The role of glia in the hypothalamus: implications for gonadal steroid feedback and reproductive neuroendocrine output

Luis Miguel Garcia-Segura, Betty Lorenz and Lydia L DonCarlos

Instituto Cajal, CSIC, Avenida Doctor Arce 37, E-28002 Madrid, Spain, Neuroscience Program and Department of Cell Biology, Neurobiology and Anatomy, Loyola University Chicago, Maywood, Illinois 60153, USA

Correspondence should be addressed to L M Garcia-Segura; Email: lmgs@cajal.csic.es

Abstract

Neuron-to-glia, glia-to-neuron, and glia-to-glia communication are implicated in the modulation of neuronal activity and synaptic transmission relevant to reproduction. Glial cells play an important role in neuroendocrine regulation and participate in the sexual differentiation of neuronal connectivity of brain regions involved in the control of reproductive neuroendocrine output. During puberty, modifications in the morphology and chemistry of astrocytes and tanycytes in the hypothalamus and median eminence influence the maturation of the neuronal circuits controlling the secretion of GnRH. During adult reproductive life, the glial cells participate in the transient remodeling of neuronal connectivity in the preoptic area, the arcuate nucleus, the median eminence, and other brain regions involved in the control of reproduction. Gonadal hormones regulate glial plasticity by direct and indirect effects and regulate various other endocrine signals, local soluble factors and adhesion molecules that also affect glial function and glia-to-neuron communication. The glial cells, therefore, are central to the coordination of endocrine and local inputs that bring about neural plasticity and adapt reproductive capacity to homeostatic signals.


Introduction

In recent years, a considerable amount of evidence has demonstrated the existence of reciprocal communication between the glial cells and the neurons, showing that the glial cells have an essential role in the regulation of the functional activity of the nervous system. The glial cells are sensitive to the activity of neighboring neurons and may influence synaptic transmission and neuronal function (Araque et al. 2001, Volterra & Meldolesi 2005, Jourdain et al. 2007, Ni et al. 2007, Perea & Araque 2007, Wigley et al. 2007). The glial cells express receptors for neurotransmitters and release several substances that act as gliotransmitters and may influence glia-to-glial and glia-to-neuron communication as well as neuronal differentiation and plasticity. In addition, modifications in glial cell morphology affect the formation and maintenance of synaptic contacts (Garcia-Segura et al. 1994, Hatton 1997, Theodosis et al. 2006). Therefore, a substantial modification in the interpretation of the function of glial cells has emerged. In this review, we will focus on recent evidence indicating that the glial cells play an important role in the control of gonadotropin-releasing hormone (GnRH) release and in the gonadal steroid feedback on GnRH neurons, either by direct interactions with GnRH neuronal somas and terminals or by the modulation of the neuronal circuits that regulate the activity of GnRH neurons.

The role of glial cells in the maturation of neuronal circuits regulating GnRH neurons

The glial cells are important mediators of the sexual differentiation of neuronal connectivity induced by gonadal hormones. This is substantiated by findings of several laboratories indicating that the morphology, immunoreactivity, enzymatic activity, and gene expression of astroglia are sexually dimorphic in several brain areas and can be modified by the postnatal actions of sex hormones. Furthermore, the glial cells express receptors for gonadal hormones, metabolize gonadal steroids, and participate in the synthesis of endogenous steroids by the nervous system (for review see Garcia-Segura & Melcangi 2006). Sex differences in the differentiation of astroglia may impact on the organization of the neuronal network that regulates the activity and secretion of GnRH neurons. Exposure of the fetal brain of guinea pigs (Connolly & Resko 1994), rats (Foecking et al. 2005), mice (Sullivan & Moenter 2004), pigs (Elsaesser & Parvizi 1979), sheep (Fabre-Nys & Venier 1991, Herbosa et al. 1996, Kim et al. 1999, Robinson 2006), and rhesus monkeys (Dumesic et al. 1997) to testosterone results in modifications in the number and function of synaptic inputs to GnRH neurons. Differences in the morphology of glial cell processes associated with GnRH neurons have been
observed in parallel to the synaptic changes (Chen et al. 1990, Kim et al. 1999, Sullivan & Moenter 2004). The GnRH neuronal network in female animals that have been exposed in utero to testosterone has an impaired, male-like response to the estrogen-stimulated GnRH surge (Sharma et al. 2002, Birch et al. 2003).

During the peripubertal period, the neuronal systems that govern the activity of the neurons that produce GnRH, including GABAergic neurons, preproenkephalinergic neurons, glutamatergic neurons, and kisspeptin-expressing neurons, show morphological and functional modifications (Perera & Plant 1997, Han et al. 2002, Navarro et al. 2004, Shahab et al. 2005, Cottrell et al. 2006). In parallel, changes occur in the morphology and chemistry of tanycytes in the median eminence, and this plasticity affects the regulation of GnRH release into the portal blood vessels of the median eminence. Tanycytes are specialized bipolar glial cells, located in the arcuate nucleus and the median eminence, that play a key role in neuroendocrine regulation. Tanycytes contribute to the regulation of GnRH release by extension and retraction of end-foot processes that are interposed between GnRH synaptic terminals and the portal vasculature of the median eminence (Kobayashi et al. 1972, Kozlowski & Coates 1985, Ugrumov et al. 1985, 1989, Silverman et al. 1991, King & Letourneau 1994, Prevot et al. 1999).

The arcuate nucleus contains several neuronal populations that are involved in the control of GnRH cells, including a subpopulation of kisspeptin neurons. In addition, the arcuate nucleus integrates other hormonal signals that regulate energy balance and food intake, such as ghrelin and leptin, which may also regulate synaptic plasticity in this hypothalamic region (Horvath 2006). Horvath et al. have shown that leptin deficiency and replacement regulate the number of excitatory and inhibitory synapses and postsynaptic currents onto astrocyte terminal processes in parallel with the increase in LH concentration in plasma. The following day, on estrus, both the surface area and the number of astrocyte processes per astrocyte return to levels similar to those on the morning of proestrus (Cashion et al. 2003). Similar findings have been observed in adult primates, where ovariectomy females, but not males, respond to the neuroplastic actions of E2 (Olmos et al. 1989, Garcia-Segura et al. 1994, Horvath et al. 1997, Csakvari et al. 2007). These sex differences are induced by the perinatal secretion of testosterone in male rats. Perinatal testosterone increases in astrocytes the expression of a cytoskeletal protein that regulates astroglia cell morphology, glial fibrillary acidic protein (GFAP), increasing the growth of astrocytic processes and the extent of neuronal membranes covered by these processes. Coincident with these changes in astrocytic morphology there is a strong reduction in the density of dendritic spines and axo-somatic synapses on arcuate neurons in males (Garcia-Segura et al. 1994, 1995b, Mong et al. 1996, 1999, Mong & McCarthy 1999; Fig. 1).

The role of glial cells in the control of GnRH release in adulthood

In rats, morphological changes in astrocytes that are directly apposed to GnRH neurons in the rostral preoptic area are associated with gonadotropin release. The surface area of astrocytes and the number of processes per astrocytic process decrease from the morning of proestrus, before the initiation of the GnRH-induced luteinizing hormone (LH) surge, to the afternoon of proestrus. During the afternoon of proestrus, there is a significant decrease in the surface area and the number of astrocytic processes in parallel with the increase in LH concentration in plasma. The following day, on estrus, both the surface area and the number of processes per astrocyte return to levels similar to those on the morning of proestrus (Cashion et al. 2003).

Figure 1 Sex differences in axo-somatic synaptic inputs and coverage of neuronal somas by glial processes in the arcuate nucleus of the rat hypothalamus. In male rats testosterone induces the growth of astrocytic processes, resulting in an increased coverage of neuronal somas by glia when compared with female rats. Conversely, there is an increased formation of axo-somatic synapses in female neurons when compared with males. Astrocytic processes may limit the amount of neuronal surface available for the formation of synaptic contacts and may be the cause of the sex difference in synaptic contacts.
increases the apposition of glial processes to GnRH neuronal perikarya and decreases the number of synaptic inputs to GnRH neurons, while ovarian hormone replacement has the opposite effects, decreasing the glial ensheathment and increasing the innervation of GnRH somas (Witkin et al. 1991). In addition, tanycytic processes in the median eminence of rodents extend and retract following hormonal changes during the estrous cycle. As mentioned before, tanycytic processes ensheathe the GnRH terminals, preventing GnRH release. However, during the preovulatory stage of the estrous cycle, tanycytic processes retract, allowing the transient contact of GnRH terminals with portal capillaries (King & Letourneau 1994, Prevot et al. 1999).

Neuron-glial remodeling associated with the regulation of GnRH release is not limited to the GnRH terminals or to the direct synaptic inputs onto GnRH neurons. Diurnal oscillation of GFAP immunoreactivity has been detected in a hypothalamic region dorsal to the suprachiasmatic nucleus and close to the third ventricle, known as the peri-suprachiasmatic area. The oscillation in GFAP is enhanced by E2 administration to ovariectomized rats, which also causes an increase in LH rhythm (Fernandez-Galaz et al. 1999b). These results suggest that the peri-suprachiasmatic area could be an important locus for structural remodeling linking circadian rhythms with the estrogen-induced LH surge. Neuron-glial remodeling occurs in other brain regions involved in the regulation of GnRH neurons, such as the infundibular neurons of monkeys (Naftolin et al. 1993) and the hypothalamic arcuate nucleus of rodents (Olmos et al. 1989).

The arcuate nucleus exhibits a natural phasic synaptic and glial remodeling that is linked to hormonal variations during the ovarian cycle. The number of axo-somatic GABAergic synapses on arcuate neurons falls between the morning and afternoon of proestrus, remains low until the morning of estrus and then rises to baseline conditions by the morning of metestrus. The fluctuation in the number of axo-somatic synaptic profiles cannot be ascribed to changes in the size of the synaptic terminals or to modifications in the perimeter of arcuate neuronal somas, but reflects a modification in the number of terminals contacting the somas (Fig. 2). On the other hand, since the changes in synapses are not accompanied by degeneration, the reduction in the number of synaptic contacts on the day of proestrus could involve a retraction of the synaptic terminal or a displacement of synapses from the soma to the neurites rather than a degenerative loss (Olmos et al. 1989, Garcia-Segura et al. 1994). In addition, synapses on dendrites also vary during the estrous cycle. Synapses on dendritic spines, probably glutamatergic, undergo a highly significant increase in number on the afternoon of proestrus, remain high on the day of estrus and return to the basal level for the next 2 days (Csakvari et al. 2007). The surge of LH on the afternoon of proestrus is thus coincident with the modification of synaptic inputs on arcuate neurons. The transient decrease in the number of inhibitory GABAergic synapses together with the transient increase in excitatory inputs on dendritic spines is consistent with the observation that E2 induces an increase in arcuate neuronal firing that is temporally correlated with the release of LH during the ovarian cycle (Yeoman & Jenkins 1989, Kis et al. 1999).

Since arcuate neurons appear to be involved in the control of GnRH secretion, it is conceivable that the observed synaptic modifications brought about in part by glia have an important relationship with the estrogen-induced gonadotropin surge. Indeed, gonadal steroids appear to play a fundamental role in the induction of synaptic remodeling in the arcuate nucleus. Studies in ovariectomized rats showed that the administration of a single dose of E2, resulting in plasma levels of the hormone similar to those detected during proestrus,
induces a reversible decline in the number of arcuate GABAergic axo-somatic synapses and a parallel increase in the number of excitatory synapses on dendritic spines (Parducz et al. 1993, 2002, Perez et al. 1993), further indicating that the final effect of E2 is to decrease inhibition and increase excitation of arcuate neurons. This was corroborated by the use of electrophysiological recordings that revealed an increased frequency of neuronal firing in a subpopulation of arcuate neurons in response to E2 (Parducz et al. 2002). These results suggest that the synaptic changes detected in arcuate neurons during the estrous cycle are driven by the rise in E2 plasma levels that occur during proestrus. Furthermore, the simultaneous administration of progesterone and E2 to ovariectomized rats, a treatment known to inhibit the ability of estrogen to evoke LH surges (Banks & Freeman 1980, Barraclough et al. 1986), inhibits the effect of E2 on arcuate synapses (Perez et al. 1993). This finding further supports the concept that estrogen-induced reorganization of synapses in the arcuate nucleus is involved in the hypothalamic control of GnRH and the preovulatory surge of gonadotropins. In addition, at least some of the arcuate neurons involved in the synaptic remodeling send axons to the median eminence and are probably neurosecretory neurons (Parducz et al. 2003) that may be involved in the release of prolactin or other pituitary hormones. Since the arcuate nucleus is a key neuroendocrine control center that is involved not only in the regulation of reproduction, but also in growth, energy balance, and food intake, changes in arcuate neuronal activity in response to modulation of astrocyte morphology during the estrous cycle may have a broad physiological impact.

As mentioned before, gonadal steroids also affect astrocytic morphology in the rostral preoptic area, but the plasticity in this region is interestingly different from that observed in the arcuate nucleus. Astrocytic processes increase in the arcuate nucleus but decrease in the rostral preoptic area on the afternoon of proestrus, in association with the increase in LH release (Cashion et al. 2003). In the rostral preoptic area, astrocytic processes contact GnRH neurons and, interestingly, the number of synapses on the soma of GnRH neurons, at least in monkeys, increases in association with the peak in LH release (Witkin et al. 1991), while the number of axo-somatic synapses decreases in the rat arcuate nucleus at this time and after E2 treatment. E2 also increases glial ensheathing of neuronal somas and reduces the number of axo-somatic in the infundibular nucleus of monkeys (Naftolin et al. 1993), which is functionally homologous to the arcuate nucleus of rodents. Therefore, glial and synaptic plasticity have similar characteristics in rodents and monkeys but different timing in the arcuate/infundibular nucleus and the rostral preoptic area. A similar situation occurs in the median eminence: on the afternoon of proestrus, tanycytic processes retract, allowing the contact of GnRH terminals with portal capillaries and the release of GnRH (King & Letourneau 1994, Prevot et al. 1999). Thus, in the median eminence, as in the rostral preoptic area, glial processes retract during the same phase of the estrous cycle quickly followed by the extension of glial processes in the arcuate nucleus. Furthermore, as mentioned before, the number of axo-somatic synapses increases in the rostral preoptic area but decreases in the arcuate nucleus during proestrus. Thus, gonadal hormones facilitate glial and synaptic plasticity in different brain regions during the estrous cycle and do so in a manner that is specific and appropriate to the region. According to this interpretation, gonadal hormones will not induce the growth or the retraction of glial processes or the connection or the disconnection of synapses per se. Rather, the role of hormones will be to serve as permissive factors allowing a coordinated plasticity in response to the functional demands that allow glia and neurons to adopt different characteristics in different brain regions.

One of the questions still under debate is whether the glial changes that are linked to E2-induced synaptic plasticity are the result of direct hormonal effects on the glial cells or if they are neurally mediated. Astrocytes are influenced by their neuronal environment, either by direct contact or by soluble factors released by neurons. Thus, the effect of E2 on arcuate astroglia may depend, at least in part, on neurons bearing hormone receptors that are abundant in the arcuate nucleus. Alternatively, the hormone may act directly on arcuate glial cells. Indeed, several laboratories have reported the expression of estrogen receptors (ERs) in the glial cells (see Garcia-Ovejero et al. 2005, for review), including astrocytes located in the hypothalamus (Langub & Watson 1992, Donahue et al. 2000, Kuijjer et al. 2002). Langub & Watson (1992) reported the existence of glia immunoreactive for ERα in the guinea pig hypothalamus, using electron microscope immunocytochemistry, and immunohistochemical analyses have revealed the existence of astrocytes immunoreactive for ERα in the human hypothalamus (Donahue et al. 2000, Kuijjer et al. 2002). In addition, Gudino-Cabrera & Nieto-Sampedro (1999) detected immunoreactivity for ERα in rat tanycytes. Double-labeled immunofluorescence for ERα or β isoforms and GFAP has also demonstrated ERα-containing astrocytes in the adult rat hypothalamic arcuate nucleus (unpublished results; Fig. 3). This finding raises the possibility that estrogen has direct effects on astrocytes. Astrocytes immunoreactive for the androgen receptor have also been previously reported in the adult rat arcuate nucleus (Lorenz et al. 2005). On the other hand, there is evidence that soluble factors, such as growth factors and neurotransmitters, and adhesion molecules may mediate neuron-to-glia communication in the hypothalamus and the median eminence. We will examine this question in detail in the next section.
Cellular and molecular mechanisms involved in the neuron–glia interactions associated with GnRH regulation

The glial cells express neurotransmitter receptors and respond to neuronal activity and to the release of neurotransmitters. Jessica Mong and her colleagues have provided evidence that GABA plays a role in the sexual differentiation of astrocytes in the arcuate nucleus (Mong et al. 2002), suggesting that GABA release by arcuate neurons may affect astrocyte morphology. In addition, E2 increases the glial expression of glutamine synthetase, which facilitates the conversion of glutamate to glutamine. Glutamine may then be used by neurons for glutamate synthesis, which in turn may affect neuronal and glial function (Blutstein et al. 2006, Mong & Blutstein 2006). The laboratory of Sergio Ojeda has provided detailed information on soluble factors released by hypothalamic glia that may affect neurons and other glial cells to regulate GnRH release and may potentially affect glial and neuronal remodeling in the arcuate nucleus. These include growth factors, such as transforming growth factors (TGFs) and the neuregulins, which are produced in hypothalamic astrocytes and act in a paracrine or autocrine manner on the same cell types to elicit the production of other soluble factors, such as prostaglandin E2, which stimulates GnRH secretion upon binding to specific receptors on GnRH neurons (Ojeda et al. 2000).

TGFα is one of the factors involved in the control of GnRH release, via the release of prostaglandin E2 and TGFβ1. During the period encompassing the preovulatory surge of gonadotropins, there is an enhanced expression of TGFα in hypothalamic astrocytes, followed by an increase in the expression of prostaglandin E2 and TGFβ (Ma et al. 1992). TGFα released by astrocytes act on tanycytes located in the arcuate nucleus and the median eminence. In vitro, E2 increases the expression of TGFα in hypothalamic astrocytes (Galbiati et al. 2002) and tanycytes in primary cultures respond to TGFα, via the activation of erb-1 receptors, by releasing prostaglandin E2 and TGFβ1 (Prevot et al. 2003). Cultured tanycytes respond to TGFα and TGFβ1 with opposite morphological modifications: TGFβ1 induces the retraction of tanycytic processes, whereas short exposure to TGFα increases the outgrowth of tanycytic processes and the migration of tanycytes, which show remarkable changes in motility, extending and retracting filopodia as they migrate outward from their initial site of seeding. However, prolonged exposure to TGFα, for more than 12 h, results in the retraction of tanycytic processes, an effect mediated by TGFβ1 (Prevot et al. 2003). Therefore, TGFα may regulate extension and retraction of tanycytic processes by direct actions and by actions mediated by TGFβ1, respectively, imitating the plastic morphological changes that occur in these cells during the estrous cycle (Fig. 4). Tanycytes can also participate in the release of GnRH to the portal blood by providing other soluble...
mediated by TGFβ. The retraction of tanycytic processes by direct actions and by actions of growth and retraction of tanycytic processes (3). Therefore, TGFβ regulates the extension and retraction of tanycytic processes by direct actions and by actions mediated by TGFβ1 respectively.

**Figure 4** TGFβ acts on neuro-glial plasticity that is under the influence of estrogen was suggested by *in vitro* studies on hypothalamic monolayer cultures. Immunostaining of these cultures with an antibody that specifically recognizes PSA-N-CAM resulted in prominent labeling of neuronal membranes. E2 induces marked changes in the shape of astrocytes in these cultures. Interestingly, the effect of E2 on the morphology of astrocytes was blocked when polysialic acid was removed from PSA-N-CAM using a bacterial endo- neuraminidase (Endo-N) that specifically removes polysialic acid from the cell surface (Garcia-Segura et al. 1995a). While these results suggested that PSA-N-CAM may be crucial for estrogen-induced neuro-glial plasticity, the direct proof came from studies carried out by Hoyk et al. (2001), showing that either the intracerebroventricular infusion of antibodies raised against PSA, or the microinjection of Endo-N directly over the arcuate nucleus, blocked the plastic remodeling of arcuate synapses induced by E2. Therefore, PSA is a necessary prerequisite for estrogen-induced phasic remodeling of synapses in the adult female arcuate nucleus. Other adhesion molecules, in addition to PSA-N-CAM, may be important for the hormonally driven neuro-glial plasticity in the arcuate nucleus. Indeed, immunoreactivity for several cell adhesion molecules, such as F3/contactin and its ligand, the matrix glycoprotein, and tenascin-C, has been detected in the adult hypothalamic-neurohypophysial system and Ojeda and his collaborators (Mungenast & Ojeda 2005, Ojeda et al. 2006) have identified several additional adhesion molecules that may participate in neuron–glia interactions. Using DNA microarrays of genes expressed in the hypothalamus of female rhesus monkey at different phases of pubertal development, these investigators have identified three families of adhesion molecules that may participate in the regulation of cell-to-cell adhesion and at the same time interact with intracellular signaling (Mungenast & Ojeda 2005, Ojeda et al. 2006). These molecules, which include components of the contactin-dependent neuronal–glial adhesiveness complex, may regulate the remodeling of tanycytes and the associated GnRH terminals in the median eminence. In addition, these investigators, using quantitative proteomics, have identified synaptic cell adhesion molecule (SynCAM), an immunoglobulin-like adhesion molecule required for synapse formation, as another important molecule involved in the communication of glial cells and GnRH neurons (Ojeda et al. 2006). SynCAM expression is decreased in DNeerbB4 mice that lack a functional component of the necessary growth factor receptor signaling complex and have delayed puberty. SynCAM molecules in the membranes of glial cells may establish homophilic interactions with other SynCAM molecules expressed by adjacent neurons. In contrast, contactin expressed by GnRH neurons and axons may establish heterophilic interactions with the short form of the receptor protein for tyrosine phosphatase β located in the glial membranes. This receptor activates signals, including the excitatory neurotransmitter glutamate (Roth et al. 2006).

Adhesion molecules may also be involved in the plastic neuron-glial remodeling in the arcuate nucleus and the median eminence during the estrous cycle. In adults, the arcuate nucleus and the median eminence express high levels of polysialylated neural cell adhesion molecule (PSA-N-CAM; Bonfanti et al. 1992), a form of N-CAM that reduces cell adhesion and allows cellular morphological plasticity. High immunoreactivity for PSA-N-CAM has also been detected in the region of the GnRH pulse generator of the monkey (Perera et al. 1993), a hypothalamic zone in which the number of axo-somatic synapses changes in response to varying gonadal steroid levels (Witkin et al. 1991, Perera & Plant 1997). PSA-N-CAM immunoreactivity increases in the median eminence in the proestrus phase of the cycle when compared with the diestrus phase in rats (Kaur et al. 2002, Parkash & Kaur 2005). A role for PSA-N-CAM in neuro-glial plasticity that is under the influence of estrogen was...
intracellular signaling and transmits contact information to the glial cells after the interaction with contactin.

The genomic and proteomic analyses carried out in the laboratories of Ojeda et al. have identified several other genes that may potentially be involved in the initiation of the structural and functional neuro-glia remodeling of the median eminence at puberty and during estrous cyclicity, although its functional significance is still uncertain. Some of these genes may act as master genes or ‘upper echelon’ genes, which coordinate the expression of a network of other regulatory genes and maintain the hierarchical structure of the network. Among the candidates to function as upper echelon genes, three are of particular interest: Oct2 (octamer binding protein-2), thyroid transcription factor-1 (TTF1), and enhanced at puberty (EAP-1). Oct2 is a transcriptional regulator of the POU domain family of homeobox-containing genes, which may regulate TGF-β and SynCAM transcription. The expression of Oct2 increases in the hypothalamus during juvenile development and the blockade of Oct2 synthesis delays the age at first ovulation. In contrast, sexual precocity is associated with increased hypothalamic expression of Oct2 (Ojeda et al. 1999, 2006). TTF1, the second candidate for upper echelon gene is, like Oct2, a homeobox gene. TTF1 enhances the transcriptional activity of genes that facilitate puberty, such as GnRH, ERBB2 (erythroblastic leukemia viral oncogene homolog 2), and KiSS1 (human melanoma metastasis suppressor), and suppresses the expression of genes inhibitory to the pubertal process, such as the preproenkephalin gene. TTF1 is expressed by GnRH neurons and tanycytes and its expression increases at puberty in the hypothalamus. TTF1 disruption is associated with delayed puberty, disruption of initial estrous cyclicity and decreased reproductive capacity (Ojeda et al. 2006). The third candidate, EAP-1, encodes a nuclear protein expressed in GnRH neurons and in neuronal subpopulations involved in the control of GnRH neurons, such as glutamatergic, GABAergic, proenkephalinergic, and KiSS1 neurons. Hypothalamic EAP-1 mRNA levels increase in both monkeys and rats during female puberty. Similar to TTF1, EAP-1 enhances the transcriptional activity of genes that facilitate the initiation of puberty and suppresses the expression of genes that inhibit the pubertal process and its knocking down in the hypothalamus delays puberty and disrupts estrous cyclicity (Ojeda et al. 2006). It is tempting to speculate on the possibility that Oct2, TTF1, and EAP-1, acting as upper echelon genes, may coordinate the plastic functional and structural neuro-glial reorganization of the median eminence and the hypothalamus, including the reorganization of synaptic connectivity in the arcuate nucleus, associated with GnRH release.

Gonadal hormones activate the reorganization of tanyctyes and GnRH axons at puberty. Tanyctyes, which express ERs (Gudino-Cabrera & Nieto-Sampedro 1999), may be one of the direct cellular targets of gonadal hormones for the initiation of these plastic changes. In addition, tanyctyes are also a target for other hormones that may influence pubertal onset. For example, tanyctyes express receptors for insulin-like growth factor-I (IGF-I), which may contribute to the initiation of puberty by the activation of GnRH release. IGF-I acts on the regulation of GnRH release both as a hormone and as a local paracrine or autocrine factor. Gore et al. have shown that IGF-I is expressed by GnRH cells and that the expression of IGF-I in GnRH ‘cells is increased at puberty (Miller & Gore 2001, Daftary & Gore 2003, 2004). Furthermore, in female rats, during the peripubertal period, IGF-I increases GnRH synthesis in the rostral preoptic area, where GnRH neuronal somas are located. These findings strongly suggest that IGF-I, produced by GnRH neurons, acts as an autocrine or paracrine factor to enhance GnRH release at puberty (Daftary & Gore 2005). On the other hand, tanyctyes take up peripheral IGF-I by a mechanism regulated by E2 and progesterone. The uptake of blood-borne IGF-I by tanyctyes is highly enhanced at puberty in male and female rats (Dueñas et al. 1994). Peripheral IGF-I may contribute to the regulation of GnRH release at puberty by acting directly on GnRH cells or in the neuronal circuits that control the activity of GnRH neurons, such as those located in the anteroventral periventricular nucleus and the hypothalamic arcuate nucleus. Indeed, IGF-I participates in the structural remodeling of glial cells and synapses in association with GnRH release in female rats. IGF-I levels fluctuate during the estrous cycle in parallel with the release of gonadotropins (Dueñas et al. 1994). High levels of IGF-I immunoreactivity in tanyctyes are detected during the afternoon of proestrus and the morning of estrus in the arcuate nucleus of cycling female rats, whereas IGF-I immunoreactivity declines during the morning of metestrus. Therefore, IGF-I immunoreactivity in tanyctyes follows the changes in plasma levels of E2 and progesterone during the estrous cycle. The rise and fall of IGF-I levels in arcuate glia is due to fluctuations in uptake of IGF-I by tanyctyes, which express IGF-I receptors and accumulate IGF-I from the cerebrospinal fluid (Fernandez-Galaz et al. 1996). Intracerebroventricular administration of an IGF-I receptor antagonist to rats blocks the accumulation of IGF-I by arcuate nucleus tanyctyes (Fernandez-Galaz et al. 1996, 1997, Garcia-Segura et al. 1999). In addition, E2 and progesterone may control IGF-I accumulation by tanyctyes by the regulation of the expression of IGF-binding protein-2 in these cells (Cardona-Gomez et al. 2000a). IGF-I immunoreactivity increases in a dose-dependent manner when ovariectomized rats are injected with E2 and this effect is blocked by the simultaneous administration of progesterone (Dueñas et al. 1994).

The synchrony in the transient fluctuations of IGF-I levels in tanyctyes with the transient remodeling of arcuate glial processes and synaptic contacts, during the estrous cycle and after ovarian hormone administration to
ovariectomized rats, suggests a causal link (Garcia-Segura et al. 1994). Indeed, results from in vivo experiments using intracerebroventricular infusion of specific receptor antagonists have shown that both ERs and IGF-I receptor are involved in the induction of synaptic and glial modifications in the arcuate nucleus during the estrous cycle. The intracerebroventricular administration of ICI 182 780, an antagonist of ERs, blocked the decrease in the number of axo-somatic synapses and the accompanying increase in glial ensheathing of neuronal somas in the arcuate nucleus between the morning of proestrus and the morning of estrus and after the administration of E2 to ovariectomized rats. This finding indicates that estrogen-induced synaptic and glial plasticity in the arcuate nucleus is mediated by the activation of ERs. In addition, the synaptic and glial changes between the morning of proestrus and the morning of estrus and after E2 administration were also prevented by the administration of an IGF-I receptor antagonist, either alone or in combination with the ER antagonist, indicating that the E2-induced neuro-glial plasticity in the arcuate nucleus of ovariectomized rats is dependent on IGF-I receptors (Fernandez-Galaz et al. 1997, 1999a, Cardona-Gomez et al. 2000a, 2000c). Interestingly, Etgen et al. have shown that the infusion of the IGF-I receptor antagonist in the third ventricle also suppresses LH surges and estrous cyclicity and partially blocks female sexual behavior (Quesada & Etgen 2002, Etgen & Acosta-Martinez 2003, Etgen et al. 2006, Todd et al. 2007). Therefore, it seems that the hypothalamic IGF-I receptors are involved in the regulation of neuro-glial plasticity, neurosecretory activity, and reproductive function of intact rats during the estrous cycle.

Arcuate neurons express estrogen and IGF-I receptors (Garcia-Segura et al. 1997, Shughrue et al. 1997, Cardona-Gomez et al. 2000b) and may be, therefore, a direct target for both E2 and IGF-I. IGF-I may affect pre- and/or postsynaptic mechanisms, since ultrastructural studies have shown that IGF-I receptor is present both in axo-somatic presynaptic terminals as well as in neuronal somas of the rat arcuate nucleus (Garcia-Segura et al. 1997). In addition, arcuate astrocytes are also a target for IGF-I, since they also express IGF-I receptor (Garcia-Segura et al. 1997). Interestingly, studies on hypothalamic tissue fragments from ovariectomized rats have shown that IGF-I receptor activation is needed for the induction of GFAP changes by estrogen in the arcuate nucleus (Fernandez-Galaz et al. 1997). These findings suggest that E2 and IGF-I interact in the facilitation of neuro-glial plasticity in the arcuate nucleus associated with the estrous cycle.

Thus, the final glial-neuronal remodeling in the arcuate nucleus, the median eminence, and the preoptic area during the estrous cycle and puberty may be regulated by a finely orchestrated bidirectional crosstalk between neurons and glial cells, mediated by growth factors, prostaglandin E2, neurotransmitters, and cell adhesion molecules. The glial cells may coordinate different endocrine and local inputs to adapt the development and plasticity of neuronal circuits controlling reproduction to homeostatic demands. The exquisite sensitivity of glial morphology and function to a host of peripheral signals, such as gonadal steroid hormones and IGF-I, and others suggests that modulation of the architecture and chemistry of these cells plays a primary role in integrating neuronal activity in the context of cyclic neuroendocrine signaling. Further studies should determine whether upper echelon regulatory genes, such as Oct2, TTF1, and EAP-1 (Ojeda et al. 2006), are involved in the coordination of hormonal signals with the plasticity of tanycytes and GnRH axons in the median eminence, the uptake of IGF-I by tanycytes, the neuro-glial plasticity of the hypothalamic neuronal circuits regulating GnRH neurons, and the plasticity and neurosecretory activity of GnRH neurons at puberty and during reproductive cycles in the adult brain.

Acknowledgements

We acknowledge support from Ministerio de Educación y Ciencia, Spain (SAF 2005-00272) and the European Union (EWA project: LSHM-CT-2005-518245) to L M G S; Arthur J. Schmitt Fellowship to B L and National Institute of Mental Health (MH62588 and MH69995) to L L D C. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References


Horvath TL 2006 Synaptic plasticity in energy balance regulation. *Obesity* (Suppl 5) 228S–233S.


Perera AD & Plant TM 1997 Ultrastructural studies of neuronal correlates of the pubertal reaugmentation of hypothalamic gonadotropin-releasing hormone (GnRH) release in the rhesus monkey (Macaca mulatta). Journal of Comparative Neurology 385 71–82.


Perez J, Luquin S, Naftolin F & Garcia-Segura LM 1993 The role of estradiol and progesterone in phased synaptic remodelling of the rat arcuate nucleus. Brain Research 608 38–44.


Received 4 December 2007
First decision 14 January 2008
Accepted 7 February 2008