hormones and receptors of the $GABA_A$ supramolecular complex. I. Benzodiazepine receptor level changes in some extrahypothalamic brain areas of the female rat following sex steroid treatment. Neuroendocrinology 57:965-973; 1993.
10. Canonaco, M.; Tavolaro, R.; Maggi, A. Steroid hormones and receptors of the $GABA_A$ supramolecular complex. II. Progesterone and estrogen inhibitory effects on the chloride ion channel receptor in different forebrain areas of the female rat. Neuroendocrinology 57:974-984; 1993.
11. Drury, R. A.; Gold, R. M. Differential effects of ovarian hormones on reactivity to electric foot shock in the rat. Physiol. Behav. 20:187-191; 1978.
12. Finn, D. A.; Gee, K. W. The estrus cycle, sensitivity to convulsants and the anticonvulsant effect of a neuroactive steroid. J. Pharmacol. Exp. Ther. 271:164-170; 1994.
13. Franklin, K. B. J.; Abbott, F. V. Pentobarbital, diazepam, and ethanol abolish the interphase diminution of pain in the formalin test: Evidence for pain modulation by $GABA_A$ receptors. Pharmacol. Biochem. Behav. 46:661-666; 1993.
14. Freeman, E. W.; Purdy, R. H.; Coutifaris, C.; Rickels, K.; Paul, S. M. Anxiolytic metabolites of progesterone: Correlation with mood and performance measures following oral progesterone administration to healthy female volunteers. Neuroendocrinology 58:478-484; 1993.
15. Freeman, L. M.; Breedlove, S. M. Steroid receptors in the central nervous system. In: Conn, P. M., ed. Methods in neurosciences, vol. 11. San Diego: Academic Press; 1993.
16. Frye, C. A.; Bock, B. C.; Kanarek, R. B. Hormonal milieu affects tailflick latency in female rats and may be attenuated by access to sucrose. Physiol. Behav. 52:699-706; 1992.
17. Frye, C. A.; Cuevas, C. A.; Kanarek, R. B. Diet and estrous cycle influence pain sensitivity in rats. Pharmacol. Biochem. Behav. 45:255-260; 1993.
18. Frye, C. A.; Duncan, J. E. Progesterone metabolites, effective at the $GABA_A$ receptor complex, attenuate pain sensitivity in rats. Brain Res. 643:194-203; 1994.
19. Frye, C. A.; Mermelstein, P. G.; DeBold, J. F. Evidence for a nongenomic action of progestins on sexual receptivity in hamster VTA but not hypothalamus. Brain Res. 578:87-93; 1992.
20. Gallo, M. A.; Smith, S. S. Progesterone withdrawal decreases latency to and increases duration of electrified prod burial: A possible rat model of PMS anxiety. Pharmacol. Biochem. Behav. 46:897-904; 1993.
21. Gintzler, A. R. Endorphin mediated increase in pain threshold during pregnancy. Science 210:193-195; 1980.
22. Gintzler, J. H.; Bohan, M. Pain thresholds are elevated during pseudopregnancy. Brain Res. 507:312-316; 1990.
23. Gynes, M.; Wang, Q.; Gibbs, T.; Farb, D. H. Phosphorylation factors control neurostransmitter and neuromodulator actions at the γ-aminobutyric acid type A receptor. Mol. Pharmacol. 46: 542-549; 1994.
24. Hamon, G.; Goetz, C.; Euvarard, C.; Pasqualini, C.; Le Dafneit, M.; Kerdelhue, B.; Cesselin, F.; Peillon, F. Biochemical and functional alterations in central GABA receptors during chronic estrogen treatment. Brain Res. 279:141-152; 1983.
25. Herzog, A. G. Reproductive endocrine considerations and hormonal therapy for women with epilepsy. Epilepsia 32:27-33; 1991.
26. Holzbauer, M. Physiological variations in the ovarian production of 5α-pregnane derivatives with sedative properties in the rat. J. Steroid Biochem. 6:1307-1310; 1975.
27. Ichikawa, S.; Sawada, T.; Nakamura, Y.; Morioka, H. Ovarian secretion of pregnane compounds during the estrous cycle and pregnancy in rats. Endocrinology 94:1615-1620; 1974.
28. Iswari, S.; Colas, A. E.; Karavolas, H. J. Binding of 5α-dihydroprogesterone and other progestins to female rat anterior pituitary nuclear extracts. Steroids 47:189-203; 1986.
29. Jung-Testas, I.; Renoir, M.; Bugnard, H.; Greene, G. L.; Baulieu, E.-E. Demonstration of steroid hormone receptors and steroid action in primary cultures of rat glial cells. J. Steroid Biochem. Mol. Biol. 41:621-631; 1992.
30. Jung-Testas, I.; Schumacher, M.; Robel, P.; Baulieu, E. E. Actions of steroid hormones and growth factors on glial cells of the central and peripheral nervous system. J. Steroid Biochem. Mol. Biol. 48:145-154; 1994.
31. Kavaliers, M.; Wiebe, J. P. Analgesic effects of the progesterone metabolite, 3α-hydroxy-5α-pregnan-20-one, and possible modes of action in mice. Brain Res. 415:393-398; 1987.
32. Klimek, A.; Durko, A.; Gluszcz-Zielinska, A. Hormonal status in cluster headache. In: Rose, F. C., ed. Progress in migraine research, vol. 2. London: Pitman; 1984.
33. Lee, S.; Panerai, E.; Bellabarba, D.; Friesen, H. G. Effect of endocrine modifications and pharmacological treatments on brain and pituitary concentrations of β-endorphin. Endocrinology 107:245-248; 1980.
34. Macdonald, R. L.; Olsen, R. W. $GABA_A$ receptor channels. Ann. Rev. Neurosci. 17:569-602; 1994.
35. Maggi, A.; Perez, J. Progesterone and estrogens in rat brain: Modulation of GABA receptor activity. Eur. J. Pharmacol. 103: 165-168; 1984.
36. Maggi, A.; Perez, J. Estrogen-induced upregulation of gamma-aminobutyric acid receptors in the CNS of rodents. J. Neurochem. 47:1793-1797; 1986.
37. Majewska, M. D.; Demirgoren, S.; Spivak, C. E.; London, E. D. The neurosteroid dehydroepiandrosterone sulfate is an allosteric antagonist of the $GABA_A$ receptor. Brain Res. 526:143-146; 1990.
38. Majewska, M. D.; Harrison, N. L.; Schwartz, R. D.; Barker, J. L.; Paul, S. M. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232:1004-1007; 1986.
39. Marshall, J. F.; Teitlebaum, P. Further analysis of sensory inattention following lateral hypothalamic damage in rats. J. Comp. Physiol. Psych. 86:375-395; 1974.
40. Martinez-Gomez, M.; Cruz, Y.; Salas, M.; Hudson, R.; Pachecos, P. Assessing pain threshold in the rat: Changes with estrus and time of day. Physiol. Behav. 55:651-657; 1994.
41. McCarthy, M. M.; Caba, M.; Komisurak, B. R.; Beyer, C. Modulation by estrogen and progesterone on the effect of muscimol on nociception in the spinal cord. Pharmacol. Biochem. Behav. 37:123-128; 1990.
42. McGowan, M. K.; Hammond, D. L. Antinociception produced by microinjection of L-glutamate into the ventromedial medulla of the rat: Mediation by spinal $GABA_A$ receptors. Brain Res. 620: 86-96; 1993.
43. Morley, J. E.; Levine, A. S.; Grace, M.; Kneip, J.; Gosnell, B. A. The effect of ovariectomy, estradiol and progesterone on opioid modulation of feeding. Physiol. Behav. 33:237-241; 1984.
44. Morley, P.; Whitfield, J. F.; Vanderhyden, B. C.; Tsang, B. K.; Schwartz, J. L. A new, nongenomic estrogen action—The rapid release of intracellular calcium. Endocrinology 131:1305-1312; 1992.
45. Morrell, M. J. Hormones and epilepsy through the lifetime. Epilepsia 33:49-61; 1992.
46. Nomikos, N.; Spyraki, C. A.; Kazandjian, A.; Sfikakis, A. Estrogen treatment to ovariectomized rats modifies morphine induced behavior. Pharmacol. Biochem. Behav. 27:611-617; 1987.
47. O'Connor, L. H.; Nock, B.; McEwen, B. S. Regional specificity of gamma-aminobutyric acid receptor regulation by estradiol. Neuroendocrinology 47:473-481; 1988.
48. Olson, G. A.; Olson, R. D.; Kastin, A. J. Endogenous opiates: 1990. Peptides 12:1407-1432; 1991.
49. Paul, S. M.; Purdy, R. H. Neuroactive steroids. FASEB J. 6: 2311-2322; 1992.
50. Paxinos, G.; Watson, C. The rat brain. New York: Academic Press; 1986.

51. Purdy, R. H.; Morrow, A. L.; Moore, J.; Paul, S. M. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. Proc. Natl. Acad. Sci. 88:4553-4557; 1991.
52. Ratka, A.; Simpkins, J. W. Effects of estradiol and progesterone on the sensitivity to pain and on morphine-induced antinociception in female rats. Horm. Behav. 25:217-228; 1991.
53. Roselli, C. E.; Fasasi, T. A. Estradiol increases the duration of nuclear androgen receptor occupation in the preoptic area of the male rat treated with dihydrotestosterone. J. Steroid Biochem. Mol. Biol. 42:161-168; 1992.
54. Rubin, D. B. Multiple imputation for nonresponse in surveys. New York: John Wiley; 1987.
55. Rupprecht, R.; Reul, J.; Trapp, T.; van Steensel, B; Wetzel, C.; Damm, K.; Zieglgansberger, W.; Holsboer, F. Progesterone receptor-mediated effects of neuroactive steroids. Neuron 11: 523-530; 1993.
56. Ryan, S. M.; Maier, S. F. The estrous cycle and estrogen modulate stress-induced analgesia. Behav. Neurosci. 3:371-380; 1988.
57. Schumacher, M.; Coirini, H.; McEwen, B. S. Regulation of high affinity $GABA_A$ receptors in specific brain regions by ovarian hormones. Neuroendocrinology 50:315-320; 1989.
58. Shoupe, D.; Montz, F. J.; Lobo, R. A. The effects of estrogen and progestin on endogenous opioid activity in oophorectomized women. J. Clin. Endocrinol. Metab. 60:178-183; 1985.
59. Smythe, J. W.; McCormick, C. M.; Rochford, J.; Meaney, M. J. The interaction between prenatal stress and neonatal handling on nociceptive response latencies in male and female rats. Physiol. Behav. 55:971-974; 1994.
60. Wardlaw, S.; Thoron, L.; Frantz, A. Effects of sex steroids on brain beta-endorphin. Brain Res. 245:327-331; 1982.
61. Westerling, P.; Lindgren, S.; Meyerson, B. Functional changes in $GABA_A$ receptor stimulation during the estrous cycle of the rat. Br. J. Pharmacol. 103:1580-1584; 1991.
62. Wilson, M. A. Influences of gender, gonadectomy, and estrous cycle on GABA/BZ receptors and benzodiazepine responses in rats. Brain Res. Bull. 29:165-172; 1992.
63. Wilson, M. A.; Biscardi, R. Effects of gender and gonadectomy on responses to chronic benzodiazepine receptor agonist exposure in rats. Eur. J. Pharmacol. 215:99-107; 1992.
64. Winfree, C. J.; Coombs, D. W.; DeLeo, J. A.; Colburn, R. W. Analgesic effects of intrathecally administered 3α-hydroxy-5α-pregnan-20-one in a rat mechanical visceral pain model. Life Sci. 50:1007-1012; 1992.