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Estrogen Effects on Neuronal Morphology

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This review focuses on the effects of estrogen on neuronal morphology. Over the last decade neuroscientists have accumulated a wealth of information confirming the trophic effects of 17β-estradiol on a variety of brain regions, including changes of hippocampal spine density and axonal outgrowth and retraction in hypothalamic nuclei, as well as other measures of structural reorganization that could underlie some of the cognitive benefits attributed to this hormone. Overall, results from a variety of investigators suggest that 17β-estradiol is a potent structural signal that can drive developmental as well as adult plastic events in a variety of brain regions, not only those implicated in reproduction, but also in a diversity of functions. Most notably, these structural modifications that subserve cyclic physiological processes, can also be activated in other brain regions to protect and even reverse structural neurodegenerative processes. The data presented here are not exhaustive, but rather meant to provide a few examples of these structural effects of 17β-estradiol that could have important implications for clinical practice.

Key Words: Outgrowth; plasticity; estrogen; regeneration; sprouting.

Introduction

Over the last 30 yr, substantial progress has been made in the understanding of the hormonal events that underlie the development of sex differences in the brain. In mammals, the brains of males and females are different: they show differences in the volume of specific brain nuclei, in the density of neurons, in the complexity of dendritic arborizations, and even in the expression of neuropeptides *(1)*. It has been known for quite some time *(2)* that these *organizational* effects of gonadal steroids are distinct from *activational* effects, which refer to hormonal actions on neural systems that mediate specific behaviors in adulthood. Nonetheless, changing levels of hormones at puberty and during adult life determine morphological and neurochemical plasticity, not only in the parts of the nervous system that are organized by gonadal hormones during development, but also in others whose developmental dependence on hormones is not that clear.

This review is focused on the wide range of structural effects of estrogen (17β-estradiol, E2) on neurons, using as examples the effects of E2 on four distinct brain regions, involved in such diverse functions as female reproductive behavior, pain, and learning and memory. The study of morphological regulation by E2 in the nervous system is of particular interest because it offers the possibility of identifying the cellular mechanisms that drive structural reorganization, as well as the functional consequences of changes in neuronal structure. Although the direct targets of E2 can be identified and localized based on the expression of the relevant receptors, it is important to acknowledge that the site of direct E2 action is not necessarily the same cell that displays morphological changes. This seems to be the case, for example, in the hippocampus, where E2 regulates structural plasticity by acting on E2-sensitive afferents, involving a neurotrophin such as brain-derived neurotrophic factor (BDNF) as a transsynaptic signal.

By and large, the most promising outcome of studies of E2-dependent structural plasticity is the potential to understand the functional consequences of changes in neuronal architecture. By placing specific E2-induced morphological changes in their appropriate physiological and behavioral context, neuroendocrinologists can gain insight into how plasticity of brain structure leads to plasticity in behavior.

Although effects of E2 on neuronal morphology can be generalized to the whole neuron, even more interesting are those effects that can be restricted to a particular compartment. In the present review we will also focus on the differential effects of E2 on axons and dendrites.

Effects of Estrogen on Structural Plasticity of Hypothalamic Neurons

Ultrastructural studies of hypothalamic nuclei during the perinatal period reveal poorly developed axons and dendrites, and decreased numbers of synapses compared to adults. Normal development leads to increased number of processes and synapses, as well as maturation of synaptic morphology *(3,4)*. Early in vitro studies employing explants from newborn rodent hypothalami *(5,6)* have confirmed E2's ability to accelerate the proliferation of hypothalamic neurites, and administration of E2 to rats during the first month of life

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results in twice the number of axodendritic synapses within the arcuate nucleus compared to controls *(3)*. The results of in vitro studies have further demonstrated that E2 can enhance the outgrowth of neurite processes from preoptic/hypothalamic explant cultures *(7)* and from dissociated hypothalamic cells *(8)*. Because these regions contain E2 receptors, it is suggested that E2 effects on neurite outgrowth may be the result of interaction with its receptors and subsequent modulation in the expression of genes crucial to neuronal development.

E2's capacity to alter synaptic density is not limited to the developmental stages. A series of studies by Matsumoto and Arai *(9,10)* have shown that, following mechanical denervation of the hypothalamus, E2 administration restores synaptic density within the denervated region. Garcia-Segura and colleagues have further characterized a process they have called "phased synaptic remodeling" in which a denervation/reinnervation cycle follows E2 administration *(11, 12)*. Phased synaptic remodeling also accompanies the preovulatory E2 surge in cycling rats, and the number of arcuate nucleus synapses thus fluctuates in a predictable fashion throughout the estrus cycle *(12)*. A summary of this finding, and a discussion of its significance can be found in ref. *13*. Similar results have been described in ventromedial nucleus (VMN) hypothalamic neurons, using Golgi techniques *(14)*. In the ovariectomized, oil-treated rat, spine density in the VMN is significantly lower than E2-treated or intact controls, and in the normal cycling rat, significantly fewer spines are found at diestrus than proestrus. This rise and fall in dendritic spine density across the estrous cycle has subsequently been confirmed by others *(15)*.

In addition, a number of studies have provided evidence that E2 differentially affects selective portions of the dendritic tree in VMN neurons *(15–17)*. Although 72 h of E2 treatment causes an overall increase in dendritic spine density, this effect is not uniform. In fact, this increase is found in short primary dendrites, which are oriented randomly within the VMN, and coexists with a 50% reduction in spine density in the middle portion of long, ventrolaterally oriented primary dendrites *(16,17)*. This spatial precision on dendritic plasticity may be a mechanism by which E2 regulates synaptic input to the VMN and modulates connectivity patterns that mediate female sexual behavior. A detailed discussion of the functional consequences of dendritic remodeling of VMN neurons can be found in a recent review *(18)*.

Although E2 has been found to modulate many proteins in the adult VMN *(19)*, of relevance in the context of sprouting is the growth-associated protein GAP-43, a protein implicated in axonal growth and motility *(20)*. *In situ* hybridization studies have demonstrated that the modulation of GAP-43 mRNA expression in the rat brain by E2 is region-specific, with a significant effect on the medial preoptic area (MPA) and VMN of the hypothalamus, but none on the cortex *(21–24)*. In the median eminence, GAP-43 protein staining is observed in the same regions where most GnRH axons and terminals were located, and dual immunocytochemistry confirms that numerous GnRH terminals are also positive for GAP-43. Furthermore, the number of GnRH neurons from the arcuate nucleus that contain GAP-43 mRNA is significantly higher at the time of proestrus, compared to either diestrus or estrous phase of the cycle *(25)*. This fluctuation suggests that increases in GAP-43 at the time of proestrus could promote the sprouting of axon terminals from GnRH neurons, and allow them to contact the pericapillary space in the region of the median eminence *(26,27)*.

Sprouting of axon terminals has been confirmed in vitro, and hypothalamic neurons maintained in culture also react to E2 in the medium with a significant neuritic outgrowth *(28)*, a response preceded by increases in the levels of tau protein, but not in tubulin, microtubule-associated protein (MAP)-1a, or MAP-2. Higher levels of tau protein suggest that as a consequence of the expression of more stable microtubules, hypothalamic neurons are able to extend longer neurites with increased stability or to stabilize neurite branches that would otherwise retract. These findings are in agreement with others showing hormonal-induced changes in mRNAs coding for specific brain proteins, including tubulin-like molecules, in the hypothalamic preoptic area *(29,30)*.

Neuritic outgrowth in hypothalamic neurons in culture also follows a sexually dimorphic pattern. Early work has shown that neurons taken from female hypothalamus differentiate axons later than the corresponding males, and also have fewer primary neurites and shorter dendrites *(31)*. Carrer and his colleagues followed this finding with a series of studies *(32–34)* that demonstrated how, in primary cultures of dissociated hypothalamic neurons taken from the VMN, the neuritogenic effect of E2 is exerted differentially depending on the genetic sex of the neurons, the age of the donor, and the presence of heterotopic glia from the target region. When taken at embryonic d 19, both male and female hypothalamic neurons would react to the presence of E2 with increased outgrowth and branching, whereas in cultures prepared from embryonic d 16, only male cells were responsive to E2. This neuritogenic effect of E2 in male neurons could not be blocked by either tamoxifen or ICI, suggesting that the effect is not exerted through the classical intracellular receptor signal transduction mechanism but rather through a membrane-mediated mechanism *(35)*. On the other hand, in cultures grown without glia, or with glia from non-target regions, E2 had no immediate effect, thus pointing toward an indirect effect of E2. Furthermore, the axogenic effect of E2 was suppressed in cultures treated with a specific TrkB antisense oligonucleotide *(36,37)* suggesting a synergistic effect of E2 and neurotrophic factors in the sprouting response of these neurons.

Estrogen Modulates Structural Plasticity of the Hippocampus

E2 regulation of dendritic spines and spine synapses on pyramidal neurons in the hippocampus is one of the most

studied examples of hormone-driven dendritic plasticity in adulthood. Gould et al. *(38)* were the first to show that dendritic spine density in the adult rat hippocampal neurons is sensitive to hormone levels. A number of groups replicated this finding in rats *(39,40)* and in monkeys *(41,42)*, and extended it to show that E2 modulates both pre- and post-synaptic proteins in the hippocampus of female macaques *(43)*. A recent report *(18)* reviews in detail the cellular mechanisms of E2-induced dendritic spine density and the corresponding changes in hippocampal connectivity as well as its behavioral correlates. They describe that rats' working memory is improved 1–4 d after E2 treatment, at the time of elevated spine density, but not before spine density increases (d 0–1) or after it returns to low levels (d 9–10).

In some of their earlier work using Golgi-impregnated tissue, Woolley and colleagues *(44)* demonstrated that apical dendritic spine density in CA1 hippocampal pyramidal cells undergo a cyclic fluctuation across the estrous cycle in the adult female rat, with spines being more numerous in the afternoon of proestrus than in estrus, which would favor a better integration of synaptic information. However, not only spine density but also spine shape is affected by E2 levels, and during proestrus mushroom spines are more abundant, while thin spines are more abundant during estrus *(45)*. In ovariectomized mice, E2 has been shown to induce similar transformations of spines into the mushroom-shaped type, at least in part through its action on cytoskeleton-associated synaptic proteins *(46)*.

Investigations to determine the mechanism of E2-induced neurotrophism point to a temporal cascade of E2-inducible effects that are mediated by separated but potentially interacting pathways. It is known that E2 induces a rise in intracellular calcium *(47)*, frequently the initiating event leading to intracellular signaling pathways that regulate neurite outgrowth and synaptic connectivity *(48)*. But several other signaling pathways have been invoked as an explanation for dendritic sprouting, and there is evidence for E2 actions on pre- and post-synaptic proteins *(43,49)*. The earliest phase of E2-induced neurotrophism, filopodial outgrowth, can occur within minutes of exposure and one mechanistic candidate could be E2-dependent activation of a member of the Rho family of GTPases, Rac 1B *(50)*. The second phase, the development of stable dendritic spines appears to be mediated by an NMDA receptor–dependent mechanism through E2 activation of a src tyrosine kinase that phosphorylates the NMDA receptor *(51)*. The third phase appears to involve E2-inducible spine stabilization via a transient suppression of GABA (A)-mediated inhibition of CA1 pyramidal cells *(52)*. Interestingly, some of these effects are mediated via cholinergic input *(53–57)*, as described below.

Effects of Estrogen on Structural Plasticity of the Basal Forebrain Cholinergic System

The physiology of the basal forebrain cholinergic system is also modulated by E2, and the effect of the hormone on the regulation of muscarinic receptors and choline acetyltransferase (ChAT) activity was already described more than 25 yr ago *(58–60)*. This finding was followed by a number of studies showing that E2 can significantly enhance the expression and activity of ChAT, the synthetic enzyme responsible for the production of acetylcholine *(61)*. We have recently described *(62)* that E2 induces a significant fourfold increase in total neurite length in basal forebrain cholinergic neurons from female rats, grown in culture. This change was accompanied by a twofold increase in the number of segments per neuron, and was found evenly distributed throughout the dendritic arborization, with no particular preference for proximal versus distal processes. We hypothesized that cytoarchitectural modifications by E2 could also participate in neuronal regeneration under pathological conditions. In particular, E2 may play a role in supporting the survival of cholinergic neurons in neurodegenerative disorders, and newly formed neurite branches may lead to the formation of new synapses. Furthermore, structural enhancement of nerve cell morphology has been postulated to be an integral step in cellular processes leading to information storage in the nervous system, and perhaps E2-induced neurite sprouting within cholinergic neurons could underlie the cognitive enhancement effects of E2 treatment. Saenz and colleagues *(63)* addressed this question using an experimental lesion model. Sixteen young adult female rats were used, eight ovariectomized (OVX) and eight intact (NOVX), and four rats from each group were treated with subcutaneous pellets releasing E2 (+E) or placebo (+P). All 16 rats received a unilateral lesion of the cholinergic-specific toxin 192 IgG-saporin as described previously *(64)*. The immunotoxin destroyed between 30% and 50% of the cholinergic neurons in the nucleus of the horizontal diagonal band of Broca (HDB), and a comparison of morphological parameters of cholinergic neurons in the lesioned versus the control basal forebrain showed a hormonal effect. E2 treatment, whether from natural sources (the NOVX+P group) or from the pellet (the OVX+E group), was able to maintain dendritic arborization in the surviving neurons at control levels. By contrast, dendritic arborization remained significantly reduced in animals totally deprived of E2 (OVX+P). The number of dendritic segments was not changed by any of these manipulations, and there was no obvious effect of the hormone on this structural parameter. These results are consistent with findings by Ferrini and colleagues *(65)* showing that in aged male mice E2 treatment increased neurite length in ChAT immunoreactive cells to levels similar to young animals and also upregulated the expression of GAP-43 mRNA in the aged mice.

Importantly, the effect of E2 on cholinergic neurons is not limited to the dendritic compartment, but also includes axons. E2 action at the level of presynaptic terminals may be responsible, in turn, for the structural changes in hippocampal pyramidal cells, and a number of in vivo experiments suggest a role for the basal forebrain cholinergic system in

the regulation of hippocampal dendritic spines. Evidence in this regard comes from work indicating that transsection of the fimbria/fornix, the fiber tract that contains the cholinergic axons from the basal forebrain to the hippocampus, blocks E2-induced spine formation *(39)*, and also from experiments showing that E2 delivered directly to the medial septum can increase spine density in the hippocampus *(55)*, as well as from electrophysiological data showing that, in animals with specific cholinergic lesions, E2 is much less effective in disinhibiting hippocampal pyramidal cells *(53)* than in controls.

Morphological Effects of E2 in the Peripheral Nervous System (PNS)

E2 affects many subpopulations of the PNS, including non-nociceptive *(66,67)* as well as nociceptive sensory nerves. This correlates with physiological findings showing that pain threshold in female rats fluctuates across the estrous cycle, and pain sensitivity is reduced by E2 *(68,69)*. Compared with gonadectomized rats, chronic elevation of plasma E2 induces a significant sprouting of calcitonin gene-related peptide (CGRP)–immunoreactive fibers innervating mammary gland arterioles *(70)*, those in the ear pinna and the mesenteric vasculature *(71)*. Vascular endothelial and smooth muscle cells express E2 receptors *(72,73)*, and also synthesize and secrete various neurotrophic factors to which sensory nociceptor neurons are responsive *(74,75)*. It is therefore possible that E2 acts on peripheral tissue to increase neurotrophic factor availability. Nonetheless, because the majority of dorsal root ganglion (DRG) neurons express E2 receptors *(76)*, a direct effect of E2 on sensory neurons cannot be ruled out. In fact, recent work *(77)* has described a robust outgrowth in DRG neurons cultured with E2. This sprouting response is not accompanied by changes in neuron number or size, but elicits an increase in CGRP-immunoreactive levels, consistent with an effect of E2 on CGRP synthesis *(78,79)*.

Another interesting example of structural actions of E2 on the PNS is the remodeling that occurs in the sympathetic innervation of the rat uterus as a function of E2 levels, a degenerative and regenerative process that occurs not only during pregnancy but also as a normal component of the estrous cycle. Low levels of E2 following ovariectomy and during the early diestrus phase of the estrous cycle are characterized by relatively high sympathetic nerve density of the uterine horns. However, when E2 levels increase (injection, sustained infusion, or during the early estrous phase), density of sympathetic nerves innervating the myometrium rapidly decreases *(80,81)*. This loss of innervation occurs within 24 h, and is the result of axonal degeneration, not of retraction of intact axons, as determined by electron microscopy *(82)*. Furthermore, this loss of innervation appears related, at least in part, to changes in the properties of the target tissue, as in vitro studies have shown that while the myometrium from low-E2 rats can induce the sprouting of superior cervical ganglion (SCG) neurons, raising E2 levels in vivo prior to harvesting the myometrium abolishes this response *(83)*. It has recently been reported that E2 regulates this sympathetic neurite outgrowth by modulating BDNF synthesis and release by the rodent uterus *(84)*.

Conclusion

The work presented in this review confirms the importance of E2 in regulating structural plasticity, through direct or indirect, positive or negative effects on dendritic and axonal morphology.

In the case of dendrites, because of their fundamental involvement in synaptic integration and synaptic plasticity, plastic properties are important for both neuronal information processing and storage. Modifications in dendritic arbors profoundly shape the computational and storage capability of neurons, and thus allow a degree of flexibility that enables the performance of the most appropriate computation under a given condition. From a behavioral perspective, this structural flexibility may be particularly useful to enable responses to fluctuating levels of hormones, although it also should be noted that it potentially renders the system more susceptible to errors, and thus pathological conditions.

E2 actions at the presynaptic level are a source of modulatory influences on target regions, and as such, affect patterns of synaptic connectivity more widely than would be expected from the distribution of E2 receptors. A particularly striking example is the basal forebrain cholinergic system, where E2 seems to elicit a sprouting response at the dendritic level in cholinergic neurons (which express E2 receptors), while also exerting a remote effect, that is, driving an increase in synaptic density in hippocampal neurons (which do not express E2 receptors) that are the target for cholinergic projections.

Documenting specific structural effects of E2 on neurons is, obviously, only the first albeit necessary step to further elucidate the molecular cascades that are activated by the hormone. A review of the available data, even as limited as the present, highlights the need for much more work to be done.

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References

1. Simerly, R. B. (2002). *Annu. Rev. Neurosci.* **25,** 507–536.
2. Harris, G. W. (1964). *Endocrinology* **75,** 627–648.
3. Arai, Y. and Matsumoto, A. (1978). *Psychoneuroend* **3,** 31–45.
4. Walsh, R. J., Brawer, J. R., and Naftolin, F. (1982). *J. Anat.* **135,** 733–744.
5. Toran-Allerand, C. D. (1976). *Brain Res.* **106,** 407–412.
6. Toran-Allerand, C. D., Hashimoto, K., Greenough, W. T., and Saltarelli, M. (1983). *Brain Res.* **283,** 97–101.

7. Toran-Allerand, C. D. (1980). *Brain Res.* **189,** 413–427.
8. Uchibori, M. and Kawashima, S. (1985). *Int. J. Dev. Neurosci.* **3,** 169–176.
9. Matsumoto, A. and Arai, Y. (1979). *Cell Tissue Res.* **198,** 427–433.
10. Matsumoto, A. and Arai, Y. (1981). *J. Comp. Neurol.* **197,** 197–205.
11. Garcia-Segura, L. M., Baetens, D., and Naftolin, F. (1986). *Brain Res.* **366,** 131–136.
12. Garcia-Segura, L. M., Hernandez, P., Olmos, G., Tranque, P. A., and Naftolin, F. (1988). *J. Neurocytol.* **17,** 377–383.
13. Naftolin, F., Garcia-Segura, L. M., Keefe, D., Leranth, C., Maclusky, N. J., and Brawer, J. R. (1990). *Biol. Reprod.* **42,** 21–28.
14. Frankfurt, M., Gould, E., Woolley, C. S., and McEwen, B. S. (1990). *Neuroendocrinol.* **51,** 530–535.
15. Madeira, M. D., Ferreira-Silva, L., and Paula-Barbosa, M. M. (2001). *J. Comp. Neurol.* **432,** 329–345.
16. Calizo, L. H. and Flanagan-Cato, L. M. (2000). *J. Neurosci.* **20,** 1589–1596.
17. Calizo, L. H. and Flanagan-Cato, L. M. (2002). *J. Comp. Neurol.* **447,** 234–248.
18. Cooke, B. M. and Woolley, C. S. (2005). *J. Neurobiol.* **64,** 34–46.
19. Lustig, R. H., Pfaff, D. W., and Mobbs, C. V. (1989). *Endocrinology* **124,** 1863–1869.
20. Benowitz, L. I. and Routtenberg, A. (1997). *Trends Neurosci.* **20,** 84–91.
21. Shughrue, P. J. and Dorsa, D. M. (1994). *Endocrinology* **134,** 1321–1328.
22. Lustig, R. H., Sudol, M., Pfaff, D. W., and Federoff, H. J. (1991). *Mol. Brain Res.* **11,** 125–132.
23. Shughrue, P. J. and Dorsa, D. M. (1993). *Neuroendocrinol.* **57,** 439–447.
24. Shughrue, P. J. and Dorsa, D. M. (1994). *J. Comp. Neurol.* **340,** 174–184.
25. Prevot, V., Bouret, S., Croix, D., et al. (2000). *Endocrinology* **141,** 1648–1657.
26. Prevot, V., Dutoit, S., Croix, D., Tramu, G., and Beauvillain, J. C. (1998). *Neurosci.* **84,** 177–191.
27. Prevot, V., Croix, D., Bouret, S., et al. (1999). *Neurosci.* **94,** 809–819.
28. Ferreira, A. and Caceres, A. (1991). *J. Neurosci.* **11,** 392–400.
29. Stanley, H. F. and Fink, G. (1986). *Brain Res.* **370,** 223–231.
30. Stanley, H. F., Borthwick, N. M., and Fink, G. (1986). *Brain Res.* **370,** 215–222.
31. Diaz, H., Lorenzo, A., Carrer, H. F., and Caceres, A. (1992). *J. Neurosci. Res.* **33,** 266–281.
32. Carrer, H. F., Cambiasso, M. J., and Gorosito, S. (2005). *J. Steroid Biochem. Mol. Biol.* **93,** 319–323.
33. Cambiasso, M. J., Diaz, H., Caceres, A., and Carrer, H. F. (1995). *J. Neurosci. Res.* **42,** 700–709.
34. Cambiasso, M. J., Colombo, J. A., and Carrer, H. F. (2000). *Eur. J. Neurosci.* **12,** 2291–2298.
35. Cambiasso, M. J. and Carrer, H. F. (2001). *J. Neurosci. Res.* **66,** 475–481.
36. Carrer, H. F., Cambiasso, M. J., Brito, V., and Gorosito, S. (2003). *Ann. NY Acad. Sci.* **1007,** 306–316.
37. Brito, V. I., Carrer, H. F., and Cambiasso, M. J. (2004). *Eur. J. Neurosci.* **20,** 331–337.
38. Gould, E., Woolley, C. S., Frankfurt, M., and McEwen, B. S. (1990). *J. Neurosci.* **10,** 1286–1291.
39. Leranth, C., Shanabrough, M., and Horvath, T. L. (2000). *Neurosci.* **101,** 349–356.
40. Adams, M. M., Shah, R. A., Janssen, W. G. M., and Morrison, J. H. (2001). *Proc. Natl. Acad. Sci. USA* **98,** 8071–8076.
41. Leranth, C., Shanabrough, M., and Redmond, D. E. Jr. (2002). *J. Comp. Neurol.* **447,** 34–42.
42. Hao, J., Janssen, W. G., Tang, Y., et al. (2003). *J. Comp. Neurol.* **465,** 540–550.
43. Choi, J. M., Romeo, R. D., Brake, W. G., Bethea, C. L., Rosenwaks, Z., and McEwen, B. S. (2003). *Endocrinology* **144,** 4734–4738.
44. Woolley, C. S., Gould, E., Frankfurt, M., and McEwen, B. S. (1990). *J. Neurosci.* **10,** 4035–4039.
45. Gonzalez-Burgos, I., Alejandre-Gomez, M., and Cervantes, M. (2005). *Neurosci. Lett.* **379,** 52–54.
46. Li, C., Brake, W. G., Romeo, R. D., et al. (2004). *Proc. Natl. Acad. Sci. USA* **101,** 2185–2190.
47. Zhao, L., Chen, S., Ming Wang, J., and Brinton, R. D. (2005). *Neurosci.* **132,** 299–311.
48. West, A. E., Chen, W. G., Dalva, M. B., et al. (2001). *Proc. Natl. Acad. Sci. USA* **98,** 11024–11031.
49. Brake, W. G., Alves, S. E., Dunlop, J. C., et al. (2001). *Endocrinology* **142,** 1284–1289.
50. Brinton, R. D. (1993). *Mol. Cell Neurosci.* **4,** 36–46.
51. Yu, X.-M., Askalan, R., Keil, G. J. II, and Salter, M. W. (1997). *Science* **275,** 674–678.
52. Rudick, C. N. and Woolley, C. S. (2001). *J. Neurosci.* **21,** 6532–6543.
53. Rudick, C. N., Gibbs, R. B., and Woolley, C. S. (2003). *J. Neurosci.* **23,** 4479–4490.
54. Lam, T. T. and Leranth, C. (2003). *Neurosci.* **116,** 491–498.
55. Lam, T. T. and Leranth, C. (2003). *Eur. J. Neurosci.* **17,** 1997–2005.
56. Kovacs, E. G., MacLusky, N. J., and Leranth, C. (2003). *Neurosci.* **122,** 807–810.
57. MacLusky, N. J., Luine, V. N., Hajszan, T., and Leranth, C. (2005). *Endocrinology* **146,** 287–293.
58. Luine, V. N., Khylchevskaya, R. I., and McEwen, B. S. (1975). *Brain Res.* **86,** 293–306.
59. McEwen, B. S., Biegon, A., Davis, P. G., et al. (1982). *Rec. Prog. Horm. Res.* **38,** 41–92.
60. Luine, V. N. (1985). *Exp. Neurol.* **89,** 484–490.
61. McMillan, P. J., Singer, C. A., and Dorsa, D. M. (1996). *J. Neurosci.* **16,** 1860–1865.
62. Dominguez, R., Jalali, C., and de Lacalle, S. (2004). *J. Neurosci.* **24,** 982–990.
63. Saenz, C., Dominguez, R., and de Lacalle, S. (2006). *Neurosci. Lett.* **392,** 198–201.
64. Hartonian, I., Mufson, E. J., and de Lacalle, S. (2002). *Neurosci.* **115,** 787–795.
65. Ferrini, M., Bisagno, V., Piroli, G., Grillo, C., Deniselle, M. C., and De Nicola, A. F. (2002). *Cell Mol. Neurobiol.* **22,** 289–301.
66. Komisaruk, B. R., Adler, N. T., and Hutchison, J. (1972). *Science* **178,** 1295–1298.
67. Robbins, A., Sato, Y., Hotta, H., and Berkley, K. J. (1990). *Neurosci. Lett.* **110,** 82–85.
68. Martinez-Gomez, M., Cruz, Y., Salas, M., Hudson, R., and Pacheco, P. (1994). *Physiol. Behav.* **55,** 651–657.
69. Dawson-Basoa, M. E. and Gintzler, A. R. (1996). *Pain* **64,** 608–615.
70. Blacklock, A. D. and Smith, P. G. (2004). *J. Neurobiol.* **59,** 192–204.
71. Blacklock, A. D., Cauveren, J. A., and Smith, P. G. (2004). *Brain Res.* **1018,** 55–65.
72. Andersson, C., Lydrup, M. L., Ferno, M., Idvall, I., Gustafsson, J., and Nilsson, B. O. (2001). *J. Endocrinol.* **169,** 241–247.
73. Orshal, J. M. and Khalil, R. A. (2004). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **286,** R233–249.
74. Krasinski, K., Spyridopoulos, I., Asahara, T., van der Zee, R., Isner, J. M., and Losordo, D. W. (1997). *Circulation* **95,** 1768–1772.
75. Kim-Schulze, S., Lowe, W. L. Jr., and Schnaper, H. W. (1998). *Circulation* **98,** 413–421.

76. Taleghany, N., Sarajari, S., DonCarlos, L. L., Gollapudi, L., and Oblinger, M. M. (1999). *J. Neurosci. Res.* **57,** 603–615.
77. Blacklock, A. D., Johnson, M. S., Krizsan-Agbas, D., and Smith, P. G. (2005). *Eur. J. Neurosci.* **21,** 2320–2328.
78. Gangula, P. R., Lanlua, P., Wimalawansa, S., Supowit, S., DiPette, D., and Yallampalli, C. (2000). *Biol. Reprod.* **62,** 1033–1039.
79. Mowa, C. N., Usip, S., Collins, J., Storey-Workley, M., Hargreaves, K. M., and Papka, R. E. (2003). *Peptides* **24,** 1163–1174.
80. Zoubina, E. V., Fan, Q., and Smith, P. G. (1998). *J. Comp. Neurol.* **397,** 561–571.
81. Zoubina, E. V., Mize, A. L., Alper, R. H., and Smith, P. G. (2001). *Histol. Histopathol.* **16,** 989–996.
82. Zoubina, E. V. and Smith, P. G. (2000). *Auton. Neurosci.* **84,** 176–185.
83. Krizsan-Agbas, D. and Smith, P. G. (2002). *Neurosci.* **114,** 339–347.
84. Krizsan-Agbas, D., Pedchenko, T., Hasan, W., and Smith, P. G. (2003). *Eur. J. Neurosci.* **18,** 2760.